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BEHAVIOURAL CIRCADIAN RHYTHMS: A NOVEL APPROACH TO MONITOR SHEEP IN EXTENSIVE SYSTEMS AND STUDY THE DIFFERENCES BETWEEN BEEF STEERS FOR METHANE EMISSION, FEED EFFICIENCY AND GROWTH

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"Instead of sleeping as our bodies dictate, we drink another cup of coffee, turn up the radio, roll down the car window and kid ourselves that we can beat a few billion years of evolution."

(Russell G. Foster and Leon Kreitzman)

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RITMOS CIRCADIANOS COMPORTAMENTAIS: UM NOVO MODO DE MONITORAR OVINOS EM SISTEMAS EXTENSIVOS E ESTUDAR A EMISSÃO DE METANO, EFICIÊNCIA ALIMENTAR E CRESCIMENTO DE BOVINOS DE CORTE[1]

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RESUMO
As tecnologias baseadas em sensores estão cada vez mais disponíveis e podem ser usadas para coletar informações detalhadas sobre o comportamento animal. Com esta informação é possível avaliar o ritmo circadiano de variáveis comportamentais e monitorar sua resposta. A identificação de variações na resposta deste ritmo tem o potencial de detectar problemas de saúde e questões de bem-estar animal. O objetivo deste trabalho foi estudar os ritmos circadianos comportamentais como uma nova abordagem para monitorar ovelhas em sistemas extensivos e estudar a emissão de metano, eficiência alimentar e crescimento de novilhos de corte. Este trabalho foi composto por dois experimentos (ovinos e bovinos). Foram utilizados cochos automatizados e sensores de atividade baseados em acelerômetro para coletar informações detalhadas do comportamento ingestivo (bovinos) e do comportamento de atividade (ovinos e bovinos), juntamente com as características de desempenho animal. Estes dados foram utilizados para calcular a percentagem de comportamento cíclico harmônico/sincronizado a cada período de 24 h. Essa porcentagem é chamada de grau de acoplamento funcional (DFC) e é calculada com uso de um período móvel de sete dias. No experimento com ovinos, um total de 29 ovelhas Scottish Blackface foram monitoradas por quatro semanas em cada estação do ano, em sistema extensivo nas terras altas da Escócia. Dados meteorológicos foram coletados diariamente. Modelos estatísticos de regressão com efeito aleatório foram utilizados para avaliar a variação da resposta entre indivíduos. Houve uma forte dinâmica criada pelas estações do ano e pelo ciclo produtivo/fisiológico das ovelhas. Durante a primavera e o verão, o desvio padrão do DFC foi um melhor estimador do ganho de peso quando comparado ao índice de moção. A combinação da análise do DFC e o agrupamento de indivíduos com base em sua resposta às variáveis ambientais oferece potencial para obter informações relevantes para o manejo do rebanho. O experimento de bovinos foi conduzido com duas dietas contrastantes (volumoso: concentrado 8:92 e 50:50) e duas raças (40 mestiços Charolês e 40 Luing). Os padrões diurnos de ingestão e atividade foram altamente sincronizados. O ritmo circadiano da atividade foi importante para explicar as diferentes emissões de metano entre indivíduos, independente da raça ou dieta, e também teve ligação com a eficiência alimentar e o crescimento dos novilhos. Este trabalho mostra a importância dos ritmos circadianos comportamentais e como essas abordagens podem melhorar a qualidade e o significado dos dados provenientes de sensores automatizados.
Palavras chave: Comportamento animal, eficiência alimentar, emissão de metano, plasticidade fenotípica, pecuária de precisão, ritmo circadiano, sensores

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BEHAVIOURAL CIRCADIAN RHYTHMS: A NOVEL APPROACH TO MONITOR SHEEP IN EXTENSIVE SYSTEMS AND STUDY THE DIFFERENCES BETWEEN BEEF STEERS FOR METHANE EMISSION, FEED EFFICIENCY AND GROWTH [2]

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ABSTRACT
Sensor-based technologies are becoming increasingly available and can be used to gather detailed information about animal behaviour. With this information it is possible to assess animal behavioural circadian rhythm and monitor its response. Identifying breakdowns of this rhythm has the potential to detect health problems and animal welfare issues. The aim of this work was to study the behavioural circadian rhythms as a novel approach to monitor sheep in extensive systems and to study the differences between beef steers production traits, in methane emission, feed efficiency and growth. This work consisted of two experiments, one dealing with sheep in an extensive system and the other with housed beef steers. Automated feed intake equipment and accelerometer-based activity sensors were used to collect detailed information on feed intake (for cattle) and activity behaviour (for sheep and cattle), alongside animal performance characteristics. These data were used to calculate the percentage of cyclic behaviour that is harmonic/synchronized to each 24 h period as Degree of Functional Coupling (DFC) shown within rolling seven day periods. In the sheep experiment, in total twenty-nine Scottish Blackface ewes were monitored for four consecutive weeks in each season across a full year, in an extensive system on Scottish upland pastures. Weather data were collected daily. Random regression statistical models were used to assess between-individual variation in response to the weather. There was a strong dynamic created by the seasons and by the production and physiological cycle in sheep in these high latitude systems. Over the spring and summer period, the variation in the response of DFC was a better estimator of BWG (Body Weight Gain) than the use of a simple motion index. The combination of circadian rhythm analysis and the clustering of individuals into groups based around their regression response to environmental variables provides considerable potential to glean information relevant for group and individual animal management. The cattle experiment was conducted with two contrasting diets (concentrate-based and mixed diet) and two breeds (40 crossbred Charolais and 40 purebred Luing). The diurnal patterns of feeding and activity behaviours were strong and highly synchronised. Activity rhythmicity was well suited to show up differences between individual methane emissions independent of breed or diet, and it was also well related to important production traits as feed efficiency and growth of beef steers. This work shows the importance of the behavioural circadian rhythms and that these approaches may enhance the quality and meaningfulness of data coming from automated sensors.

Keywords: animal behaviour, circadian rhythm, feed efficiency, methane emission, phenotypic plasticity, precision livestock farming, sensors

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LIST OF ABBREVIATIONS

BW - Body Weight

BWG - Body Weight Gain

BV - Bunker Visits

DFC - Degree of Functional Coupling

DMI – Dry Matter Intake

FCR - Food Conversion Ratio

FD – Feeding Duration

FD $12^{th}/13^{th}$ - Ultrasonic fat depth at the 12th/13th rib

FR – Feeding Rate

ICT - Information and Communication Technology

MI - Motion Index

MBW - Metabolic Body Weight

RFI – Residual Feed Intake

STD - standard deviation

CHAPTER I

INTRODUCTION

The world's livestock systems cover a major range in level of intensity from feedlot systems to extensive grazing systems and each one has particular challenges and needs of improvement. Extensive systems have challenging environments, great difficulties to mechanise and a need of improved efficiency. Intensive systems are also continuously pushed for improved efficiency of feed and growth, reduced environmental impact with lower methane emission and improved animal welfare.

In Scotland, sheep production is mostly carried out in extensive systems. Flocks remain for a long time on natural or semi-natural pasture in the hills and uplands. Thereby, the daily monitoring and control process of flocks becomes a difficult task and the early perception of disease or stress is hardly possible. In the same context, the extensive system is largely used in Brazil and others countries. New technologies such as accelerometer-based sensors enable stockmen to gather detailed information about animal behaviour for long periods. However, there is a need of developing decision support systems which are not only capable of evaluating animal behaviour, but to convert this behavioural data into useful data to support flock management. This raises the question if circadian rhythm analysis could be used to identify changes in the behavioural circadian rhythms and therefore to detect health or stress problems before disease affects animal productivity. New analysis techniques in conjunction with sensor technology should optimize monitoring processes, reduce labour and assist decision-making in the flock management. Identifying breakdowns in behavioural circadian rhythms is one area that has been hypothesized to have potential to identify health problems and animal welfare (BERGER et al., 2003), but this has not been evaluated in extensive farming systems. Nevertheless, at first it is necessary to understand the differences between individuals within a flock and in relation to environmental factors in terms of activity rhythm throughout the year.

By contrast, finishing cattle are mainly raised in feedlot systems, which rely heavily on mechanised feeding systems. Feed efficiency and methane emissions by ruminants are currently amongst the most important factors to be studied by researchers. It is known that different diets alter ruminal methanogenic community (ZHOU; HERNANDEZ-SANABRIA; LUO GUAN, 2010) and rumen fermentation, decreasing or increasing methane emissions (DUTHIE et al., 2017; PATRA, 2014). Likewise, it is known that feed efficiency varies with animal genotype and that behaviour traits may contribute to the variation in animal efficiency (CHEN et al., 2014; NKRUMAH et al., 2007). However, this is the first work that was done to assess behavioural circadian rhythms and its relationships with feed efficiency, methane emission and growth.

Another key question is whether behavioural circadian rhythms are linked to the variation of between-animals responses, or whether they are linked to a particular period for a particular animal and its methane emission level, feed efficiency and growth. Could new sensor technology identify inefficient animals, either due to a short period of disrupted feeding or activity patterns, or more generally due to their less structured feeding or activity behaviour?

Therefore, one of the goals of this study was to evaluate the behaviour and circadian rhythms of activity and feeding of beef steers in an intensive system, using two contrasting breed types and diets, to understand the differences between individuals and its relation with production traits, such as methane emission, feed efficiency and growth. Regarding sheep in extensive systems, the goal was to analyse the activity circadian rhythm and to identify natural changes associated with environmental influences that impact on this rhythm throughout the year, as well as disturbing factors; and to study if there are links between activity circadian rhythm and sheep performance. Thus, the overall aim of this work was to study the behavioural circadian rhythms as a novel approach to monitor sheep in extensive systems and to study the differences between beef steers production traits, in methane emission, feed efficiency and growth.

The conceptual model of this Thesis is presented in the Figure 1 and involves both cattle and sheep research. Five chapters compose this Thesis. The Chapter I consist of the introduction, hypothesis, objectives and the literature review about the issues discussed throughout the Thesis. Chapter II contains a journal paper regarding the evaluation of the circadian rhythm of activity of ewes kept on semi-natural pasture in the Highlands of Scotland. The objective of this chapter is to understand the differences between individuals within a flock and in relation to environmental factors in terms of activity rhythm and body weight change throughout a full year. The journal paper in Chapter III is focused on intensive cattle finishing and it is about whether behavioural traits and circadian rhythm of activity and feeding are linked to the variation of between-animals responses, or whether they are linked to a particular period for a particular animal and its feed efficiency, methane emission level and growth. Chapter IV consists of the final considerations, references, appendices and the author curriculum vitae.

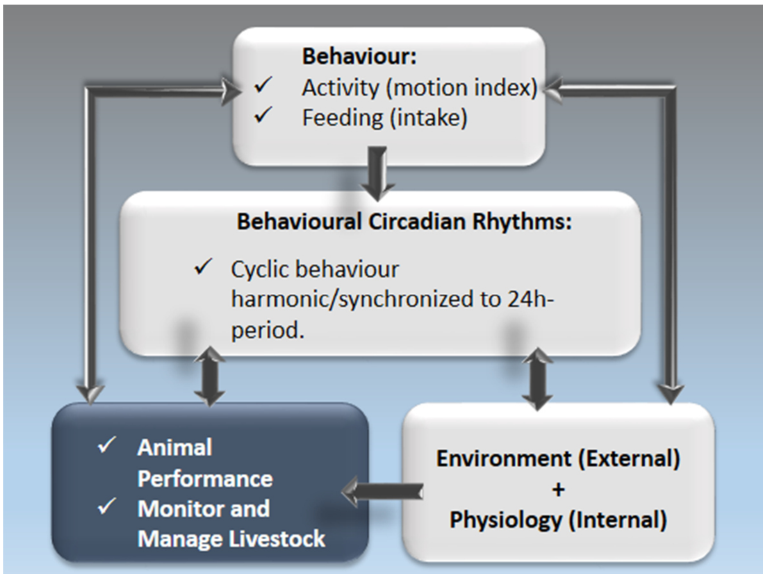

Figure 1. Thesis Conceptual Model

LITERATURE REVIEW

Precision Livestock Farming

Precision livestock farming is the use of information science and information technology to optimise management process of livestock production (BERCKMANS, 2008). Technologies such as sensors enable the gathering of detailed information about individual animals. These data are used in a computer modelling process, usually in real time, and the information can then be used for livestock production management. According to WATHES et al. (2008) precision livestock farming requires (i) continuous outputs; (ii) a mathematical model to analyse the output that respond to the input variation (biological response) and it is best if estimated at real time; (iii) a target value for each output; (iv) model-based predictive controller (Figure 2).

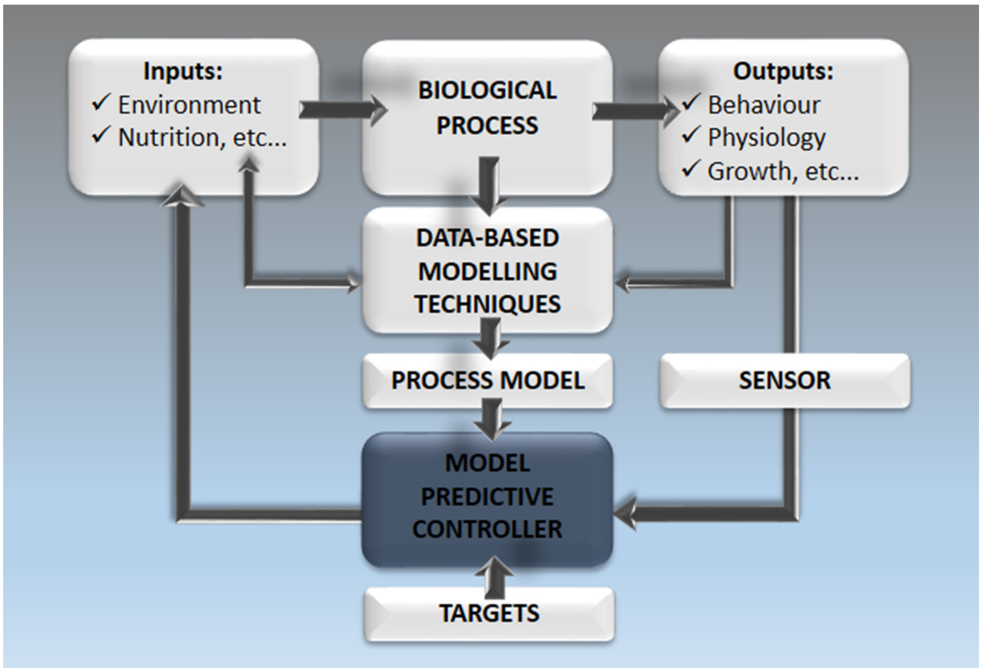

Figure 2. Schematic overview of the precision livestock farming key components to control biological processes (WATHES et al., 2008)

The model-based predictive controller would involve a number of control methods for the process input, using continuous feedback of the process output. Target values will help to control the animal processes and these values will be set according to the production focus that could be growth, feed efficiency, methane emission, health, among others environmental and economic issues. The trajectory to achieve the target is also very important in precision livestock management and it is the track of the process output that is controlled with the inputs and their adjustments in a day-by-day level and it may be even a minute-by-minute level (WATHES et al., 2008).

Data collection with the use of sensors can be simple, low cost and might be used on animals in both intensive and extensive systems. The collection is continuously improved through better devices and communication. However, the entire scheme of the precision livestock farming is complex and requires computer-modelling techniques in regards to the biological processes. Therefore, the electronic data can be collected and converted into useful information linked to biological processes. Thus, a substantial research and development is need to analyse the concurrently collected sensor data and biological or production data before any uptake can be achieved.

Contrasting systems, such as intensive and extensive systems bring different challenges and quantities of input and output process data. For example, to measure individual feed intake using automated feeders, the data collected is mostly restricted to housed animals and requires specialized instrumentation (POMAR; LÓPEZ; POMAR, 2011). Data are more easily collected in intensive systems, with a rich data basis of growth rates, feed intake, methane emission, feed efficiency and even detailed behavioural data. In contrast, the traditional extensive systems are data poor and animal variables (data outputs), such as growth rates, feed intake, methane emission among others are difficult to measure, as data collection opportunities are highly limited. In addition, other inputs, such as environmental and/or nutritional inputs need to be measured. These difficulties make extensive systems an even bigger challenge.

Precision farming offers the potential to explore multiple levels of heterogeneity and nonlinear responses in the animal production processes to provide improved animal health and welfare, decrease labour, increase profitability, and reduce environmental impacts (LACA, 2009). Other advantages of electronic motoring systems are that data can be continuously collected without the disturbance of human handling and it is less stressful for the animals. These technologies potentially allow management of livestock on an individual animal level, although its application may be more general focusing on the pen, flock or herd.

The use of activity sensors to measure animal behaviour

Researchers start to use electromechanical and electronic tools to measure animal behaviour since five decades ago, with failure and success. POWELL (1968) was the first work that used pedometers to study the value of these instruments for measuring distances walked, and to investigate the importance of weather on the distance travelled by sheep. The author observed that the pedometers gave rise to inaccurate records of about 50% of the instruments studied and concluded that the disadvantages of the pedometer outweigh its advantages. However, some decades latter sensors were being developed with potential to gather a wide range of information for example climatic conditions, feed intake, growth rate, animal behaviour and product quality (FROST et al., 1997). Despite of the sensors development the practical applications were slow and researchers start to notice the importance on developing systems to process and utilise the information, considering that the raw data, on its own, is of limited value (FROST et al., 1997; MOTTRAM, 1997). For example, MOTTRAM (1997) studied the automatic monitoring of the health and metabolic status of dairy cows and concluded that although the technology to sense particular conditions could be developed, it was not clear whether veterinary and nutritional models were sufficiently well developed to allow decisions to be made based on the sensor data alone.

UMSTÄTTER; WATERHOUSE; HOLLAND (2008) demonstrated the potential of sensor-based to measure sheep behaviour. The authors observed that with the sensor raw data was possible to distinguish between two categories of behaviour “active” and “inactive”. ALVARENGA et al. (2016) studied the accuracy, sensitivity, specificity and precision with which tri-axial accelerometers (attached to a halter on the under-jaw of each animal) can identify sheep behaviour at pasture and concluded that the model predicted grazing and ruminating behaviour highly accurately. Sensor-based technology has been largely used in current researches and has shown to be an accurate method to measure animal behaviour as motion index, steps, lying bouts, time spending lying and standing (ALVARENGA et al., 2016; BERGER et al., 2003; KOKIN et al., 2014; MARSCHOLLEK, 2015; RICE et al., 2016; UMSTÄTTER; WATERHOUSE; HOLLAND, 2008).

Timing mechanism of the biological cycle

The living organisms exhibit a timing mechanism of the biological cycle of life and have plenty of ultradian, circadian and seasonal rhythms (FOSTER; KREITZMAN, 2005; WOOD; LOUDON, 2014). This rhythmicity is represented by a change that is repeated with a similar pattern during a determined period (rhythmic cycle). When the period is shorter than 20 h it is an ultradian rhythm If the period is about 24 h (varying between 20 and 28 h) it is considered a circadian rhythm and if it is greater than 28 h it is called an infradian rhythm (KOUKKARI; SOTHERN, 2006). The presence of functional rhythmic variations is a well-documented phenomenon in all physiological levels of organization and especially for the operation of the cellular components, tissues and organ systems (FOSTER; KREITZMAN, 2005; KOUKKARI; SOTHERN, 2006). The circadian rhythm of physiological variables follows a “free-running” rhythm with approximately 24 hours pattern if the organism is kept in constant light or darkness. The “free-running” rhythm occurs because the physiological timing is genetically programed and this rhythmicity was an outcome of the evolutionary process, responding primarily to the photoperiod of light and darkness (Foster and Kreitzman, 2005; Wood and Loudon, 2014). However, this dynamic is not just a physiological issue, as it is also influenced by environmental stimuli such as changes in light, temperature, precipitation, tides and winds; and thus the circadian rhythm brings into harmony the entire organism with its environment, allowing adaptation (BLOCH et al., 2013; KOUKKARI; SOTHERN, 2006; WOOD; LOUDON, 2014). Thus, healthy and adapted organisms are believed to have a good coordination between different physiological variables and behaviour (BERGER et al., 2003; MARTINO et al., 2008).

Degrees of Functional Coupling (DFC) as a measure of behavioural circadian rhythms

Healthy organisms are believed to have a good coordination between different physiological variables and behavioural functions (BERGER et al., 2003; MARTINO et al., 2008). The Degree of Functional Coupling (DFC) is a parameter for measuring rhythmicity of behavioural variables. The DFC expresses the percentage of cyclic behaviour that is harmonic/synchronized to each 24 h period.

Therefore, DFC expresses the percentage of the circadian and harmonic ultradian components in relation to all rhythmic components of a spectrum (SCHEIBE et al., 1999). In other studies, DFC is calculated within rolling seven-day periods and may vary from 0% up to 100%, where low DFCs indicate lower synchronization between the organism's physiology and its environment and high DFCs shows higher synchronization (BERGER et al., 2003; SCHEIBE et al., 1999).

Breakdowns in the DFC response may indicate that the animal is passing through a disturbing period. The disturbance may be a physiological issue (illness or changes in physiological status – internal stressor) or an environmental problem (external stressor) and any of these stressors may affect the intensity and time structure of behaviour (BERGER et al., 1999, 2003, SCHEIBE et al., 1998, 1999). Berger et al. (2003) observed that the DFC based on activity measurements has the potential to identify and evaluate disturbances in behavioural rhythmicity. In their study, different species of herbivores presented significantly lower DFCs in periods of adaptation (translocation from a zoo to a semi-reserve), stress, changes in physiological status (such as parturition), injury, parasitic infection or other illnesses and even under social interaction (SCHEIBE et al., 1999). The same authors found that the DFC is high in well-adapted, healthy and undisturbed individuals.

Between individual variation on behavioural traits

Environmental influences may regulate the behavioural responses and within a population the animals may differ from each other in the way they deal with momentary changes in environment conditions (DINGEMANSE; WOLF, 2013; FAVREAU et al., 2014; NUSSEY; WILSON; BROMMER, 2007). This ability of individuals (or genotypes) to adapt phenotypic traits in response to their environment is called phenotypic plasticity and has been largely studied as a mediator of evolutionary changes (FOSTER; SIH, 2013; PIGLIUCCI, 2001). In a review, DINGEMANSE and WOLF (2013) assert that environmental variables in combination with competition amongst individuals, as well as social interactions and animal state, may build up differences between individuals in their plasticity. Behavioural plastic changes can be short or long-term (KOLB; GIBB, 2014). Animal phenotypic plasticity can be maladaptive (non-adaptive) or adaptive and the variation between individuals plasticity may affect the entire population and consequently its stability and persistence (DINGEMANSE; WOLF, 2013; NUSSEY; WILSON; BROMMER, 2007; WILSON, 1998).

Random regression models to access the between-individual variation

Most evolutionary ecology studies have used simple linear regression model to understand and identify population level response to the environment at both phenotypic and genetic levels. The coefficient of the linear regression of the phenotype with the environmental variable of interest is described as reaction norm (PIGLIUCCI, 2001). In a study, NUSSEY; WILSON; BROMMER (2007) asserted that little is known about the prevalence, and evolutionary and ecological causes and consequences of variation in life history phenotypic plasticity in the wild. Thus, these researchers underlined a framework using random regression models to access not only the population level responses, but also the between-individual variation in life history plasticity. To understand the population level responses to the environment and simultaneously to identify animals that deviated from the population response is of great value to understand the within- and between-animal variation.

The approach made by NUSSEY; WILSON; BROMMER (2007) was to model individual reaction norms considering variation at the level of the individual phenotype, and contributions to this from both genetic and non-genetic effects. Considering that a population-level response to the environment will depend on individual-level plasticity, the population response is modelled by all animal responses and then each animal response (as a random effect) is compared with the population response and its deviation is calculated. Thus, the population-level phenotypic response to the environment can be broken down based first on whether or not there is variation in plasticity among individuals at a phenotypic level, and secondly, the relative contribution of genetic and non-genetic sources to variation to phenotypic variation in reaction norms (NUSSEY; WILSON; BROMMER, 2007). No study involving farmed animal was found in the literature.

Sheep behaviour and weather influences

Sheep are diurnal animals and, as well as all herbivores, these animals exhibit higher activity during daylight and it can be assumed that most of the activity behaviour is associate with grazing. UMSTÄTTER; WATERHOUSE; HOLLAND (2008) studied behaviour types associated with grazing of extensively managed ewes in Scotland and observed that ewes were active for over 60.5% of the daytime and spent 59.9% of the activity time grazing and only 0.6% walking (without grazing). Therefore, the authors state that it is possible to define two behaviour categories, active (mainly grazing) and inactive (mainly recumbent).

WARREN; MYSTERUD (1991) studied activity patterns of sheep on coniferous forest range in southern Norway over the summer and observed that most of the flock was inactive for 2-5 hours in the middle of the day and rumination occurred over lay idling time. The authors also observed that overnight sheep stood in groups with a few night activity. The activity pattern was rhythmic and consistent throughout the summer season, though it was affected by both weather conditions and day length. Cold (< 10°C), wet weather or foggy conditions reduced the flock overall activity. When the days became shorter there was a delay in the starting activity in the morning and earlier camping in the evening. The study of BERGGREN-THOMAS; HOHENBOKEN (1986) agree that cool, cloudy days have less distinct grazing periods by sheep than warm, sunny days. In a literature review, DWYER; BORNETT (2004) explain that in hot weather sheep adjust the diurnal patterns to the coolest times of the day. When temperatures are low, wind speed is high and/or it is raining, lambs and lactating ewes make use of shelter. These are important mechanisms that help these animals to deal with thermal extremes and decrease thermoregulation risk. In addition, the authors emphasis that sheep exposed to pastures lacking in shelter may experience thermal distress. The energy costs of cold exposure must eventually be reflected in a poor animal performance or increased food intake (SLEE, 1971).

CHAMPION et al. (1994) also observed the effects of rainfall in the daily pattern of grazing behaviour of sheep. These authors noticed that heavy rain (greater than 1 mm/h) reduced eating activity during the hours of the day that without rainfall eating activity would have been high. However, if rainfall occurred when the eating activity was normally low, it had little effect. Other environmental factor that the authors imply to affect grazing behaviour in this study was the declining sward height, but the authors had no firm evidence about it. In addition, others researchers observed that weather had a greater effect than forage availability on grazing time (BERGGREN-THOMAS; HOHENBOKEN, 1986).

Methane emission and feed efficiency of beef cattle

The level of methane emission by ruminants rely on many factors, where different levels of energy alter the ruminal methanogenic community (ZHOU; HERNANDEZ-SANABRIA; LUO GUAN, 2010) and rumen fermentation, decreasing or increasing methane emissions (DUTHIE et al., 2017; PATRA, 2014). Methane production also depends on Dry Matter Intake (DMI) and Gross Energy Intake (GEI), but not on these alone, as the type of energy-supply and animal physiological state differences are very important and also affect increasing or decreasing methane emission (STERGIADIS et al., 2016). Different types of diets, crudely means different routes of fermentation. High concentrate diets have high starch and thus, higher production of propionate, which leads to less hydrogen production and less hydrogen available for methanogenesis. While, forage diets have higher neutral detergent fibre than high concentrate diets, which leads to a higher production of acetate in relation to the other SCFA, consequently higher production of hydrogen increases the methanogenesis (ROOKE et al., 2014). Feeding behaviour also plays a role in this dynamic and the methane emission is positive correlated with feeding duration (NKRUMAH et al., 2014).

Genetic parameters are also important. Roehe et al. (2016) observed large phenotypic ranges in methane emissions between the extremely low and high emitting animals within the same breed. The authors found a significant differences on methane yield and on archaea:bacteria ratios between sire progeny groups and some of these differences were even larger than the differences between the diets. Thus, the authors concluded that these results indicate a substantial genetic influence of the host animal and that the mechanisms behind genetic influences of the host on the microbial community composition are expected to be based on many different biological factors. Many factors affecting the methane emission creates a strong dynamic that provides a big challenge to an accurate prediction of methane emissions. No study involving relations between methane emission and behavioural circadian rhythms was found in the literature.

Residual feed intake (RFI) is the difference between the actual and expected feed intake of an animal based on its body weight and growth over a specified period. Likewise, animal RFI depends on many physiological mechanisms and the reasons for variation in animal efficiency are still not well understood (RICHARDSON; HERD, 2004). Studies have been shown that high efficiency steers have similar growth rates, final live weights and carcass traits, but have lower DMI and lower RFI than low efficiency steers (GOMES et al., 2012; RICHARDSON; HERD, 2004). Feed efficiency varies with the animal phenotype and genotype and feeding behaviour traits may contribute to the variation in animal efficiency (CHEN et al., 2014; NKRUMAH et al., 2007). No study involving relations between feed efficiency and behavioural circadian rhythms was found in the literature.

HYPOTHESES AND OBJECTIVES

Hypotheses

1. The use of the accelerometer-based sensor to evaluate the activity behaviour of sheep carried out in extensive systems make it possible to analyse activity circadian rhythm and to identify the natural changes associated with environmental influences that act on this rhythm throughout the year, as well as, the disturbing factors. Activity circadian rhythm is linked to sheep performance.
2. There are relationships between behavioural traits and the variation of between-animal responses, such as feed efficiency, methane emission and growth of beef steers.
3. There are relationships between behavioural circadian rhythms and the variation of between-animal responses, such as feed efficiency, methane emission and growth of beef steers.

Objectives

a. To evaluate the activity circadian rhythm of ewes carried out in extensive systems and identify the natural changes of this rhythm;
b. To potentially identify links between the behavioural circadian rhythm and performance of the ewes;
c. To potentially identify links between behavioural traits and feed efficiency, methane emission and growth of beef steers;
d. To potentially identify links between behavioural circadian rhythms and feed efficiency, methane emission and growth of beef steers;
e. To understand whether the behavioural circadian rhythms are linked to the variation of between-animals responses, or whether it is linked to a particular period for a particular animal and its feed efficiency, methane emission and growth.
f. To determine if it is possible to develop a decision support system which is capable of evaluating animal behaviour, identifying changes in the behavioural circadian rhythm and in real time detecting health or stress problems before it affects animal productivity;
g. To determine if a decision support system could evaluate the animal behavioural circadian rhythm and identify inefficient animals, either due to a short period of disrupted feeding or activity patterns, or more generally to their less structured feeding or activity behaviour.

CHAPTER II

Article prepared according to the norms of the journal Applied Animal Behaviour Science (Appendices)

The use of circadian rhythm of activity and random regression model as a novel approach to monitor sheep in an extensive system

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Abstract

Sensor-based technologies are becoming increasingly available and can be used to gather detailed information about animal activity. With this information it is possible to assess the animal activity circadian rhythm and monitor its response. Identifying breakdowns of this rhythm has the potential to detect health problems and animal welfare issues. The aim of this study was to identify the natural changes due to environment influences that act on this rhythm throughout the year, as well as, disturbing factors; and links between this rhythm and sheep performance. Scottish Blackface ewes of different ages and body condition scores were used. The animals were monitored for four consecutive weeks in

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each season, in extensive systems on Scottish upland pastures, and without human handling. The number of ewes were: winter (21), spring (24), summer (22), Autumn (24). An accelerometer-based sensor was fitted with a collar to collect the motion index for every minute (uninterrupted) during each seasonal period. These data were used to calculate the percentage of cyclic behaviour that is harmonic/synchronized to each 24 h period as Degree of Functional Coupling (DFC) shown within rolling seven days periods. Low DFCs indicate lower synchronization. Weather data were collected daily. Reaction norm concept and random regression statistical models were used to assess between-individual variation. During the winter period, the level of the activity DFC dropped dramatically for some ewes in response to a high level of precipitation combined with the low temperatures of the winter. However, some ewes showed a reduced variation and others a greater drop than the population level. These differences in the between-individual DFC response were regarding to the individual phenotypic plasticity for the precipitation level. The overall mean for activity DFC was highest in the autumn (95.4%), however it did not differ between summer and spring (90.2% and 88.1%, respectively), but was significantly lower for the winter period (81.7%, $P<0.001$) compared with summer and autumn. Over the spring and summer periods, the variation in the response of DFC was a good estimator of BWG. It is concluded that the analysis of activity circadian rhythms using the DFC mathematical model enables a better understanding of sheep responses to weather and environmental influences, compared to the use of simple activity behaviour (motion index) alone. There was a strong dynamic created by the seasons and by the production/physiological cycle in sheep in high latitude systems. The random regression model method was effective in identifying animals that deviated from population responses.

Keywords: animal behaviour, circadian rhythm of activity, phenotypic plasticity, precision livestock, random regression, sensors

1. Introduction

In extensive production systems, animals are often kept in semi-natural habitats without close supervision for long periods of time. A telemetric monitoring system could be very important to ensure health and welfare, and provide management information for the animals. Use of ICT (Information and Communication Technology) to optimise management processes in livestock production has increased in recent years. Major developments can be found in intensive production systems and with the direct use of remotely collected behavioural traits, e. g. the use of activity to detect oestrus in dairy cows (Firk et al., 2002). However, valuable information may also be obtained by looking at synchronicity of behavioural and environmental rhythms. Monitoring the synchronicity between physiology of the individual animal and its environment could provide valuable information for management. Technologies such as accelerometer-based sensors enable us to measure detailed information about animal behaviour, such as activity and these continuous data can be used to calculate parameters describing the rhythmic structure of the behaviour.

Scheibe et al. (1999) developed a framework named Degree of Functional Coupling (DFC) for animal activity data to quantify and investigate the circadian rhythm of behavioural variables. A number of studies then showed a link between environmental influences or health issues and DFC of free ranging animals (Berger et al., 2003, 1999, Scheibe et al., 1999, 1998). However, the rapid development of new technologies that enable the acquisition and use of automatically collected activity and feeding data from individual animals makes it highly appropriate to look anew at this different way of using this type of data. Since the first series of publications, all published papers focused on wild animals (BERGER et al., 1999, 2003, SCHEIBE et al., 1998, 1999) and thus, there is a need of more studies using the DFC method in a different environment, with managed animals and looking at the relationship between DFC and production traits.

The rhythmicity of activity of each animal is unique and may vary according to short- and long-term environmental influences or its physiological status (Berger et al., 2003; Scheibe et al., 1999). Therefore, an in-depth look at the animal behavioural rhythmicity is important in order to understand whether there are relationships between activity, environmental influences and production traits.

Other recent research has been investigated how to study the complex behavioural variation between individuals within a single population (Dingemanse et al., 2010; Dingemanse and Wolf, 2013; Herczeg and Garamszegi, 2012; Nussey et al., 2007; Sih et al., 2004). Nussey et al. (2007) outlined an analytical framework using the reaction norm concept and random regression statistical models to assess the between-individual variation of 'labile' traits. In order to understand the between-individual variation of activity rhythmicity, our work, for the first time, combines DFC analysis for each animal and random regression statistical models. This enable assessment of between-individual variation of the response of DFC with respect to weather variables, and to ultimately link DFC with production traits.

The circadian rhythm of activity is a variable that has high potential to be a key indicator for the general physiologic state of animals, as its rhythmicity is an outcome of a large number of physiological processes (Piccione et al., 2005). Moreover, a strong rhythmicity of activity is known to be a characteristic of healthy and adapted organisms (Berger et al., 2003, 1999; Bloch et al., 2013). Thus, our study has the novel approach of addressing the key issue of whether the calculation and analysis of circadian rhythm through DFC of activity data from accelerometers be either complementary or better in explaining sheep behaviour and performance compared to a simple analysis of activity data through an activity motion index.

Sheep have highly seasonal production cycles and often occur in bio-geographic zones with large shifts in grass availability and light/dark cycles. Therefore, this experiment was designed to evaluate the circadian rhythm of activity of ewes in the same flock in an extensive system across all seasons without significant human intervention. Thus, the aim of this study was to understand the differences between individuals within a flock and in relation to environmental factors in terms of activity rhythm and body weight change throughout the year.

2. Material and Methods

2.1. Animal measurements

Activity was monitored in a group of Scottish Blackface ewes. The ewes covered a range of ages (2 to 6 years old) with a body condition score range of 2 to 3 points at the start of study. The ewes were kept on 23.6 ha of semi-natural pasture in the West Highlands of Scotland (SRUC, Hill & Mountain Research Centre, Scotland), latitude and longitude 56.438180 and -4.6684170. The annual precipitation is 2800 mm. Activity data were collected over four consecutive weeks, uninterrupted, in each season, across a full annual cycle, commencing in the winter. A three-way accelerometer (IceTag Pro, IceRobotics Ltd., Edinburgh, Scotland) was attached to a collar and fitted to all ewes. The collars were changed every period and ewes were therefore wearing different Icetag Pro loggers during different seasons. The IceTag Pro logger is programmed to record the g-force in three dimensions (IceRobotics Ltd, Product Guide 2010). In addition, the motion index is the average of the magnitude of acceleration on each of the three axes for each minute. A low motion index corresponded with lower activity behaviour and a higher motion index corresponded with higher activity. Weight and condition scores of ewes were taken at the beginning and end of each measuring period. A UK Meteorological Office Weather Station, based on the research farm and a distance of < 2 km away from the field location, measured the precipitation and temperatures every day.

Twenty-four ewes were measured in each of the four seasons and ewes were drawn from the same production flock kept under a similar, but larger scale, extensive system. An additional seven ewes without collars were also included in the monitoring group to ensure replacement when necessary. As some data were missing, the number of collected data sets differed between seasons. In the winter period twenty-four ewes commenced the study with loggers, however there was missing logger data for one ewe (technical failure). During the winter period two ewes died during the experimental period and three ewes died during the interval between winter and spring (non- experimental period). Replacement ewes were then used in the spring experimental period. Over the summer period there were missing logger data for five ewes (due to technical failures). Over the entire experiment data from twenty-nine ewes were used and fifteen ewes had data from all four seasons.

Winter measurements resulted in twenty-three data sets from ewes during mid-pregnancy (12^{th} of January to 10^{th} of February), under natural winter conditions, with sunrise between 07:57 – 08:46 h and sunset between 16:08 – 17:09 h. During the winter period, ewes were supplemented with two large round hay bales, one provided on 17th of January and the other on the 5th of February. Feed blocks (Rumevite Sheep, Rumenco) were also provided, at a rate of one per week, each weighing 20 kg. Human contact was avoided, except for the feed provision and daily visual checks. In spring, summer and autumn, the ewes were exclusively grazed on semi-natural pasture and there was no human contact over the experimental periods, except the regular checking of the flock. Spring measurements were performed on 22th of May to 18th of June, after parturition around the 1st of May. Out of the twenty-four ewes, sixteen were rearing lambs (six with twin and ten with single lambs) and eight ewes were without lambs. Ewes and lambs were under natural spring conditions, with sunrise between 04:28 – 04:51 h and sunset between 21:39 – 22:11 h. Summer measurements were done during late lactation (1th to 28th of August). Data were successfully collected from nineteen ewes, of which twelve were rearing lambs (six ewes with twin and six with single lambs). Ewes and lambs were under natural summer conditions, with sunrise between 05:20 – 06:14 h and sunset between 20:26 – 21:30 h. Lamb weaning took place at the end of the summer period. Autumn measurements were done one week after weaning and before mating (5th to 30th of October). Twenty-four data sets were collected from ewes in autumn, with sunrise between 07:30 – 08:22 h and sunset between 17:42 – 18:45 h.

2.2. Data analysis

2.2.1. Activity circadian and ultradian rhythms

Degrees of Functional Coupling (DFC) is a parameter, developed by Scheibe et al. (1999), used here to measure the circadian rhythmicity of behavioural variables. DFC expresses the percentage of cyclic behaviour that is harmonically synchronized to each 24 h period. To carry out the DFC analyses, preliminary analysis using different time intervals (e.g. 1, 5, 10, 15, 20 and 30 minute intervals) and different day intervals (e.g. 7 and 10 days) were performed. The data showed that 15 minute time intervals and a seven day periods of data were the most appropriate and have been used throughout this paper. Therefore, time series with 15 minute intervals were created, by taking the motion index sum of the 1 minute motion index averages within each 15 minute period, and then using the method of Scheibe et al. (1999), these data were analysed for the rhythmic components.

To reduce the noise component, a process equivalent to a moving average was used: autocorrelation functions were calculated for each seven day interval, with a shift by one day and overlap of six days. So the first seven-day interval included the first day to the seventh day of measurement; the second seven-day interval included the second day to the eighth day of measurement and so on. A power spectrum was drawn for each seven day interval using autocorrelation functions. The periodogram ordinates were calculated for all Fourier frequencies: $\omega=2\pi j/n, j=1,...,q$ with $q=n/2$ (n even) or $q=n/2-1$ (n odd), with n being the number of data points in the sample (Berger et al., 1999; Scheibe et al., 1999). The periodogram ordinates were tested for statistical significance according to the R. A. Fisher test for periodicity (Andel, 1984). The significant ordinates represent the significant periodic components (Supplementary Figure 1, a).

Harmonic periods are defined as periods which are synchronized with the day length (24 h) in relation to an integral number (1, 2, 3… etc.). Thus, 24 h divided by an integer number gives the harmonic periods (the period length of 24, 12, 8, 6, 4.8, 4, 3.4, 3, 2.6, etc. hours are harmonic). An example of a power spectrum and its significant harmonic periods is shown in Supplementary Figure 1, b. DFC is here expressed as the percentage of the circadian component and harmonic ultradian components in relation to all rhythmic components of a spectrum. Therefore, DFC demonstrates the relationship between the total intensity of significant periods that were harmonic to the circadian period (SI_{Harm}) and the total intensity of all periods that were significant (SI_{Total}) (Berger et al., 1999; Scheibe et al., 1999):

DFC (%) = (SI_{Harm}* 100)/SI_{Total}

Where, SI_{Harm} = Σ of intensities of significant periods that are harmonic to 24 hour period;

SI_{Total} = Σ of intensities of significant periods.

DFC% varies from 0% to 100%, where 0% means that only non-harmonic periods were significant and 100% means that only harmonic periods were significant (Berger et al., 2003, 1999). Low DFC indicates lower synchronization and high DFC higher synchronization to the 24 hour period. DFC was continuously calculated for all ewes, during all experimental days.

2.2.2. Differences between-individual and population response

Using the methods and analytical framework published by Nussey et al. (2007), random regression statistical models were used to assess between-individual variation (random effects) and evaluate the population responses (fixed effects) to environmental parameters. The random regression statistical models were modelled using the Mixed procedure with the Restricted Maximum Likelihood (REML) method and COVTEST statement in SAS 9.3. Thus, considering the relationship between trait y (in our case DFC) and environmental variable E (in our case precipitation and/or temperature), both measured on occasion j, at individual level y_{ij}, the response of individual I on occasion j was specified as:

$$y_{ij} = \underbrace{\mu + \beta E_j}_{\text{Fixed effects}} + \underbrace{p_i + p_{Ei} E_j}_{\text{Random effects}} + \varepsilon_{ij}$$

Where, μ = population mean on E;

β = population mean slope of y on E.

p_i = deviation from the population average intercept (response that is independent of E, represent the individual elevation);

p_{Ei} = deviation from the population average slope (response that is dependent of E, represent the individual plasticity).

E_j = Environmental variable;

ε_{ij} = residual error.

In each season, the ewes were divided into three groups based on the results of the covariance parameters for the fixed effects (DFC population intercept and slope for weather variables) and for the random effects (data for each ewe's deviation from the population intercept and slope for weather variables). The three groups were: 1) Medium Consistency: represented by ewes that did not present a significant deviation from the population intercept and slope ($P > 0.05$); 2) Low Consistency: represented by ewes that negatively deviated from the population intercept or slope ($P < 0.05$); and 3) High Consistency: represented by ewes that positively deviated from the population intercept or slope ($P < 0.05$). All ewes could be classified in this way except, over the winter period, ewe G58. Data for this ewe, for winter only, deviated from the population intercept and slope, but exceptionally the deviations were in different directions, negatively deviated the intercept (-11.0; $P < 0.001$) and positively deviated the slope (0.74; $P < 0.03$). In this case, the higher and most significant deviation was used to determine in which group this animal would be and thus, this ewe was included in the Low Consistency group. Thereafter, we will refer to the term individual phenotypic plasticity (as originally used by Nussey et al. (2007)), to support our division of individual sheep put into groups based upon the responses of their DFC data to the weather variables.

2.2.3. Others statistical analyses

The differences between the averages of the variables were analysed using the Mixed Procedure of SAS 9.3 and the *P* value of the differences of least square means were adjusted for Tukey-Kramer. DFC and its standard deviation were not normally distributed; hence, angular transformation was used. Motion index and its standard deviation were also not normally distributed; thus, square root transformation was used. Regressions with overall means were modelled in SAS 9.3 using the REG procedure. In order to allow the juxtaposition of the linear regression between DFCs and weather variables, the precipitation and temperature, daily values were displayed as moving averages for the same seven-day intervals as the DFC data.

3. Results

Body Weight Gain (BWG), Motion Index (MI), Degree of Functional Coupling (DFC%), MI and DFC% standard deviation (STD) for each season are shown in Table 1. DFC% was higher and with high consistency (as shown by STD) during the autumn whereas it was lower and with lower consistency during the winter. Moreover, BWG was lowest during the winter period compared to the other seasons.

Table 1 Body Weight Gain (BWG), Motion Index (MI), Degree of Functional Coupling (DFC), MI and DFC standard deviation (STD) for each season.

	Seasons				SEM[1]	P^2
	Winter mid-pregnancy	**Spring** after parturition	**Summer** late lactation	**Autumn** after weaning		
BWG, kg/day	0.05^b	0.18^a	0.20^a	0.20^a	0.03	0.0007
MI	3895^{ab}	4596^a	3422^{ab}	2907^b	374	0.0016
STD of MI	1866^a	1122^b	818^{bc}	564^c	224	<.0001
DFC, %	81.66^c	88.06^{bc}	90.22^{ab}	95.38^a	1.65	<.0001
STD of DFC, %	17.39^a	9.77^b	8.90^{bc}	5.39^c	1.28	<.0001

[a,b,c] Means with different superscripts along the same line are significantly different with $P<0.05$ for differences of least squares means. [1]Standard error of the mean. [2]P value for season fixed effects.

The activity behaviour pattern within the 24-hour periods changed over the year corresponding with different daylight/dark cycle lengths. Over the winter period, the day length was short and ewes showed activity bouts during both the daylight and dark phases. Ewe activity was higher during the daylight, with several activity peaks. During the dark phase, two resting bouts occurred, interrupted by one shorter activity bout with lower intensity (Figure 1, a). The length of the daylight period was longer during spring and ewe activity was consistently found only during daylight with no activity bout during the dark phase. There were several activity peaks, with all being peaks during the daylight period (Figure 1, b). Similarly, the daylight period was very long during the summer. Ewe activity was higher during the daylight, with several activity peaks and there was no activity bout during the dark phase (Figure 1, c). Over the autumn period, when the daylight began to shorten, ewes presented a pattern similar to the winter period (Figure 1, d). As there were large differences between seasons in the 24-hour activity patterns and activity rhythmicity (DFC%), the results are displayed for each of the seasons separately.

Only where significant relationships occurred are these described or shown in tables and figures.

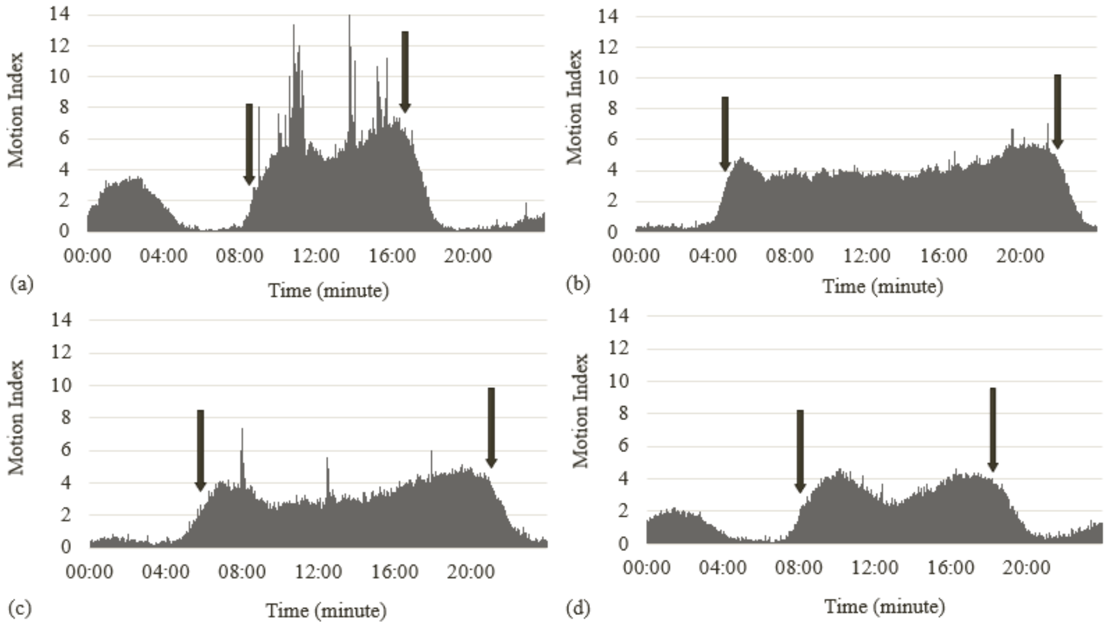

Figure 1. Motion index patterns of ewes during 24-hour over the seasons.

One bar per minute and every minute value is an overall average of all ewes. Arrows show average time of sunrise and sunset. The threshold to consider the motion index per minute as an activity bout was the value of 1. **(a) Winter period:** Jan/Feb, 30 days, 23 ewes, sunrise was between 07:57 – 08:46 h and sunset between 16:08 – 17:09 h. **(b) Spring period:** May/Jun, 28 days, 24 ewes, sunrise was between 04:28 - 04:51 h and sunset between 21:39 - 22:11 h. **(c) Summer period:** August, 28 days, 19 ewes, sunrise was between 05:20 - 06:14 h and sunset between 20:26 - 21:30 h. **(d) Autumn period:** October, 26 days, 24 ewes, sunrise was between 07:30 - 08:22 h and sunset between 17:42 - 18:45 h.

3.1. Winter

The ewes experienced a severe and challenging change in the weather as the precipitation level reached up to 39 mm per day. At the same time as a change in weather occurred, the activity of ewes, expressed as motion index, decreased showing a linear response with precipitation (Figure 2, a). The STD of motion index also showed a linear response with precipitation (Figure 2, b); however, it followed the motion index average pattern, decreasing along with the increasing precipitation.

Examining the data in a different way, by analysing the circadian rhythm of activity and the variation among its values enabled us to have a better understanding of the population response to the environmental influences. The population presented a stronger linear regression between DFC% and precipitation moving average (Figure 2, c). The DFC% of ewes decreased with the increasing precipitation, descending to lower values with higher levels of precipitation. Thus, ewes showed lower activity rhythmicity when they experienced this environmental disturbance. Looking at the STD of DFC%, it is also possible to observe that the variation of the DFC% increased along with the precipitation increase, followed by a plateau of the DFC% variation, with a quadratic relationship providing the best relationship (Figure 2, d). This suggests the beginning of an adaptation pattern.

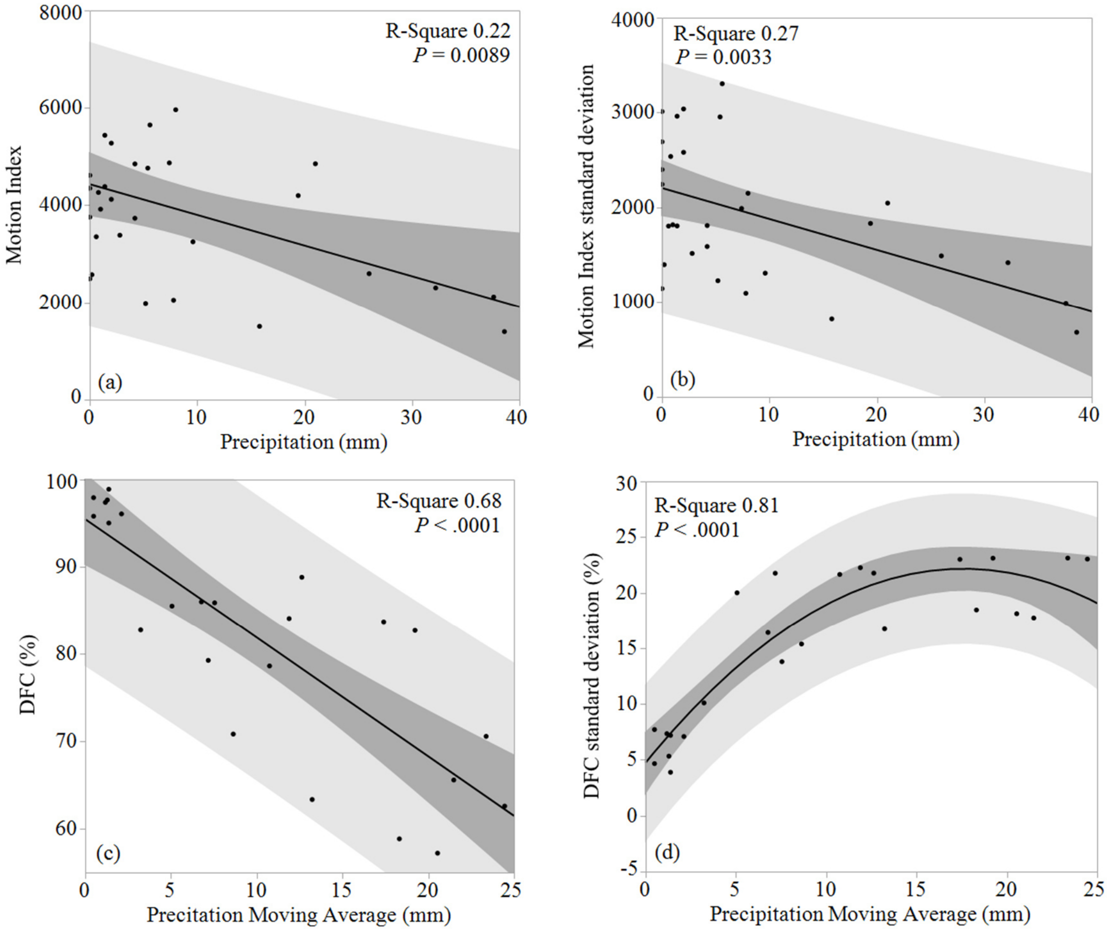

Figure 2. Linear regressions of activity behaviour and rhythmicity with the weather variable, precipitation. The dark grey area represents 95% confidence limits and the light grey area represents 95% prediction limits. **(a)** Linear regression between the overall daily average of motion index and daily precipitation. **(b)** Linear regression between the overall daily average of STD of motion index and daily precipitation. **(c)** Linear regression between overall moving averages of DFC% and moving averages of precipitation (DFC% was calculated within moving seven day intervals, thus, to allow juxtaposition, the moving average of precipitation was also set up in seven day intervals). **(d)** Quadratic regression between the overall moving averages of STD of DFC% and the moving averages of precipitation.

The absolute daily weather values over the winter period are shown in Figure 3, a. The results for random regression between DFC% and precipitation moving average are presented in Supplementary Table 1. The covariance parameter of the intercept was significant suggesting that some differences between individuals were independent of weather variables; however, only one ewe deviated from the population intercept, while data of eight ewes deviated from the slope for precipitation. Therefore, the differences between individuals were mostly linked to their different slope for precipitation. Considering this result the ewes were divided into three groups (Supplementary Table 2). The responses of DFC% are split in two figures to promote a better visualization of the deviations presented by ewes (Figure 3, b and c). However, the DFC% of all ewes together represent the population response.

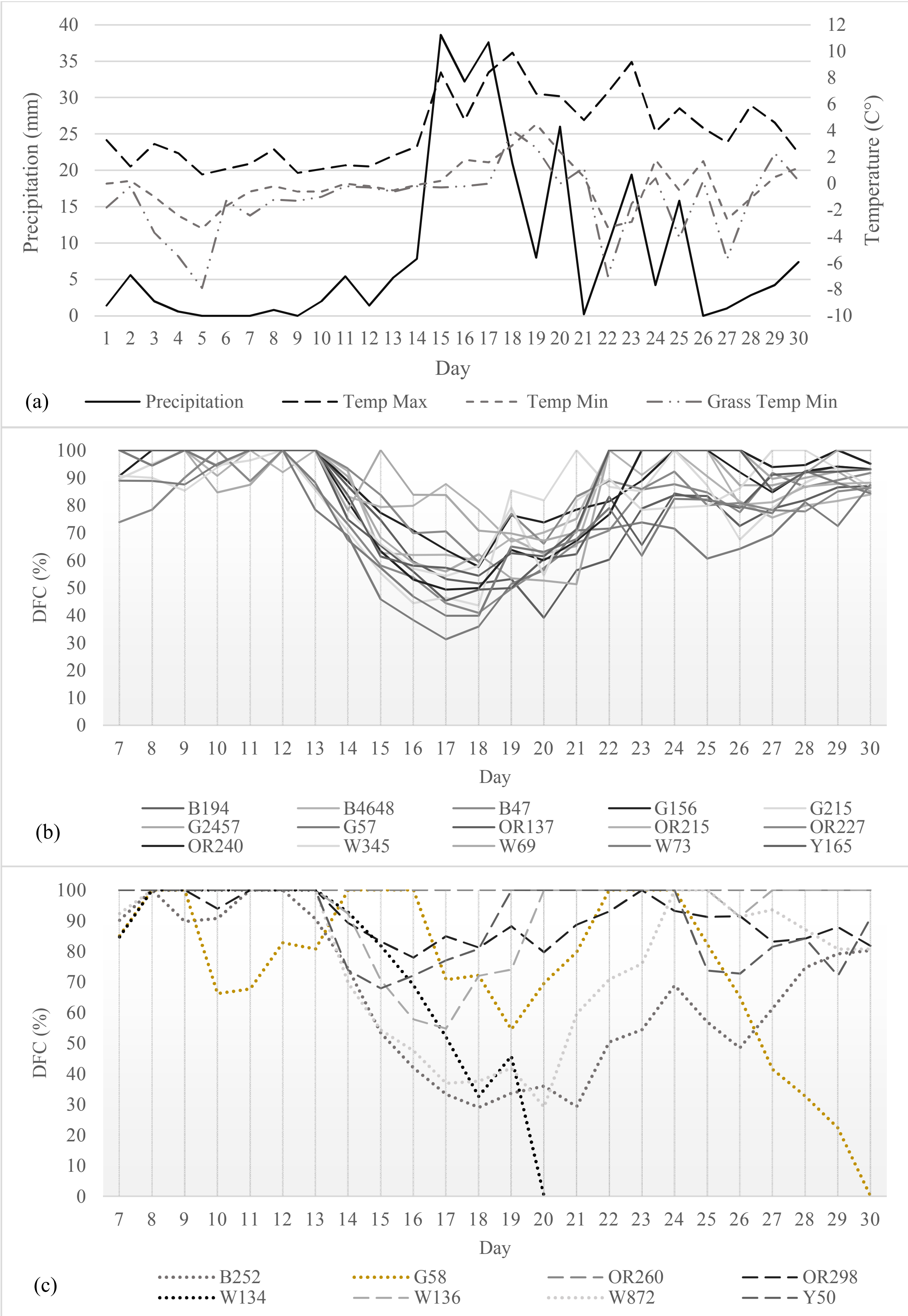

Figure 3. Degrees of functional coupling (DFCs) of activity of ewes over the winter period.

DFC% was calculated for moving seven day intervals, thus, the graphic starts at the 7th day. **(a)** Absolute values for weather variables over the winter period. **(b)** Response of DFC% of fifteen ewes that did not present deviation from the population intercept and slope over the winter period (Medium Consistency group). DFC% for these ewes declined until the 17th day and from this day started to show an adaptation pattern represented by an increase of DFC% levels, even with high levels of precipitation. **(c)** Response of DFC% of eight ewes that presented deviation from the population slope over the winter period. The four ewes represented by dashed lines showed a reduced decrease of DFC% (High Consistency group) whereas the four ewes represented by dotted lines showed a greater decrease of DFC% (Low Consistency group). The ewes W134 and G58 died on 20th and 30th. The ewe G58 also showed deviation from the population intercept.

The DFC% values started to drop dramatically from the 13th and 14th days when the precipitation level increased. For ewes that did not deviate from the population slope in the Medium Consistency group, their DFC% level dropped until the 17th day and from this day started to show an adaptation pattern represented by rising of DFC% levels, even with high levels of precipitation. Ewes in the High Consistency group presented a reduced variation of the DFC% response. In the Low Consistency group, ewes showed greater decrease in DFC% response. Thereafter, the DFC% level of two ewes started to rise, but ewe W134 did not show a recovering DFC% and died on the 20th day. Ewe G58 in the Low Consistency group showed deviation from the population slope and intercept. The positive deviation for precipitation slope showed that this ewe had a good capacity to cope with the precipitation level, however, it had a high negative deviation from the population intercept. It is important to say that the ewe B252 in the Low Consistency group also died some days after the end of measurements. Looking at the ewe mortality in Low Consistency group, 3 ewes of 4 died over the winter period, while there were no deaths for the others 19 ewes in the Medium and High Consistency Groups. A Fisher exact test for probability gives the probability of death for ewes in Low Consistency group of less than 1 ewe. The probability of the actual distribution of deaths/survivals amongst Low and combined Medium/High Consistency groups is $P = 0.002$. Thus, there is a very low probability of the distribution of deaths (3 out of 4) from the low Consistency Group compared to the other groups.

The DFC% averages of ewes in the Medium Consistency group varied in a range of 71.5 up to 91.8%. The DFC% overall average for Medium Consistency group was 82.7% and this average was higher ($P < 0.0001$) compared with the Low Consistency group that showed an overall average of 66.5% and lower ($P < 0.0001$) compared with the High Consistency group with an overall average of 93.1%. The groups also presented different STDs of DFC% ($P = 0.0002$). Ewes in the High Consistency group showed the lower variation on DFC% response, with a STD of DFC% of 8.6%. The overall average of STD of DFC% for ewes in Medium Consistency group was 16.4% and for the Low Consistency group was 30.1%. There were no differences between groups regarding to BWG ($P = 0.14$). The BWG overall averages were respectively 0, 0.08, -0.07 for High Consistency, Medium Consistency and Low Consistency. Over the winter period, the population in general showed reductions in the DFC% response, as well as low ranges of BWG. However, there were no significant regressions between BWG and DFC% (Supplementary Table 3). Considering the entire population (all ewes), there was no significant regression between BWG and simple motion index.

3.2. Spring

The daily precipitation level was the lowest of all four periods and varied from 0 up to 16.2 mm (Figure 4, a). The overall average of temperature varied between 5.4 and 16.1°C. There were significant relationships between DFC% and the weather variables.

The multiple regression and covariance parameters are shown in Supplementary Table 4. Covariance values were significant for the squared precipitation slope and temperature slope, indicating that the ewe’s responses of DFC% varied between individual in regard to their different ways to cope with precipitation and temperature levels. Responses of three ewes deviated from the population squared precipitation slope and nine ewes deviated from the population temperature slope, suggesting that the main variation between individual responses of DFC% were in regard to temperature. Thereby, the ewes were split into three groups (Supplementary Table 5). The response of DFC% of fourteen ewes that did not present deviation from the population intercept and slopes over the spring period (Medium Consistency group) are show in Figure 4, b; and the response of DFC% of ten ewes that presented deviation from the population temperature or squared precipitation slope over the spring period are shown in Figure 4, c.

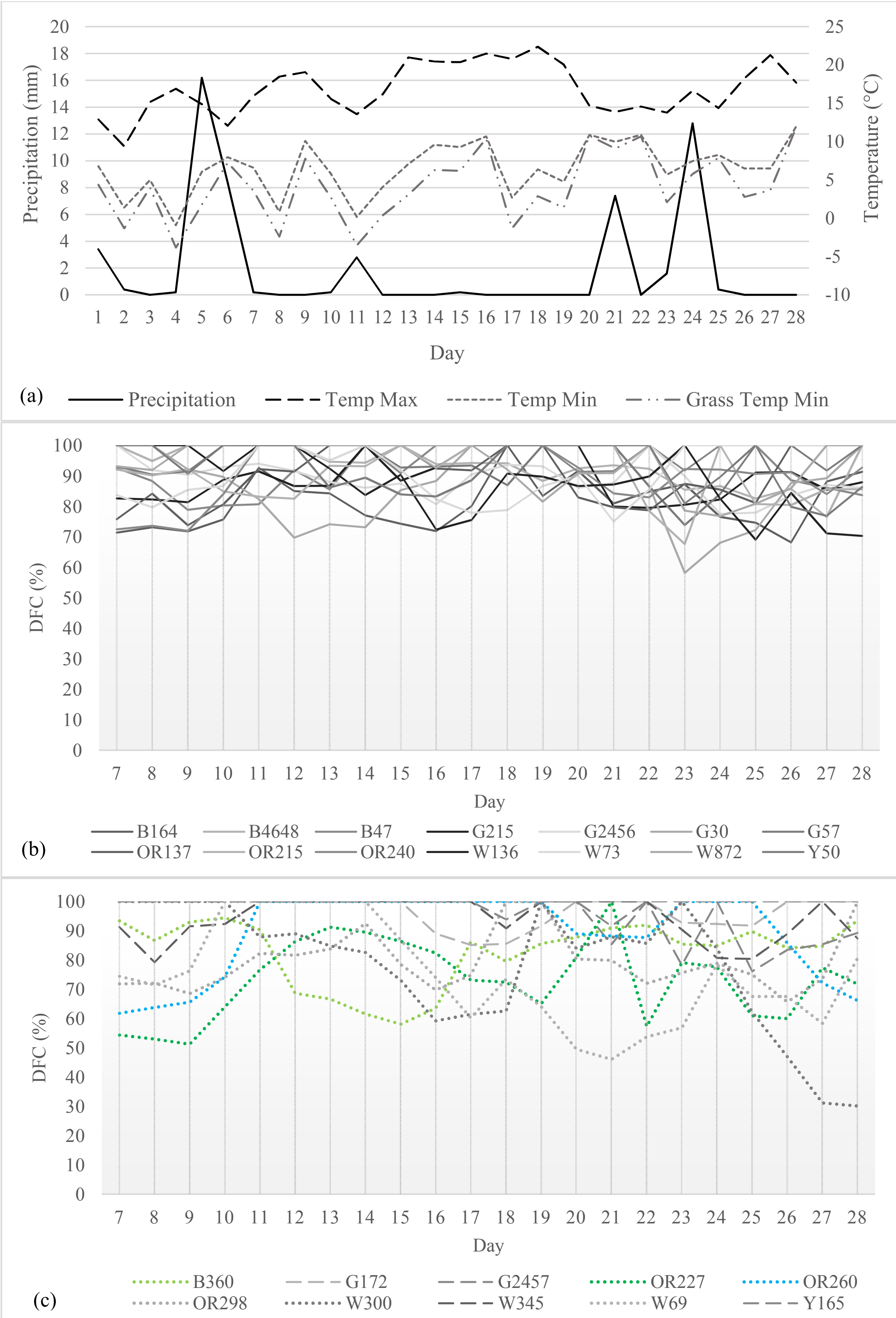

Figure 4. Degrees of functional coupling (DFCs) of activity of ewes over the spring period.

DFC% was calculated for moving seven day intervals, thus, the graphic starts at the 7th day. **(a)** Absolute daily values for weather variables over the spring period. **(b)** Response of DFC% of fourteen ewes that did not present deviation from the population intercept and slopes over the spring period (Medium Consistency group). **(c)** Response of DFC% of ten ewes that presented deviation from the population temperature or squared precipitation slope over the spring period. Four ewes represented by dashed lines presented higher consistency of DFC% (High Consistency group), whereas six ewes represented by dotted lines showed lower consistency of DFC% (Low Consistency group). Ewes with grey lines showed deviation from the population slope for temperature. Ewe with blue line showed deviation from the population slope for squared precipitation. Ewes with green lines showed deviation from both temperature and squared precipitation slopes.

The overall averages for DFC% were different between the three groups ($P <$ .0001) with means of 96.42%, 90.35% and 76.98%, respectively for the High, Medium and Low Consistency groups. Across the spring monitoring days, ewes in the Low Consistency group showed higher variation in the response of DFC% than ewes in other groups, with SDT of DFC% of 14.29% (P = 0.0006). Regressions between BWG, the STD of DFC% and the presence and number of lambs are shown in Supplementary Table 6. Considering the entire population (all ewes), there was a significant negative multiple regression for BWG with STD of DFC% and number of lambs; and a negative linear regression between STD of DFC% and BWG (Figure 5, a). To gain a better knowledge of this relationship and study the differences between the individual responses of DFC% for weather variables, linear regressions were also displayed within groups. The BWG of ewes in the Medium Consistency group was linked to the number and presence of lambs, with BWG decreasing with the presence and number of lambs. Moreover, BWG of ewes in the Low Consistency group was linked to the variation in the response of DFC%, as there was a strong negative regression between BWG and STD of DFC%, with the BWG decreasing while STD of DFC% increased (Figure 5, b). In addition, the number of lambs was not related to the high variation in the response of DFC% shown by ewes in the Low Consistency group. Within the High Consistency group, the variation between ewes DFC% and its STD seems to be linked to the presence and number of lambs (Supplementary Figure 2). Considering the entire population (all ewes), there was no significant regression between BWG and simple motion index.

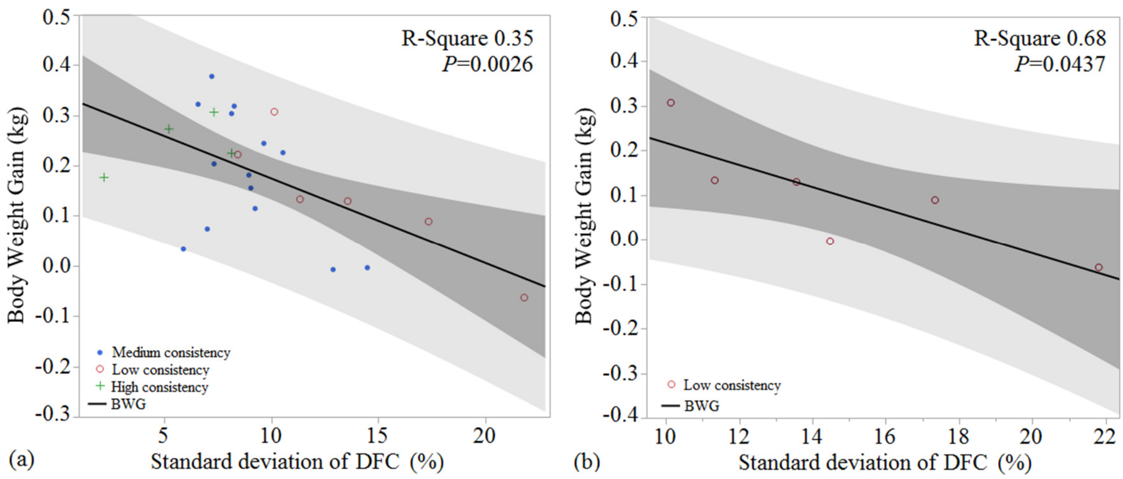

Figure 5. Linear regressions between BWG and STD of DFC%.

The dark grey area represents 95% of confidence limits and the light grey area represents 95% of prediction limits. **(a)** Linear regression between BWG and STD of DFC%, for all ewes. **(b)** Linear regression between BWG and STD of DFC% for Low Consistency group.

3.3. Summer

Throughout the summer period, the daily precipitation varied from 0 up to 21.4 mm and the overall average of temperature varied between 11.9 and 17.3°C (Figure 6, a). The multiple regressions between DFC% and weather variables were not significant. As in spring, during summer the activity DFC% showed a quadratic response with the moving average of precipitation. The DFC% dropped as the precipitation increased, followed by a plateau (Supplementary Figure 3, a). Moreover, as the precipitation increased, the STD of DFC% tended to increase (Supplementary Figure 3, b). Regarding temperature, the

DFC% for the population increased while temperature increased (Supplementary Figure 4, a). Furthermore, with higher temperatures, the STD of DFC% was lower (Supplementary Figure 4, b).

To assess the differences between the DFC patterns of activity, the individual deviations from the population intercept and slope were modelled by the random effects structure using the linear regression between activity DFC% and temperature (Supplementary Table 7). The differences between ewe responses of DFC% were independent of temperature, considering that there was not enough variation between individual slopes for temperature. However, the intercept covariance was significant showing that there were differences between ewe responses of DFC% that were independent of the weather variables. Thus, ewes were split into three groups (Supplementary Table 8). Ewes in the Medium Consistency group are shown in Figure 6, b. Five ewes showed a significant deviation from the population intercept over the summer period, with three ewes in the High Consistency group and two in the Low Consistency group (Figure 6, c).

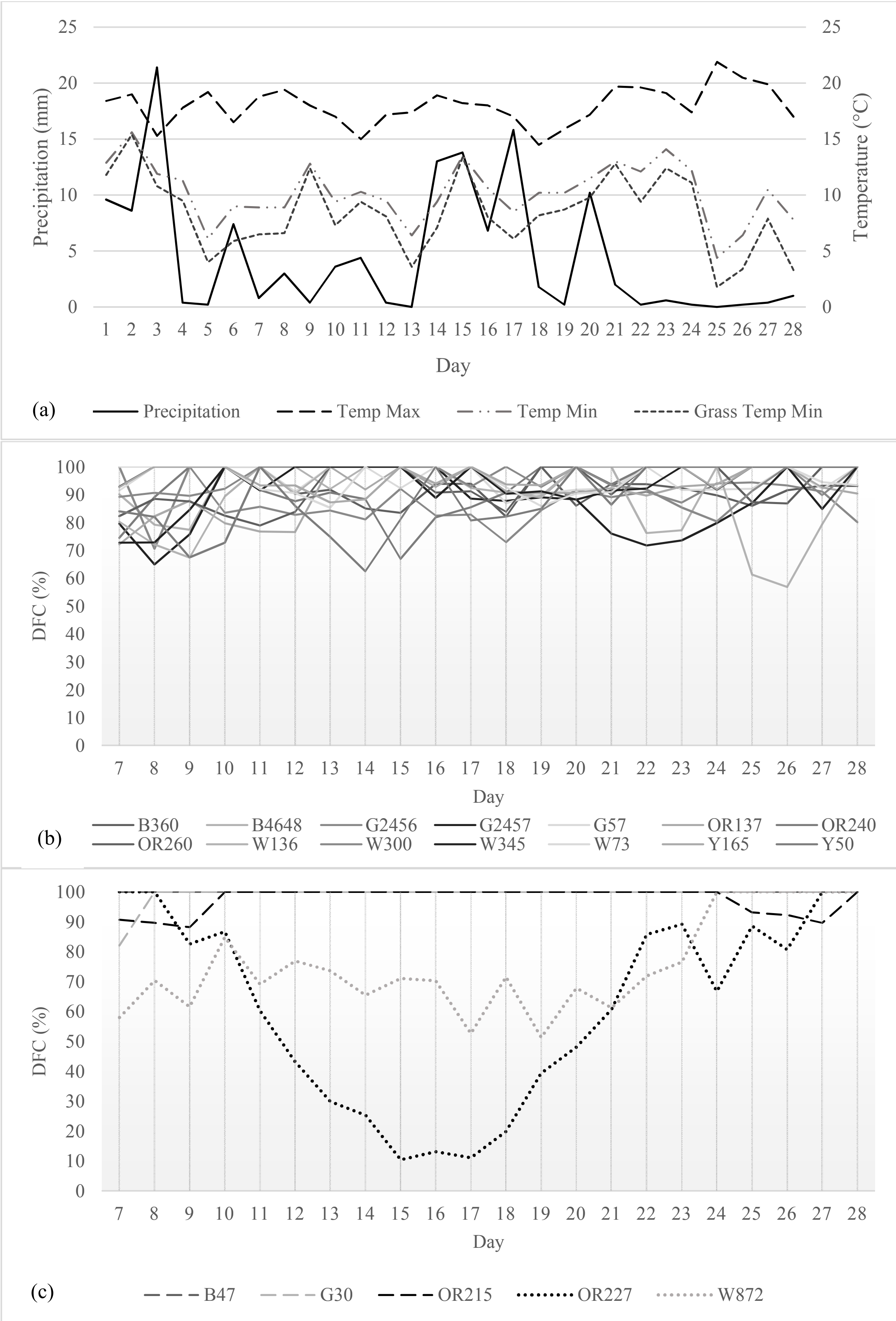

Figure 6. Degrees of functional coupling (DFCs) of activity of ewes over the summer period.

DFC% was calculated for moving seven day intervals, thus, the graphic starts at the 7th day. **(a)** Absolute values for the weather over the summer period. **(b)** Response of DFC% of fourteen ewes that did not present deviation from the population intercept and slope over the summer period (Medium Consistency group). **(c)** Response of DFC% of five ewes that showed a deviation from the population intercept over the summer period. The three ewes represented by dashed lines presented higher consistency of DFC% (High Consistency group), whereas the two ewes represented by dotted lines showed lower consistency of DFC% (Low Consistency group).

Compared with the other groups, ewes in the High Consistency group showed the lowest variation in the response of DFC% (STD of DFC% of 4.8%; $P < .0001$) and the highest DFC% average (97.6%; $P = 0.003$). Ewes in the Low Consistency group presented the greatest variation in the response of DFC% (STD of DFC% of 22%) and showed the lowest DFC% average (69.3%). Ewes in the Medium Consistency group had a DFC% average of 91.87% and STD of DFC% of 8.5%. Over the summer period, the rhythmicity of activity was related to ewe performance and the regressions between BWG, number of lambs and DFCs considering all ewes and by groups are displayed in Supplementary Table 9. Considering all ewes, there was a negative linear relationship between BWG and STD of DFC%, indicating that ewes with lower variation in the response of DFC% showed higher BWG (Figure 7, a). The same negative linear relationship was present within the Medium Consistency group, suggesting that there is a close relationship between BWG and STD of DFC% (Figure 7, b). In addition, considering all ewes, the DFC% and STD of DFC% were well explained by the BWG and number of lambs. Regarding to the Low Consistency group, just two ewes negatively deviated over the summer period, therefore it was not possible to display regression analysis, but it is important to show that the ewe OR227, with the highest breakdown in rhythmicity showed the lowest BWG of the flock, with -0.114 kg. Considering the entire population (all ewes), there was no significant regression between BWG and simple motion index.

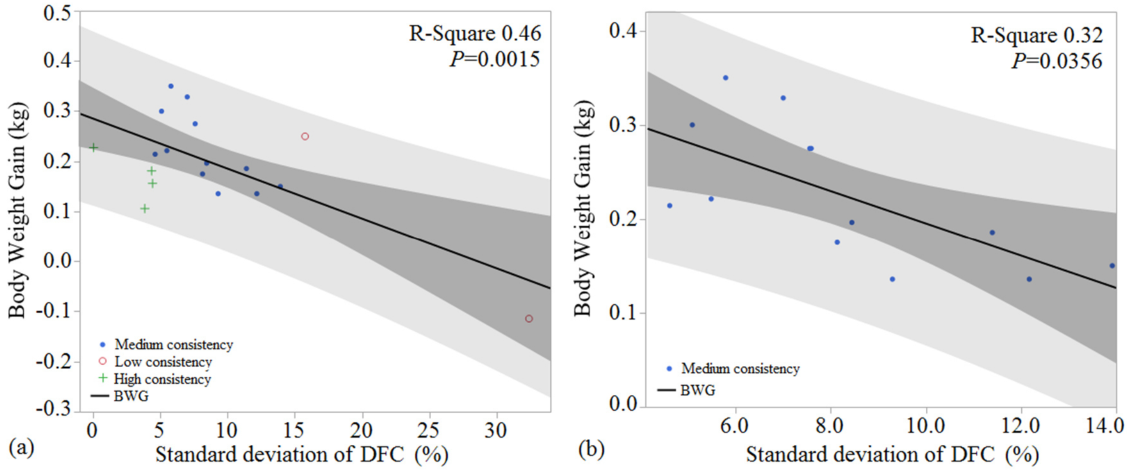

Figure 7. Linear regressions between BWG and STD of DFC%.

The dark grey area represents 95% of confidence limits and the light grey area represents 95% of prediction limits. **(a)** Linear regression between BWG and STD of DFC%, for all ewes. **(b)** Linear regression between BWG and STD of DFC%, for Medium Consistency group.

3.4. Autumn

The absolute daily weather values during the autumn period are shown in Figure 8, a. The daily precipitation level varied from zero up to 23.8 mm and the overall average of temperature varied from 4.8 and 15.4 °C during experimental days. Over the autumn period, the precipitation level did not affect the activity DFC%, since none of the regressions were significant. The activity DFC% showed a quadratic regression with the moving average of temperature (Supplementary Figure 5, a). Furthermore, there was also a quadratic response between STD of DFC% and temperature (Supplementary Figure 5, b). The individual deviations from the population intercept and slope were modelled using a quadratic regression between activity DFC% and temperature (Supplementary Table 10). Six ewes presented significant deviation from the population intercept (Figure 8, c).

There were no differences between individual ways to cope with temperature. Thereby, the ewes were split into three groups (Supplementary Table 11). Response of DFC% of eighteen ewes that did not present deviation from the population intercept and slope (Medium Consistency group) are shown in Figure 8, b. The ewe OR227 in the Low Consistency group again showed a high breakdown in the responses of DFC%, reaching low values.

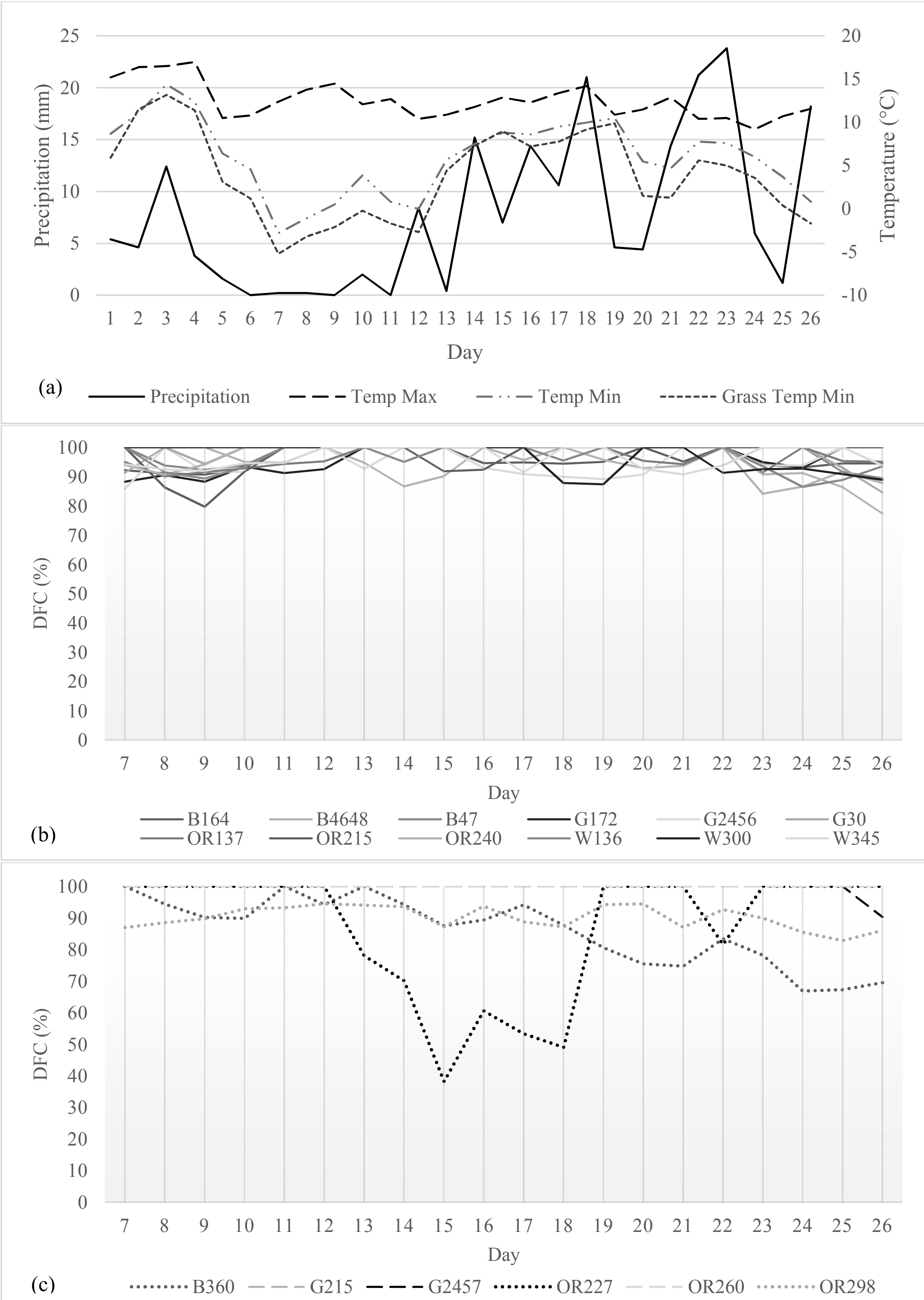

Figure 8. Degrees of functional coupling (DFCs) of activity of ewes over the autumn period.

DFC% was calculated for moving seven day intervals, thus, the graphic starts at the 7th day. **(a)** Absolute values for weather over the autumn period. **(b)** Response of DFC% of eighteen ewes that did not present deviation from the population intercept and slope over the autumn period (Medium Consistency group). **(c)** Response of DFC% of six ewes that presented deviation from the population intercept over the autumn period. These ewes differed from the population by an individual level that is independent of the temperature. The three ewes represented by dashed lines presented higher consistency of DFC% (High Consistency group), whereas the three ewes represented by dotted lines showed lower consistency of DFC% (Low Consistency group).

As in the other seasons, over the autumn period there were statistically significant differences between groups for the overall average of DFC% and its STD ($P < .0001$). Ewes in the High Consistency group showed the highest consistency of DFC% (STD of DFC% of 0.72%) and the highest average of DFC% (99.8%), whereas the Low Consistency group presented the lowest consistency of DFC% (STD of DFC% of 11.75%) and the lowest average of DFC% (87.55%). Ewes in the Medium Consistency group had an overall average of DFC% of 96.52% and STD of DFC% of 4.89%. During the autumn period, the population showed the highest DFC% values, the highest consistency of DFC% and a good BWG. Thus, there were no significant regressions between BWG and DFC% (Supplementary Table 12). Considering the entire population (all ewes), there was no significant regression between BWG and simple motion index.

4. Discussion

Activity behaviour patterns over 24 h were different between seasons and this variation was mainly in response to the number of hours of daylight and the physiological and production cycles of the sheep. Diurnal animals such as herbivores exhibit higher activity during daylight and it can be assumed that most of the activity behaviour was associated with grazing. Umstätter et al. (2008) observed that extensively managed ewes in Scotland were active for over 60.5% of the daytime and spent 59.9% of the active time grazing and only 0.6% walking (without grazing). Thus, the authors state that it is possible to define two behaviour categories, active (mainly grazing) and inactive (mainly recumbent). Longer periods of daylight, as observed during the spring and summer, allowed the ewes in our study enough time to spend grazing. Accordingly, days with short daylight length, such as observed over the winter and autumn probably were not enough to satisfy nutritional needs and, thus, some grazing overnight was required. Our findings have shown that sheep in this extensive system exhibit a circadian rhythm of activity directed by the periods of light/dark and food supply, agreeing with Piccione et al. (2010) who concluded that the daily rhythm of locomotor activity in sheep is entrained by the light/dark cycle together with food availability.

Environmental influences such as temperature and precipitation also affect activity behaviour and circadian rhythm. For example, studies of extensively managed sheep have shown they reduce grazing during heavy winter rainfall, but are less affected by rain in the summer months (Champion et al., 1994; Hunter, 1954). However, to have a better understanding of these effects it has been beneficial to look at activity data in a different way. The analyse of DFC% (activity rhythmicity) and its variation (STD of DFC%) instead of the simple activity behaviour (motion index) enabled this better understanding of animal response with respect to the weather influences. Abrupt environmental changes, such as high precipitation levels with low temperatures faced by ewes over the winter, may have a direct negative effect on activity rhythmicity and this lack of rhythmicity may persist until the end of the environmental disturbance, animal adaptation or death. However, the degree of this negative effect clearly can differ between individuals and this difference in response has been postulated to be an outcome of the animal ability to cope with short-term environmental change, the individual phenotypic plasticity. Analysing the individual response of DFC combined with the use of random regression models to classify the differences between animals was a novel approach which we showed was a feasible way to understand between-individual differences.

This knowledge of individual plasticity is important, as the population level response to the environment will depend on individual level of phenotypic plasticity (Dingemanse et al., 2010; Nussey et al., 2007; Wilson, 1998). Weather variables influenced ewe activity rhythmicity over all seasons. However, only during the winter and spring did the weather drive the variation between animals. During the summer and autumn periods, between-individuals variation in DFC data was not related with temperature or precipitation. These findings suggest that the inconsistent rhythmicity of ewes in the Low Consistency group during these seasons could be linked with some other factor.

When animals are experiencing an environmental disturbance, the DFC is low. Over the winter period, all ewes had some breakdown in the circadian rhythm of activity, even ewes in the High Consistency group. However, the ewes in the High Consistency group showed a reduced variation of the response of DFC%, evidencing that their plastic response was positive and enabled them to cope better with higher levels of precipitation and consequently showing a faster adaptation. On the other hand, ewes in the Low Consistency group showed greater decrease in DFC% reaching values lower than 30%, as well as a more difficult recovery of the response of DFC%, suggesting an adaptation problem. Some of these ewes had a maladaptive plasticity, with a greater lack of rhythmicity and an indication of poorer welfare, and consequently a great risk of poor health and death. There was a very low probability of the distribution of deaths (3 out of 4) from the low Consistency Group to the other groups, but these are very low numbers of animals and the deaths were for different reasons including one that appeared accidental rather than linked to health. For these reasons no firm conclusion of DFC data predicting death is being made.

A lack of rhythmicity caused by an environmental disturbance might be temporary and not affect animal health, however, it also might be harmful, as the circadian rhythm disorganization might predispose animals to poor health, considering that other authors have reported that stress factors may reduce immune function and parasite resistance (Dwyer and Bornett, 2004; Martino et al., 2008). Over the spring period, the quadratic response between DFC% and precipitation suggest that sheep were less affected by rain when the temperature was higher. Hunter (1954) also observed that ewes seem to be more sensitive to rainfall when exposed to cooler air over winter. Besides, precipitation was lower over the spring period.

Berger et al. (1999) observed similar results in a study with the Przewalski horse. Przewalski horses showed lowered DFCs in periods of adaptation after translocation from a zoo to a semi-reserve. Lowered DFCs of Przewalski horse were also observed during the period of hunting and shooting in the surrounding area of the semi-reserve (Scheibe et al., 1999). These findings show that not only weather variables can be the cause of circadian rhythm disorganization, but any environmental disturbance. Thus, any other environmental data set with daily observations could potentially be used to evaluate the differences between individual rhythmicity and the way that animals cope with their environment. Moreover, health problems, injury, immobilization, social interactions and even some normal physiological changes such as parturition may also be the cause of a lack of behavioural rhythmicity (Berger et al., 2003, 1999, Scheibe et al., 1999, 1998).

Our study, in a different environment from these earlier wildlife studies, has confirmed the potential value of their mathematical approach to analysing rhythmicity.

The relationship between BWG and variation of the DFC% was noteworthy over the spring and summer periods. Over the spring period, the BWG of the flock was well explained by a combination of presence and number of lambs and by variation of the DFC%. The spring period measurements were after parturition and ewes with lambs were in early lactation, and under higher metabolic demand for milk production, and thus showed lower BWG. During the summer measurement period, ewes with lambs were in late lactation, and thus the BWG was no longer directly influenced by the presence and number of lambs, but was associated with the variation of DFC%. However, variation in the response of DFC% was also an outcome of the presence and number of lambs, with the social interaction with lambs potentially implicated in the variation of the DFC%. In addition, we can clearly see that over the autumn, after weaning, almost all ewes were quite consistent in the response of DFC% and showed a good BWG.

Analysing the DFC% and the differences between individuals using the random regression model enabled the detection not only of environmental disturbances, but showed that it is also possible to detect animals with particular problems. The combination of the two frameworks (Nussey et al., 2007; Scheibe et al., 1999) could be used in practice to understand whether the differences between animals were in response to the environment or whether the difference was shown in relation to a particular problem of a particular animal. Our study was conducted with only a small population of initially healthy sheep and more studies done with healthy compared with unhealthy sheep would be useful.

Measurements of individual DFCs may not only be a source of knowledge about responses to the environment, but may have potential to be a tool in order to help the selection of healthy and adapted animals to provide a warning system for health and welfare problems. The current study was conducted with raw data that was created and saved on a memory card, and analysed (post processed) sometime later. With further technological developments, on-animal sensors could communicate with researchers, veterinarians or farmers in real, or near-real, time. Additionally, studies of circadian rhythmicity could be a useful contributor to the better understanding of biology.

5. Conclusions

This study confirmed the strong dynamic created by the seasons and by the production/physiological cycle in sheep in high latitude systems. The analysis of activity circadian rhythms using the DFC mathematical model enables a better understanding of sheep responses to weather and environmental influences, compared to the use of simple activity behaviour (motion index) alone. Over the spring and summer periods, the variation in the response of DFC was a better estimator of BWG than the use of simple motion index. The random regression model method was effective in identifying animals that deviated from population responses.

The combination of circadian rhythm analysis and the clustering of individuals into groups based around their regression response to environmental variables provides considerable potential to glean information relevant for group and individual animal management. With increasing availability of such data captured through automated telemetric systems, this work shows that these approaches may enhance the quality and meaningfulness of data coming from automated sensors. It also provides a potential means of using such data acquisition technology to obtain useful information.

6. Acknowledgment

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SUPPLEMENTARY MATERIAL

The use of circadian rhythm of activity and random regression model as a novel approach to monitor sheep in an extensive system

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2. Material and Methods

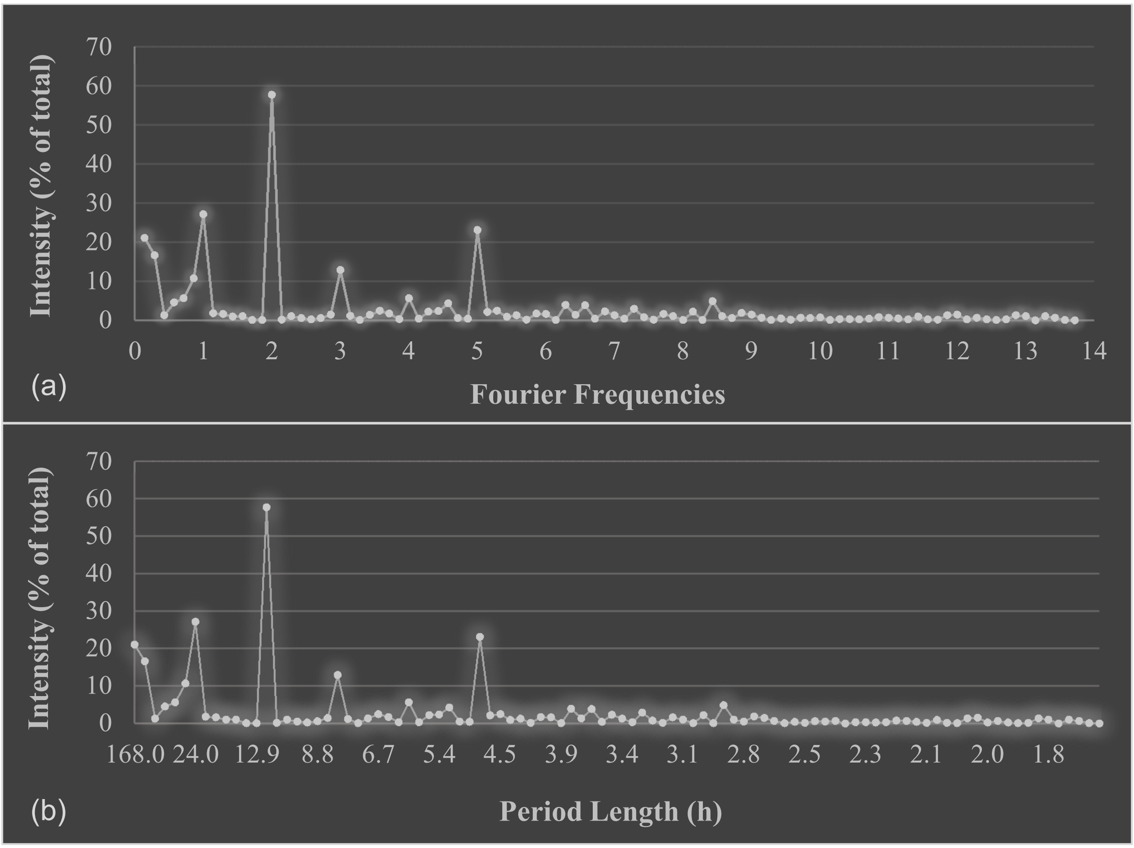

Figure 1. Power spectrum for activity drawn over a period of seven days interval of one ewe. **(a)** The Power spectrum showed seven significant ordinates, all ordinates with intensity higher than 10 were significant according to R. A. Fisher, and represent the significant periodic components. **(b)** Four of the seven significant ordinates correspond to harmonic periods. The significant harmonic periods for this power spectrum were 24, 12, 8 and 4.8.

3. Results

3.1. Winter

Table 1. Population response of activity rhythmicity (DFC) for precipitation over the winter period, and the covariance parameters modelled by the random effects structure with twenty-three ewes.

DFC (%)					
	Population response (fixed effects)			**Covariance**	
Effect	**Parameter estimate**	**SE[1]**	***P***	**Subject**	***P***
Intercept	95.31	1.06	<.0001	Ewe	0.0429
Precipitation	-1.35	0.19	<.0001	Ewe	0.0015
Residual					<.0001

[1] SE = Standard Error.

Table 2. Activity rhythmicity grouping by the standard deviations from the mean, modelled by the random effects structure with twenty-three ewes over the winter period.

Standard deviations from the mean (random effects)															
High Consistency Group															
Ewe	**OR260**	**OR298**	**W136**	**Y50**											
Intercept	3.48 (3.22)	0.65 (3.22)	2.43 (3.22)	-1.72 (3.22)											
Precipitation	1.34*** (0.33)	0.79* (0.33)	0.71* (0.33)	1.01** (0.33)											
Medium Consistency															
Ewe	**B194**	**B4648**	**B47**	**G156**	**G215**	**G2457**	**G57**	**OR137**	**OR215**	**OR227**	**OR240**	**W345**	**W69**	**W73**	**Y165**
Intercept	2.14 (3.22)	0.19 (3.22)	0.96 (3.22)	2.93 (3.22)	-2.61 (3.22)	1.60 (3.22)	-3.03 (3.22)	1.99 (3.22)	1.56 (3.22)	-2.14 (3.22)	2.53 (3.22)	-0.04 (3.22)	3.73 (3.22)	-2.23 (3.22)	2.46 (3.22)
Precipitation	-0.62 (0.33)	0.13 (0.33)	-0.42 (0.33)	0.29 (0.33)	0.36 (0.33)	-0.04 (0.33)	-0.60 (0.33)	-0.43 (0.33)	0.54 (0.33)	0.40 (0.33)	-0.11 (0.33)	0.12 (0.33)	0.49 (0.33)	-0.28 (0.33)	0.11 (0.33)
Low Consistency Group															
Ewe	**B252**	**G58**	**W134**	**W872**											
Intercept	-2.19 (3.22)	-11.00** (3.22)	-3.57 (3.22)	1.87 (3.22)											
Precipitation	-1.35*** (0.33)	0.74* (0.33)	-2.43*** (0.33)	-0.73* (0.33)											

Standard errors are reported in parentheses.
P*<0.05, ** *P*<0.01, * *P*<.0001.

Table 3. Regressions for Body Weight Gain (BWG) with activity rhythmicity (DFC) and its standard deviation (STD) over the winter period.

	BWG (kg/day)			
	All ewes	High Consistency	Medium Consistency	Low Consistency[2]
Intercept	0.167 (0.287)	-0.818 (0.302)	0.091 (0.458)	
DFC, %	-0.001 (0.004)	0.009 (0.003)	-0.0002 (0.006)	
R-Square	0.01	0.78	0.00	
P of the model	0.6886	0.1141	0.9763	
No. Observations[1]	17	4	12	1
	BWG (kg/day)			
	All ewes	**High Consistency**	**Medium Consistency**	**Low Consistency[2]**
Intercept	0.027 (0.067)	0.051 (0.029)	-0.035 (0.139)	
STD of DFC, %	0.001 (0.004)	-0.006 (0.003)	0.0069 (0.0084)	
R-Square	0.01	0.69	0.06	
P of the model	0.7156	0.1720	0.4320	
No. Observations[1]	17	4	12	1

Standard errors are reported in parentheses.
[1]Numeber of observations are different from number of ewes regarding to missing BWG values.
[2]It was not possible to do the regression analyse for Low Consistency group.

3.2. Spring

Table 4. Population response of activity rhythmicity (DFC) for precipitation and temperature over the spring period; and the covariance parameters modelled by the random effects structure with twenty-four ewes.

DFC (%)					
	Population response (fixed effects)			**Covariance**	
Effect	**Parameter estimate**	**SE[1]**	***P***	**Subject**	***P***
Intercept	105.07	6.07	<.0001	Ewe*	
Precipitation	-5.36	1.36	0.0006	Ewe*	
Precipitation[2]	1.06	0.35	0.0128	Ewe	0.0102
Temperature	-1.00	0.47	0.0475	Ewe	0.0016
Residual					<.0001

*There was not enough variation in the response to attribute any variation to the random effect, controlling for everything else in the model.
[1]SE = Standard Error. [2]Squared.

Table 5. Activity rhythmicity (DFC) grouping by the standard deviations from the mean, modelled by the random effects structure with twenty-four ewes over the spring period.

Standard deviations from the mean (random effects)														
High Consistency Group														
Ewe	**G172**	**G2457**	**W345**	**Y165**										
Intercept														
Precipitation														
Precipitation^2	0.42	0.16	-0.17	0.34										
	(0.27)	(0.27)	(0.27)	(0.27)										
Temperature	0.44*	0.44*	0.51**	0.68**										
	(0.22)	(0.22)	(0.22)	(0.22)										
Medium Consistency														
Ewe	**B164**	**B4648**	**B47**	**G215**	**G2456**	**G30**	**G57**	**OR137**	**OR215**	**OR240**	**W136**	**W73**	**W872**	**Y50**
Intercept														
Precipitation														
Precipitation^2	-0.32	0.23	-0.23	-0.17	0.18	0.11	0.23	-0.26	-0.13	-0.46	0.36	0.28	0.16	0.29
	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)
Temperature	-0.40	0.24	0.37	0.13	-0.05	-0.13	0.35	0.22	0.19	0.41	-0.25	0.06	0.31	0.18
	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)
Low Consistency Group														
Ewe	**B360**	**OR227**	**OR260**	**OR298**	**W300**	**W69**								
Intercept														
Precipitation														
Precipitation^2	-0.57*	-0.93***	-0.75**	-0.49	0.26	0.29								
	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)	(0.27)								
Temperature	-0.66**	-0.75***	0.37	-0.45*	-0.96***	-1.24***								
	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)	(0.22)								

Standard errors are reported in parentheses.
*P<0.05, ** P<0.01, *** P<.0001.

Table 6. Regressions for Body Weight Gain (BWG), activity rhythmicity (DFC) and its standard deviation (STD) over the spring period.

	BWG (kg/day)			
	All ewes	**High Consistency**	**Medium Consistency**	**Low Consistency**
Intercept	0.388*** (0.043)	0.755 (0.773)	0.291*** (0.033)	0.465* (0.130)
STD of DFC, %	-0.014** (0.004)	-0.062 (0.099)	#	-0.025* (0.008)
Lamb, n°	-0.079** (0.021)	-0.209 (0.276)	-0.109** (0.029)	#
R-Square	0.60	0.60	0.54	0.68
P of the model	<.0001	0.6292	0.0028	0.0437
	STD of DFC (%)			
	All ewes	**High Consistency**	**Medium Consistency**	**Low Consistency**
Intercept	13.31*** (1.29)	7.75** (0.29)	8.51*** (2.04)	17.48** (1.44)
BWG, kg/day	-20.43** (6.00)	#	-2.24 (6.52)	-27.39* (9.41)
Lamb, n°	#	-2.75** (0.26)	0.51 (0.97)	#
R-Square	0.35	0.98	0.11	0.68
P of the model	0.0026	0.0091	0.5115	0.0437
	DFC (%)			
	All ewes	**High Consistency**	**Medium Consistency**	**Low Consistency**
Intercept	81.34*** (4.46)	94.74*** (0.40)	88.37*** (4.05)	76.78** (6.91)
BWG, kg/day	29.14 (14.70)	#	7.18 (12.94)	-3.52 (25.33)
Lamb, n°	2.05 (2.25)	2.24* (0.36)	0.37 (1.92)	2.60 (4.32)
R-Square	0.16	0.95	0.04	0.14
P of the model	0.1557	0.0250	0.8187	0.7971
N° total ewes	24	4	14	6
N° no lamb	8	2	5	1
N° single lamb	10	1	6	3
N° twin lambs	6	1	3	2

Standard errors are reported in parentheses.
Not significant, excluded from the model.
*P<0.05, ** P<0.01, *** P<.0001.

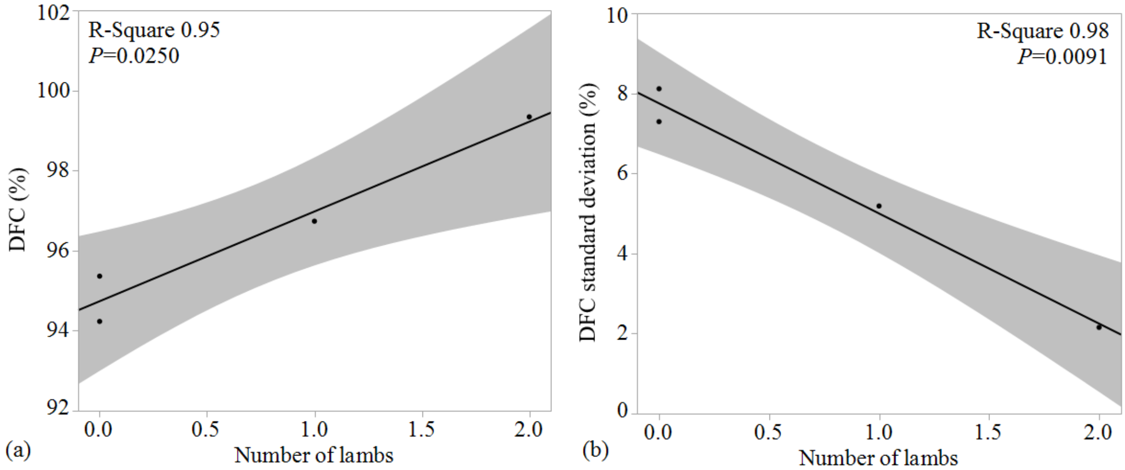

Figure 2. Lamb effects within High Consistency Group.
(a) Ewes in the High Consistency group presented a positive linear regression between DFC% and number of lambs. **(b)** Ewes in the High Consistency group presented a negative linear regression between STD of DFC% and number of lambs.

3.3. Summer

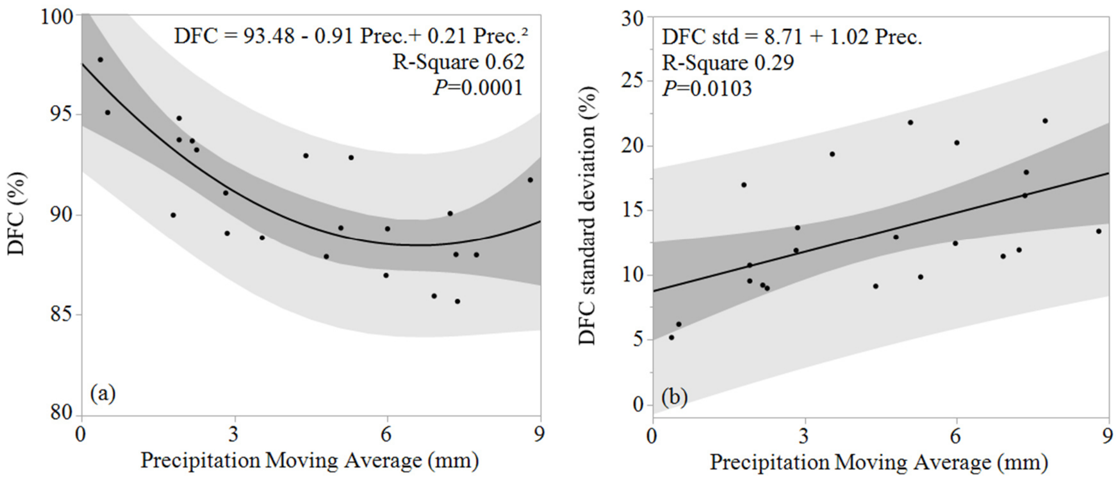

Figure 3. Quadratic regressions between circadian rhythm variables and precipitation daily moving average.
(a) Quadratic regression between activity DFC% overall daily average and precipitation daily moving average. **(b)** Quadratic regression between activity STD of DFC% overall daily average and precipitation daily moving average.

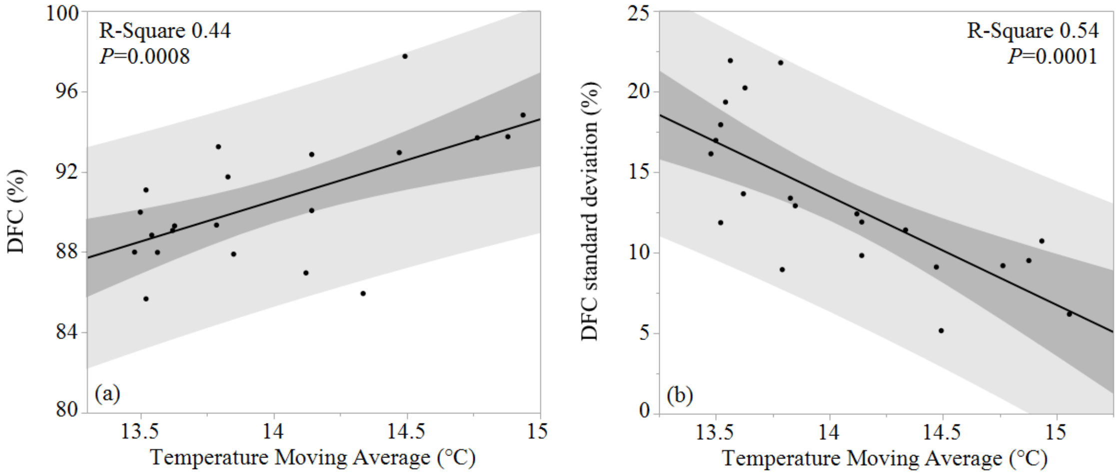

Figure 4. Linear regressions between circadian rhythm variables and temperature daily moving average.
(a) Linear regression between activity DFC% overall daily average and temperature daily moving average.
(b) Linear regression between activity STD of DFC% overall daily average and temperature daily moving average.

Table 7. Population response of activity rhythmicity (DFC) for temperature over the summer period; and covariance parameters modelled by the random effects structure with nineteen ewes.

DFC (%)					
	Population response (fixed effects)			Covariance	
Effect	**Parameter estimate**	**SE[1]**	***P***	**Subject**	***P***
Intercept	33.72	14.76	0.0347	Ewe	0.0025
Temperature	4.06	1.04	0.0010	Ewe[*]	
Residual					<.0001

[*] There was not enough variation in the response to attribute any variation to the random effect, controlling for everything else in the model.
[1]SE = Standard Error.
[2]Squared.

Table 8. Activity rhythmicity (DFC) grouping by the standard deviations from the mean, modelled by the random effects structure with nineteen ewes over the summer period.

Standard deviations from the mean (random effects)

High Consistency Group

Ewe	B47	G30	OR215
Intercept	8.68** (2.98)	7.92** (2.98)	6.27* (2.98)
Temperature			

Medium Consistency

Ewe	B360	B4648	G2456	G2457	G57	OR137	OR240	OR260	W136	W300	W345	W73	Y165	Y50
Intercept	1.57 (2.98)	4.79 (2.98)	-3.44 (2.98)	-1.80 (2.98)	4.92 (2.98)	-0.51 (2.98)	1.91 (2.98)	1.60 (2.98)	-3.12 (2.98)	3.54 (2.98)	1.90 (2.98)	4.76 (2.98)	5.61 (2.98)	-2.17 (2.80)
Temperature														

Low Consistency Group

Ewe	OR227	W872
Intercept	-27.87*** (2.98)	-14.56*** (2.98)
Temperature		

Standard errors are reported in parentheses.
P*<0.05, ** *P*<0.01, * *P*<.0001.

Table 9. Regressions for Body Weight Gain (BWG), activity rhythmicity (DFC) and its standard deviation (STD) over the summer period.

	BWG (kg/day)			
	All ewes	**High Consistency[1]**	**Medium Consistency**	**Low Consistency[2]**
Intercept	0.285*** (0.029)	0.224 (0.044)	0.331*** (0.048)	
STD of DFC, %	-0.010*** (0.002)	-0.022 (0.013)	-0.014* (0.006)	
Lamb, nº	#	#	#	
R-Square	0.46	0.73	0.32	
P of the model	0.0015	0.3455	0.0356	
	STD of DFC (%)			
	All ewes	**High Consistency[1]**	**Medium Consistency**	**Low Consistency[2]**
Intercept	20.25*** (2.32)	8.22 (3.46)	14.47*** (1.59)	
BWG, kg/day	-40.27*** (10.06)	-33.43 (20.16)	-19.85** (6.70)	
Lamb, nº	-3.70** (1.20)	#	-2.13** (0.54)	
R-Square	0.66	0.73	0.72	
P of the model	0.0002	0.3455	0.0010	
	DFC (%)			
	All ewes	**High Consistency[1]**	**Medium Consistency**	**Low Consistency[2]**
Intercept	77.46*** (3.70)	-1.69 (4.21)	86.60*** (2.68)	
BWG, kg	44.72** (16.04)	0.02 (0.04)	17.41 (11.32)	
Lamb, nº	4.68* (1.92)		1.73 (0.91)	
R-Square	0.51	0.16	0.38	
P of the model	0.0033	0.7350	0.0700	
N° total ewes	19	3	14	2
N° no lamb	7	0	5	2
N° single lamb	6	2	4	0
N° twin lambs	6	1	5	0

Standard errors are reported in parentheses.

[1]Regarding to the small number of animals that deviated in the High Consistency group was not possible to do the multiple regression analyse. Lamb effect was not significant with low R-Square.

[2]Regarding to the small number of animals that deviated in the Low Consistency group was not possible to do the regression analyse.

Not significant, excluded from the model.

*P<0.05, ** P<0.01, *** P<.0001.

3.4. Autumn

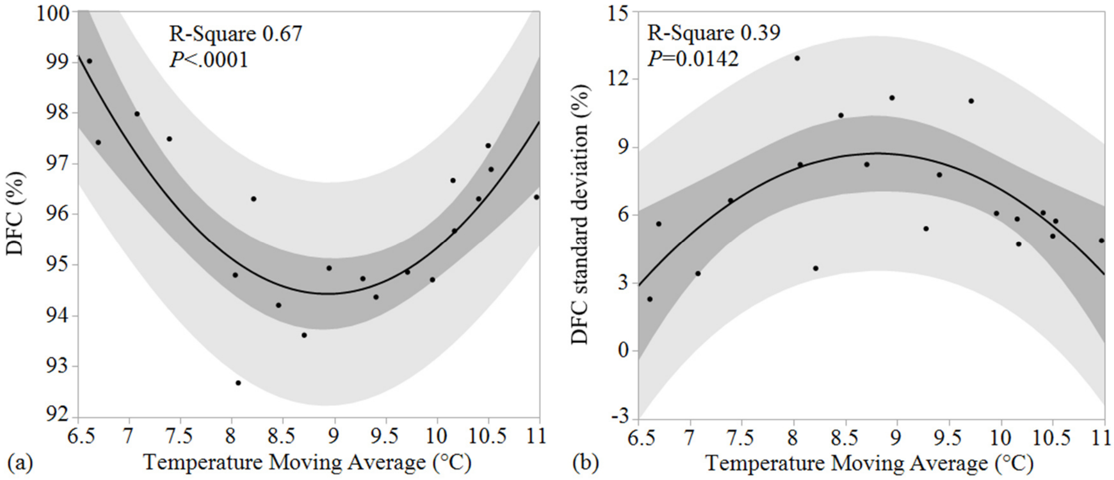

Figure 5. Quadratic regression between circadian rhythm variables and temperature daily moving average.
(a) Quadratic regression between activity DFC% overall daily average and temperature daily moving average. **(b)** Quadratic regression between activity STD of DFC% overall daily average and temperature daily moving average.

Table 10. Activity rhythmicity (DFC) population response for the weather variables over the autumn period; and covariance parameters modelled by the random effects structure with twenty-four ewes.

DFC (%)					
	Population response (fixed effects)			**Covariance**	
Effect	**Parameter estimate**	**SE[1]**	***P***	**Subject**	***P***
Intercept	157.95	14.33	<.0001	Ewe	0.0022
Temperature	-14.22	3.32	0.0003	Ewe*	
Temperature[2]	0.80	0.19	0.0003	Ewe*	
Residual					<.0001

*There was not enough variation in the response to attribute any variation to the random effect, controlling for everything else in the model.
[1]Standard error.
[2]Squared.

Table 11. Activity rhythmicity (DFC) grouping by the standard deviations from the mean, modelled by the random effects structure with twenty-three ewes over the winter period.

Standard deviations from the mean (random effects)

High Consistency Group

Ewe	G215	G2457	OR260
Intercept	3.52* (1.46)	3.11* (1.46)	3.52* (1.46)
Temperature			
Temperature^2			

Medium Consistency

Ewe	B164	B4648	B47	G172	G2456	G30	OR137	OR215	OR240	W136	W300	W345	W69	W73	W872	Y165	Y384	Y50
Intercept	0.04 (1.46)	-1.12 (1.46)	1.88 (1.46)	0.67 (1.46)	-0.57 (1.46)	1.97 (1.46)	0.59 (1.46)	1.07 (1.46)	0.35 (1.46)	0.45 (1.46)	0.64 (1.46)	0.59 (1.46)	2.42 (1.46)	2.09 (1.46)	1.12 (1.46)	-0.49 (1.46)	0.45 (1.46)	-1.46 (1.46)
Temperature																		
Temperature^2																		

Low Consistency Group

Ewe	B360	OR227	OR298
Intercept	-8.33*** (1.46)	-7.78*** (1.46)	-4.74** (1.46)
Temperature			
Temperature^2			

Standard errors are reported in parentheses.
*P<0.05, ** P<0.01, *** P<.0001.

Table 12. Regressions for Body Weight Gain (BWG), activity rhythmicity (DFC) and its standard deviation (STD) over the autumn period.

	BWG (kg/day)			
	All ewes	**High Consistency**	**Medium Consistency**	**Low Consistency**
Intercept	0.213 (0.053)	0.174 (0.056)	0.239 (0.139)	0.259 (0.056)
STD of DFC, %	-0.003 (0.008)	-0.104 (0.044)	-0.004 (0.027)	-0.008 (0.004)
R-Square	0.01	0.85	0.00	0.79
P of the model	0.7319	0.2563	0.8924	0.3044
No. Observations	24	3	18	3

Standard errors are reported in parentheses.
*P<0.05, ** P<0.01, *** P<.0001.

CHAPTER III

Article prepared according to the norms of the journal Animal - An International Journal of Animal Bioscience (Appendices)

The use of behavioural circadian rhythms as a novel approach to study the differences between beef steers methane emission, feed efficiency and growth

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Short title: behavioural circadian rhythms and performance of beef cattle

Abstract

Automated feeders and sensor-based technologies are becoming increasingly available and can be used to gather detailed information about animal behaviour.

With this information it is possible to assess animal behavioural circadian rhythm and monitor its response. The aim of this study was to evaluate the behaviour and circadian rhythms of activity and feeding of beef steers in an intensive system, to understand the differences between individuals and its relation with production traits, such as methane emission, feed efficiency and growth. Two contrasting diets and two breeds were used in a factorial design 2x2. 40 crossbred Charolais (CHx) and 40 purebred Luing (LU) assigned to *ad libitum* diets: 1) Mixed diet (MI) with a forage to concentrate ratio of 50:50 consisting of (DM basis) 28.6% whole crop barley, 20.5% grass silage, 39.7% barley, 10.3% maize dark grains, 0.9% minerals; 2) High concentrate diet (CO) with a forage to concentrate ratio of 8:92 consisting of (DM basis) 72% barley, 17.3% maize dark grains, 7.6% barley straw, 2.0% molasses, 1.1% minerals. Automated feed intake equipment and accelerometer-based activity sensors were used to collect detailed information on feed intake behaviour, feed intake and cattle activity, alongside animal performance characteristics. These data were used to calculate the percentage of cyclic behaviour that is harmonic/synchronized to each 24 h period as Degree of Functional Coupling (DFC) shown within rolling seven days periods. Low DFCs indicate lower synchronization. The diurnal patterns of feeding and activity behaviours were strong and highly synchronised. Feeding rate and feeding duration were shown to be the most important simple behavioural variables to help explain methane emission levels of cattle. However, the additional analysis of activity circadian rhythms using the DFC mathematical model enables a better understanding of production traits. Activity rhythmicity suited to visualise differences between individual methane emissions independent of breed or diet. Activity rhythmicity was also well related with important production traits such as feed efficiency and growth of beef steers.

Keywords: animal behaviour, circadian rhythm of activity, feed efficiency, methane emission, sensors

Implications

The analysis of activity circadian rhythms using the DFC mathematical model suite to visualise differences between individual methane emissions independent of breed or diet. It is also well related with important production traits such as feed efficiency and growth of beef steers. With increasing availability of such data captured through automated telemetric systems, this work shows that these approaches may enhance the quality and meaningfulness of data coming from automated sensors.

Introduction

Understanding the factors that influence ruminant methane emission and feed efficiency (Residual Feed Intake – RFI) is currently an important issue, as methane is a greenhouse gas with considerable environmental impact and feed efficiency determine the differences among animals in converting feed into body tissue. The level of methane emission by ruminants rely on many factors, where different diets alter the ruminal methanogenic community (ZHOU; HERNANDEZ-SANABRIA; LUO GUAN, 2010) and rumen fermentation, and the decreasing or increasing methane emissions (DUTHIE et al., 2017; PATRA, 2014). Roehe et al. (2016) observed that a host genetic variation also influences rumen microbial and methane production.

In fact, a healthy ruminal metabolism is an important part of the mechanism of cattle efficiency in feed utilization and production. Feeding behaviour may have an important contribution on a stable relationship between the production, absorption and passage of short-chain fatty acids in the rumen. Recent studies have reported considerable genetic and phenotypic variation in feeding behavioural traits, and important relations between feeding behaviour and cattle feed efficiency (CHEN et al., 2014; NKRUMAH et al., 2007). Chen et al. (2014) observed that feeding behaviour traits have considerable variability among steers and that some feeding behaviour traits are heritable, with 0.11 to 0.31 for different measures of feeding duration and 0.38 and 0.56 for feeding rate (measured in different breeds). In the same study, researchers found that these two feeding behaviour variables showed strong phenotypic and genetic correlations with RFI. Ruminants, as any herbivore, show a strong daily rhythmicity in its behaviour. However, feeding rate undergoes large variations throughout the day. Thus, behavioural rhythmicity may have potential to understand better this close relation between feeding behaviour and feed efficiency. In addition, feedlot systems rely heavily on mechanised feeding systems with access to feeding and activity data that can be easily collected and may have a potential use. Scheibe et al. (1999) developed a framework named Degree of Functional Coupling (DFC) for animal activity and feeding data to quantify and investigate the circadian rhythm of behavioural variables. A number of studies then showed a link between environmental influences or health issues and DFC of free ranging animals

(BERGER et al., 1999, 2003, SCHEIBE et al., 1998, 1999). However, the rapid development of new technologies that enable the acquisition and use of automatically collected activity and feeding data from individual animals makes it highly appropriate to look anew at this different way of using this type of data. Since this first series of publications, all published papers have focused on wild animals (BERGER et al., 1999, 2003, SCHEIBE et al., 1998, 1999) and thus, there is a need for more studies using the DFC method in a different environment, with managed animals. In addition, this is the first work that is also looking at the relationship of DFC with methane emission and feed efficiency of beef steers.

The key question is whether the behavioural circadian rhythms are linked to the variation of between-animals responses, or whether it is linked to a particular period for a particular animal and its methane emission level, feed efficiency and growth. Could new technology based on sensor data identify inefficient animals, either due to a short period of disrupted feeding or activity patterns, or more generally to their less structured feeding or activity behaviour? Therefore, the aim of this study was to evaluate the behaviour and circadian rhythms of activity and feeding of beef steers in an intensive system, using two contrasting breed types and diets, to understand the differences between individuals and its relation with production traits, such as methane emission, feed efficiency and growth.

Material and Methods

Animals measurements

This experiment was conducted at the Beef and Sheep Research Centre at SRUC, Scotland, latitude and longitude 55.8640 and -3.2150 respectively.

Measurements were made over the months May, June and July, with sunrise between 03:30 – 03:50 h and sunset between 20:30 – 21:00 h. The experimental design was 2 x 2 factorial comprising two contrasting breeds, 40 crossbreds Charolais-sired averaged with 504 kg of Body Weight (BW) and 40 purebred Luing averaged with 435 kg of BW. The steers were assigned to two contrasting diets, fed daily and offered *ad libitum*: 1) Mixed diet with a forage to concentrate ratio of 50:50 consisting of (DM basis) 28.6% whole crop barley, 20.5% grass silage, 39.7% barley, 10.3% maize dark grains, 0.9% minerals. 2) High concentrate diet (Concentrate) with a forage to concentrate ratio of 8:92 consisting of (DM basis) 72% barley, 17.3% maize dark grains, 7.6% barley straw, 2.0% molasses, 1.1% minerals.

It is of great value to undertake research work with contrasting breeds and diets. Charolais is an important beef breed type in the UK, of large mature weight and typically fast growth rates and are commonly considered suited for intensive systems. Luing is a Scottish breed considered more adapted to extensive rangeland systems, and well suited to lower quality diets. All cattle were born and reared by their mother at grass until weaning and were housed through winter until the study commenced. After completion of the experiment cattle were sent to slaughter.

Steers were kept in 4 large pens, with bedding of sawdust to avoid intake of straw affecting intake measurement. Each pen contained 10 Charolais and 10 Luing. The experiment consisted of three phases. The first phase was an adaptation period of 4 weeks followed by Phase 2 lasting 8 weeks measuring all needed parameters of feed intake and growth to calculate RFI, from now referred to as

RFI period. In the final phase, steers where moved in batches of six, each week, to a different shed containing the respiration chambers in order to measure methane emissions. From now on referred to as Chamber Period. During this period, steers were allocated to individual training pens (12 m^2) using a replicated randomised block design so that allocation was balanced for live-weight, breed and treatment. Steers spent 6 days in the individual training pens and subsequently were moved for 4 days into the respiration chambers. The chambers contained an internal pen identical to training pen, with an internal volume of 76 m^3. The training pens and the respiration chambers were in the same building. The methodology for housing, feeding and methane measurement is provided in Rooke et al. (2014) and Troy et al. (2015).

Data acquisition of activity behaviour for this study were done during the RFI period. During this period all steers were housed and fed at the same time. Whereas during the Chamber Period steers were removed from and returned to the group pens in batches of six. Three-way accelerometer data loggers (IceTag Pro, IceRobotics Ltd., Edinburgh, Scotland) were fitted to the hind left legs of the animals to collect detailed information on activity behaviour, including the parameters Motion Index; % of time standing, % of time lying, number of steps, and number of lying bouts. The Motion Index consists of a summary of the three-way accelerometer data for each minute using the mean of the maximum amplitude for each axis for that minute (IceRobotics Ltd, Product Guide 2010). A low Motion Index corresponds with less active behaviour and a higher Motion Index corresponds with higher activity.

Feeding behaviour was collected over the RFI period using automated feed intake equipment (HOKO, Insentec, Marknesse, The Netherlands) with 8 feeders available to all steers per pen. Fresh feed was supplied daily at approximately 9 to 9:30 h (offered *ad libitum*). Each feed was supplied to all steers per pen, i.e. there was no mixing of diet treatment within pens, as early studies with these types of diets had shown significant behavioural interaction linked to feed being presented in different feeders at different times for two diets in the same pen. The automated weighing mechanisms were used to collect detailed information of individual feeding behaviour including the following parameters: Bunker Visits (events/day), Feeding Duration (minutes/day), Dry Matter Intake (DMI; kg/day), and Feeding Rate (grams of DM/minute). Body Weight Gain (BWG) was measured weekly and growth was modelled by linear regression of BW against days on trial, to describe average BWG, BW after four weeks and Metabolic Body Weight ($MBW=BW^{0.75}$) after four weeks. Ultrasonic fat depth at the $12^{th}/13^{th}$ rib (FD $12^{th}/13^{th}$) was measured at the end of measuring period. RFI was calculated as deviation of actual DMI from DMI predicted (kg/day) based on linear regression of actual DMI on BWG, MBW and FD $12^{th}/13^{th}$. Food Conversion Ratio (FCR) was calculated as FCR = DMI (kg/day)/ BWG.

Data analysis

Activity and feeding circadian and ultradian rhythms. Degree of Functional Coupling (DFC) is a parameter, developed by Scheibe et al. (1999), used here to measure the rhythmicity of behavioural variables. DFC expresses the percentage of cyclic behaviour that is harmonically synchronized in each 24 h period. To carry out the DFC analyses, preliminary analysis using different time intervals (e.g. 1, 5, 10, 15, 20 and 30 minute intervals) were performed. The data showed that 15 minute time intervals were the most appropriate and have been used throughout this paper. Therefore, activity time series with 15-minute intervals were created, by taking the motion index sum of 1-minute motion index, and averaged within each 15-minute period. Feeding time series with 15-minute periods were created, by taking the DMI in grams of each animal over each 15-minute period. Automated feeders recorded the time that animals started and stopped feeding, as well as the intake. Therefore, these original data were used to create these time series. Using the method of Scheibe et al. (1999), activity and feeding time series were analysed for rhythmic components.

To reduce the noise component, a process equivalent to moving average was used: autocorrelation functions were calculated for each seven-day interval, with a shift by one day and overlap of six days. The first seven-day interval includes the first day to the seventh day of measurement; the second seven-day interval includes the second day to the eighth day of measurement and so on. A power spectrum was drawn for each seven-day interval using autocorrelation functions. The periodogram ordinates were calculated for all Fourier frequencies: $\omega=2\pi j/n$, $j=1,...,q$ with $q=n/2$ (n even) or $q=n/2-1$ (n odd), with n being the number of data points in the sample (BERGER et al., 1999; SCHEIBE et al., 1999). The periodogram ordinates were tested for statistical significance according to the R. A. Fisher test for periodicity (ANDEL, 1984). The significant ordinates represent the significant periodic components.

Harmonic periods were defined as periods which are synchronized with the day length (24 h) in relation to an integral number (1, 2, 3… etc.). Thus, 24 h divided by an integer number gives the harmonic periods (the period length of 24, 12, 8, 6, 4.8, 4, 3.4, 3, 2.6, etc. hours are harmonic). DFC is here expressed as the percentage of the circadian component and harmonic ultradian components in relation to all rhythmic components of a spectrum. Therefore, DFC demonstrates the relationship between the total intensity of periods that were harmonic and significant to the circadian period (SI_{Harm}) and the total intensity of all periods that were significant (SI_{Total}) (BERGER et al., 1999; SCHEIBE et al., 1999):

$$DFC\ (\%) = (SI_{Harm} * 100)/SI_{Total}$$

Where, SI_{Harm} = Σ of intensities of periods that are harmonic and significant;

SI_{Total} = Σ of intensities of periods that are significant.

DFC% varies from 0% to 100%, where 0% means that only non-harmonic periods were significant and 100% means that only harmonic periods were significant (BERGER et al., 1999, 2003). Low DFC indicates lower synchronization and high DFC higher synchronization. DFC was continuously calculated for all steers, during all experimental days and a set of DFC daily averages was built for each steer.

Others statistical analyses. Considering breed and diet effects, the differences between variables were analysed using the Mixed Procedure of SAS 9.3 and the *P* value of the differences of least squares means were adjusted for Tukey-Kramer. The variables of bunk visits, feeding duration, feeding rate and lying bouts were not normally distributed, thus square root transformation was used. The variables activity DFC% and its Standard Deviation (STD) were not normally distributed, thus logit transformation was used. Regression analyses were modelled in SAS 9.3 using REG procedure.

Results

Behavioural patterns over 24-hour periods

The activity and feeding patterns within 24-hour periods for the different groups are visualised in Figure 1 and 2, respectively, and statistical analysis is shown in Table 1. The activity behaviour patterns within 24-hour periods showed a breed influence over two time-intervals, at 9-12 h and 18-21 h. The motion index was higher for Charolais. The feeding behaviour patterns within 24-hour periods showed effects of breed, diet and interaction.

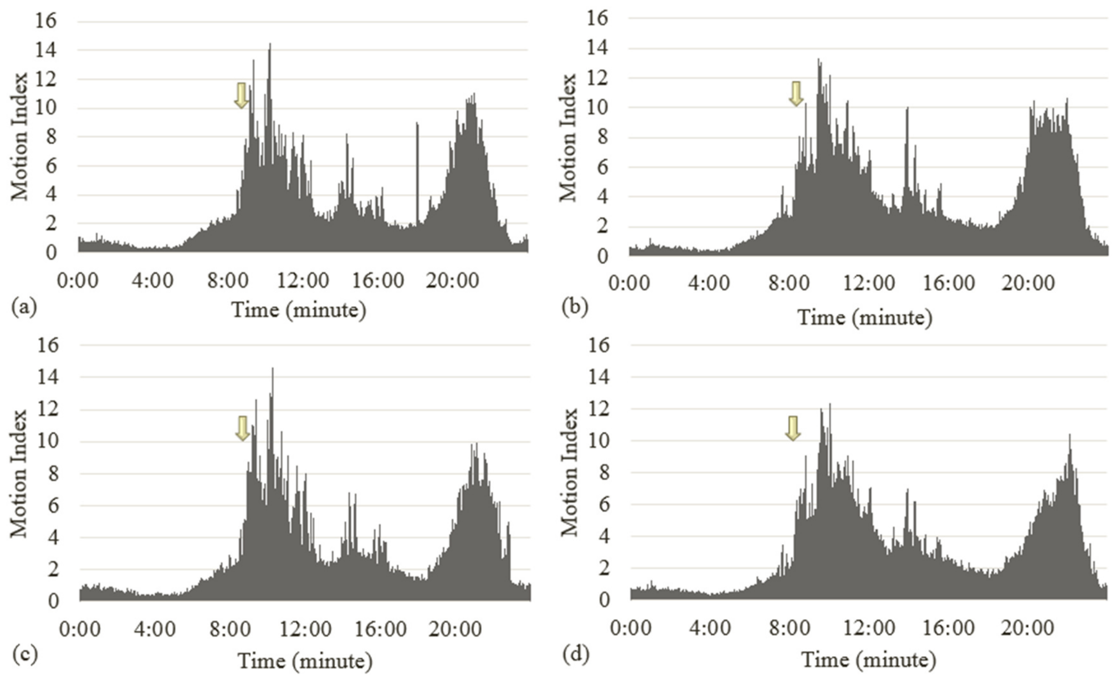


Figure 1. Motion Index patterns (24-hour) of housed steers during RFI period. Arrows show average time when fresh feed was supplied. Sunrise occurred between 03:30 – 03:50 h and sunset between 20:30 – 21:00 h. One bar

represented a minute and every minute value is an overall average of: (a) Charolais-sired steers fed with concentrate diet (n=19); (b) Charolais-sired steers fed with mixed diet (n=20); (c) Purebred Luing steers fed with concentrate diet (n=18); (d) Purebred Luing steers fed with mixed diet (n=18).

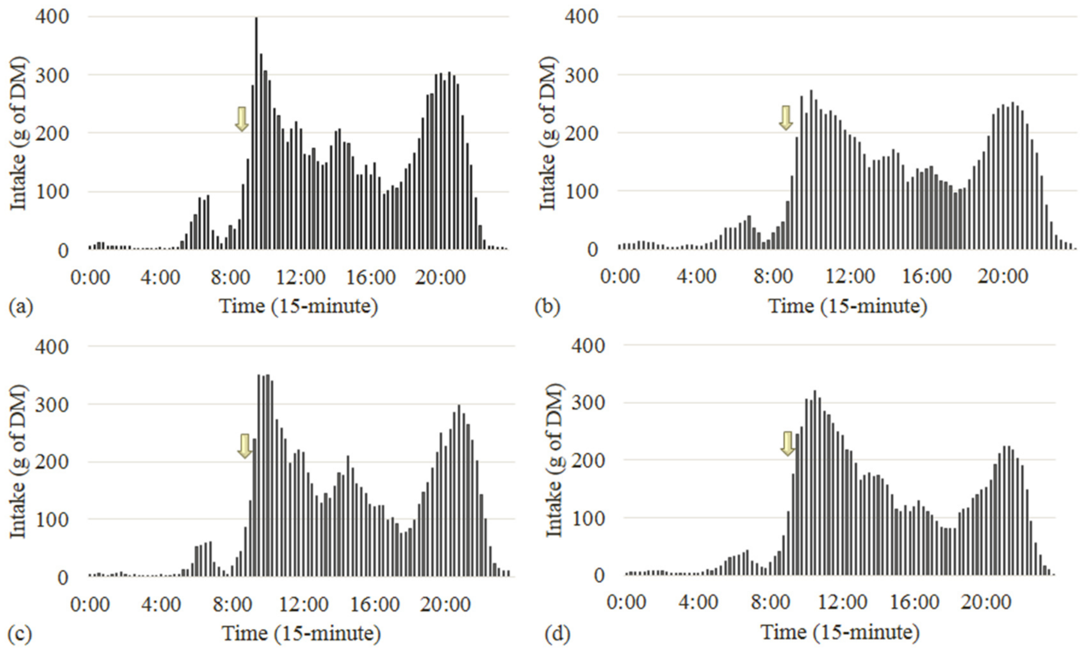


Figure 2. Intake patterns (24-hour) of housed steers during RFI period.
Arrows show average time when fresh feed was supplied. Sunrise occurred between 03:30 – 03:50 h and sunset between 20:30 – 21:00 h. One bar represents a 15-minute interval and every bar is an overall average of DMI of: (a) Charolais-sired steers fed with concentrate diet (n=20); (b) Charolais-sired steers fed with mixed diet (n=20); (c) Purebred Luing steers fed with concentrate diet (n=20); (d) Purebred Luing steers fed with mixed diet (n=20).

Table 1 *Effect of breed (B), diet (D) and their interaction (B*D) on Motion Index (MI) and Feeding Rate (FR, kg of DM/180 minutes) patterns over 24-hour periods of Charolais-sired and purebred Luing steers fed either with a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diet during RFI period*

	Mixed		Concentrate			Significance		
	Charolais	Luing	Charolais	Luing	SEM[1]	B	D	B*D
MI, 0-3 h	100.39	115.60	115.59	130.08	7.29	0.14	0.14	0.97
MI, 3-6 h	9.14	9.09	8.45	8.85	0.42	0.74	0.47	0.67
MI, 6-9 h	551.06	458.67	420.46	392.91	31.56	0.05	0.08	0.29
MI, 9-12 h	1325.24	1295.73	1248.15	1263.04	64.46	0.91	0.61	0.73
MI, 12-15 h	699.61	660.25	594.65	556.61	38.79	0.32	0.12	0.98
MI, 15-18 h	426.12	393.72	396.97	397.27	19.06	0.54	0.62	0.53
MI,18-21 h	920.76^{a}	591.92^{b}	950.89^{a}	703.28^{b}	53.34	***	0.31	0.41
MI, 21-0 h	845.56	840.51	619.13	712.95	77.75	0.39	0.15	0.63
FR, 0-3 h	0.12	0.07	0.08	0.06	0.01	0.11	0.28	0.69
FR, 3-6 h	0.01	0.01	0.01	0.01	0.00	0.33	0.72	0.78
FR, 6-9 h	0.56^{a}	0.46^{b}	0.75^{a}	0.55^{b}	0.05	**	0.09	0.39
FR, 9-12 h	2.78^{b}	3.23^{a}	3.09^{b}	3.24^{a}	0.12	**	0.46	0.19
FR, 12-15 h	1.89	2.07	2.05	1.97	0.07	0.43	0.84	*
FR, 15-18 h	1.45^{a}	1.26^{b}	1.47^{a}	1.33^{b}	0.04	**	0.46	0.65
FR, 18-21 h	2.48^{b}	1.78^{c}	3.03^{a}	2.53^{b}	0.09	***	***	0.44
FR, 21-0 h	0.88^{b}	1.19^{a}	0.72^{b}	1.06^{a}	0.07	**	0.12	0.89

***<0.0001, **<0.01, *<0.05. [a,b,c] Means with different superscripts along the same line are significantly different with $P<0.05$ for differences of least squares means. [1]Standard error of the mean for breed effect.

Behavioural variables and circadian rhythms

The overall averages for circadian rhythm and behavioural variables are presented in Table 2. Besides the differing activity patterns within the 24-hour periods, breed did not influence the overall means of Motion Index. However, diet and breed had an effect on the behavioural variables standing, lying and number of steps. Luing spent a higher percentage of time standing, but showed also a higher number of lying bouts than Charolais. Steers fed with a concentrate diet spent a higher percentage of time lying and had lower number of steps than steers fed with a mixed diet. The number of bunker visits was influenced by diet; while feeding duration and feeding rate had both breed and diet effects. Steers fed with a mixed diet had higher number of bunker visits and spent more time feeding than steers fed with a concentrate diet. Charolais showed a higher feeding rate than Luing; and steers fed with a concentrate diet had a higher feeding rate than steers fed with a mixed diet.

The activity DFC% for each steer within breed and diet groups is shown in Figure 3. The activity DFC% and its STD, which represents the DFC% variation across the experimental days, were influenced by diet and showed significant interaction between breed and diet. Steers fed with a concentrate diet had lower activity DFC% and higher variation on its response than steers fed with a mixed diet. In addition, activity DFC% was even lower for Luing fed with concentrate, showing an interaction between breed and diet. The high STD of activity DFC% for Luing fed with a concentrate diet can be clearly seen in Figure 3, c. In contrast, Luing fed with a mixed diet showed high consistency of activity DFC% (Figure 3, d). The response of feeding DFC% for each steer within breed and diet groups are shown in Figure 4. Regarding the feeding DFC%, all groups showed high values and no effect of breed and diet; however, steers fed with a concentrate diet showed a lower consistency of feeding DFC% across experimental days (high STD of feeding DFC%) than steers fed with a mixed diet.

Production traits

Effects of breed and diet on performance, efficiency and methane emission of housed steers are shown in Table 3. Body Weight Gain was not affected by diet but showed a breed effect; and the BWG of Charolais was higher than the BWG of Luing. Dry Matter Intake was influenced by diet, being higher for steers fed with a concentrate diet. However, DMI based on BW and metabolic BW showed a breed effect with lower intake for Charolais. Breed and diet did not influence the FCR, whereas the RFI showed a breed effect. Charolais were more efficient by having a lower RFI than Luing. Methane emission measurements showed a diet effect, and animals fed with a mixed diet had higher emissions than animals fed with a concentrate diet.

Table 2 *Effect of breed (B), diet (D) and their interaction (B*D) on behavioural variables and circadian rhythms of Charolais-sired and purebred Luing steers fed either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diet during the RFI period*

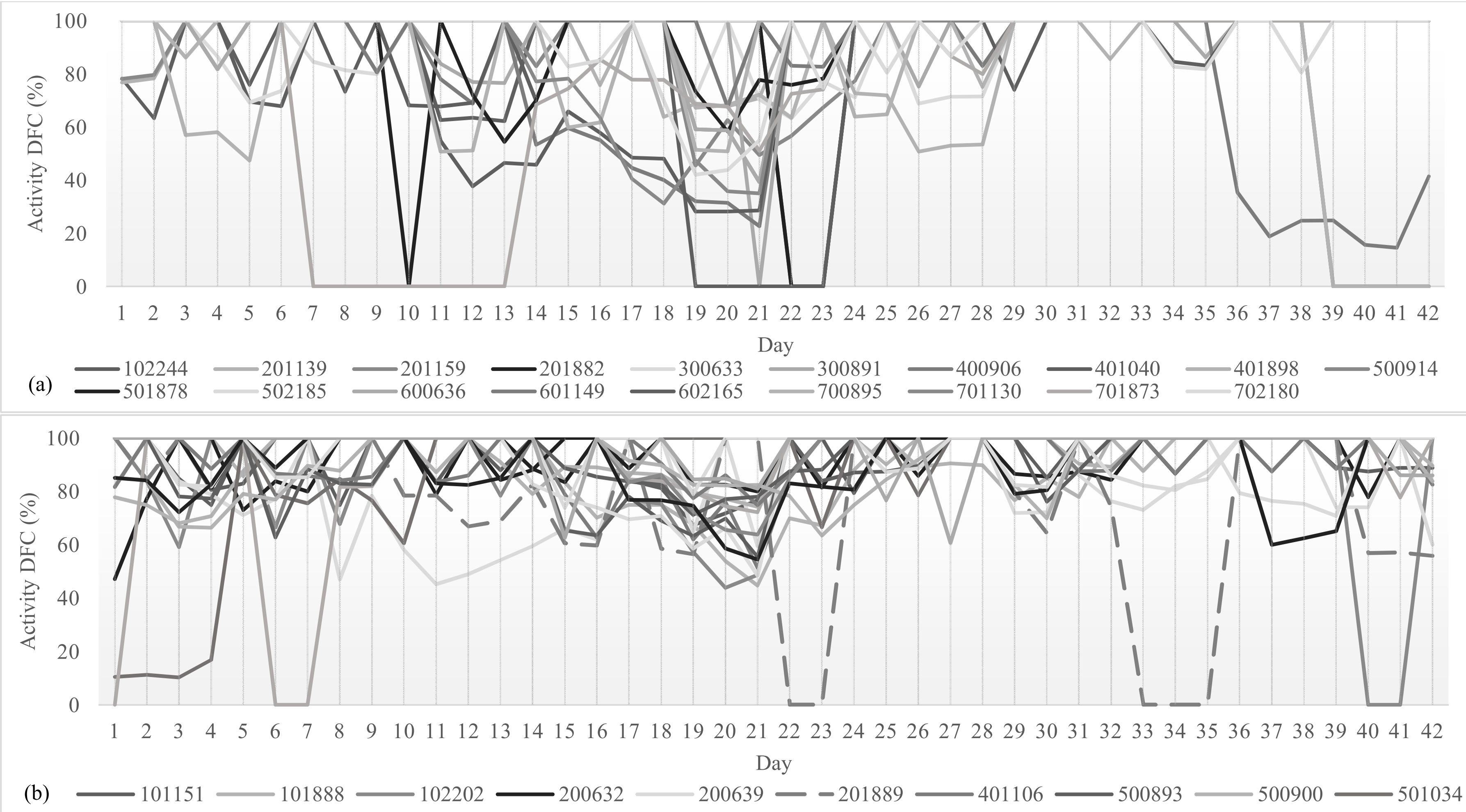
	Mixed		Concentrate		SEM[1]			Significance		
	Charolais	Luing	Charolais	Luing	B	D	B*D	B	D	B*D
Motion Index, total/day	4962.89	4433.55	4418.51	4238.28	187.16	184.62	268.66	0.17	0.15	0.50
Standing, % time of day	37.70^{b}	41.33^{a}	36.07^{b}	37.26^{b}	0.66	0.66	0.93	**	**	0.18
Lying, % time of day	62.16^{a}	58.42^{b}	63.88^{a}	62.70^{a}	0.68	0.68	0.96	**	**	0.18
Steps, count per day	1424.47^{a}	1249.70^{ab}	1253.16^{ab}	1066.53^{b}	49.31	49.31	69.83	**	**	0.93
Lying bouts, count per day	81.11	83.77	76.77	130.75	11.81	13.49	16.71	0.05	0.41	0.09
Bunker visits, events/day	52.34^{a}	55.81^{a}	32.59^{b}	32.66^{b}	1.47	1.47	2.13	0.44	***	0.57
Feeding duration, minutes/day	159.13^{a}	172.81^{a}	93.26^{b}	101.10^{b}	4.32	5.37	6.14	**	***	0.63
Feeding rate, g of DM /minute	71.01^{c}	62.82^{c}	130.30^{a}	114.93^{b}	3.31	3.82	4.72	**	***	0.54
Activity DFC, %	88.83^{a}	91.08^{a}	84.82^{ab}	81.58^{b}	2.80	3.76	3.93	0.67	**	**
STD of activity DFC, %	15.07^{bc}	11.70^{c}	22.17^{ab}	28.31^{a}	2.76	3.59	3.88	0.49	***	**
Feeding DFC, %	90.55	92.28	92.70	91.19	0.90	0.81	1.16	0.91	0.68	0.11
STD of feeding DFC, %	11.93^{b}	9.79^{b}	14.14^{a}	14.35^{a}	0.75	0.81	1.06	0.31	**	0.21

***<.0001, **<0.01, *<0.05. ^{a,b,c} Means with different superscripts along the same line are significantly different with *P*<0.05 for differences of least squares means. [1]Standard error of the mean

Table 3 *Effect of breed (B), diet (D) and their interaction (B*D) on growth, feed intake, feed efficiency and methane emission of Charolais-sired and purebred Luing steers fed either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diet during the RFI period*

	Residual feed intake period									
	Mixed		Concentrate		SEM[1]			Significance		
	Charolais	Luing	Charolais	Luing	B	D	B*D	B	D	B*D
Age start, days	394	392	391	391	5.05	4.99	7.24	0.91	0.75	0.92
Mid-test body weight, kg	540^{a}	476^{b}	560^{a}	477^{b}	9.63	9.51	13.80	***	0.43	0.50
Mid-test metabolic body weight, kg	112^{a}	102^{b}	115^{a}	102^{b}	1.53	1.51	2.19	***	0.44	0.50
Body weight gain, kg/day	1.58^{a}	1.47^{b}	1.69^{a}	1.60^{b}	0.06	0.08	0.09	*	0.06	0.90
Fat depth, mm[2]	6.60^{b}	7.74^{a}	5.98^{b}	7.05^{a}	0.25	0.24	0.35	**	0.05	0.91
Dry Matter Intake, kg/day	10.58^{b}	10.53^{b}	11.72^{a}	11.15^{a}	0.18	0.18	0.27	0.33	**	0.22
DMI g/kg Mid-test BW	19.21^{b}	22.11^{a}	19.90^{b}	22.45^{a}	0.64	0.86	0.88	***	0.35	0.57
DMI g/kg Mid-test $BW^{0.75}$	92.26^{b}	102.58^{a}	96.28^{b}	103.89^{a}	2.73	3.68	3.79	***	0.26	0.27
FCR, kg DMI/kg BWG	6.74	7.26	6.84	6.97	0.15	0.15	0.22	0.12	0.65	0.35
Residual feed intake, kg/day	-0.64^{b}	0.09^{a}	0.15^{b}	0.43^{a}	0.27	0.36	0.37	***	0.18	0.08
	Chamber period									
	Mixed		Concentrate		SEM[1]			Significance		
	Charolais	Luing	Charolais	Luing	B	D	B*D	B	D	B*D
Age at entry to chamber, days	491	500	498	497	4.56	4.63	6.82	0.61	0.73	0.42
Body weight, kg	642^{b}	568^{c}	679^{a}	589^{c}	6.44	6.36	9.37	***	**	0.38
Metabolic body weight, kg$BW^{0,75}$	127^{b}	116^{c}	133^{a}	119^{c}	0.98	0.96	1.42	***	**	0.42
Body weight gain, kg/day	1.51^{a}	1.24^{b}	1.56^{a}	1.39^{b}	0.05	0.05	0.07	**	0.14	0.47
DMI in chamber, kg/day	9.55^{b}	8.94^{b}	10.98^{a}	10.24^{a}	0.29	0.28	0.42	0.09	***	0.86
GE intake in chamber, MJ/day	180.48^{b}	169.03^{b}	204.23^{a}	190.42^{a}	5.40	5.33	7.86	0.09	**	0.87
ME intake in chamber, MJ/day	114.59^{b}	107.32^{b}	139.45^{a}	130.02^{a}	3.56	3.52	5.19	0.09	***	0.83
Methane, g/day	192.70^{a}	183.86^{a}	144.14^{b}	150.19^{b}	6.21	6.13	9.04	0.87	***	0.39
Methane, g/ kg DMI	22.15^{a}	21.96^{a}	15.05^{b}	16.12^{b}	0.80	0.72	0.94	0.49	***	0.30
Methane, KJ/ MJ GE	64.96^{a}	64.39^{a}	44.77^{b}	47.97^{b}	2.37	2.13	2.79	0.48	***	0.29
Methane, KJ/ MJ ME	102.05^{a}	101.25^{a}	65.92^{b}	70.53^{b}	3.63	3.27	4.27	0.51	***	0.33
CH_4/CO_2 molar ratio	0.07^{a}	0.07^{a}	0.05^{b}	0.06^{b}	0.001	0.001	0.002	0.17	***	0.30

***<.0001, **<0.01, *<0.05. [a,b,c] Means with different superscripts along the same line are significantly different with $P<0.05$ for differences of least squares means. [1] Standard error of the mean.


Activity DFC (%)
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
Day
(a)
102244 201139 201159 201882 300633 300891 400906 401040 401898 500914
501878 502185 600636 601149 602165 700895 701130 701873 702180
Activity DFC (%)
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
Day
(b)
101151 101888 102202 200632 200639 201889 401106 500893 500900 501034

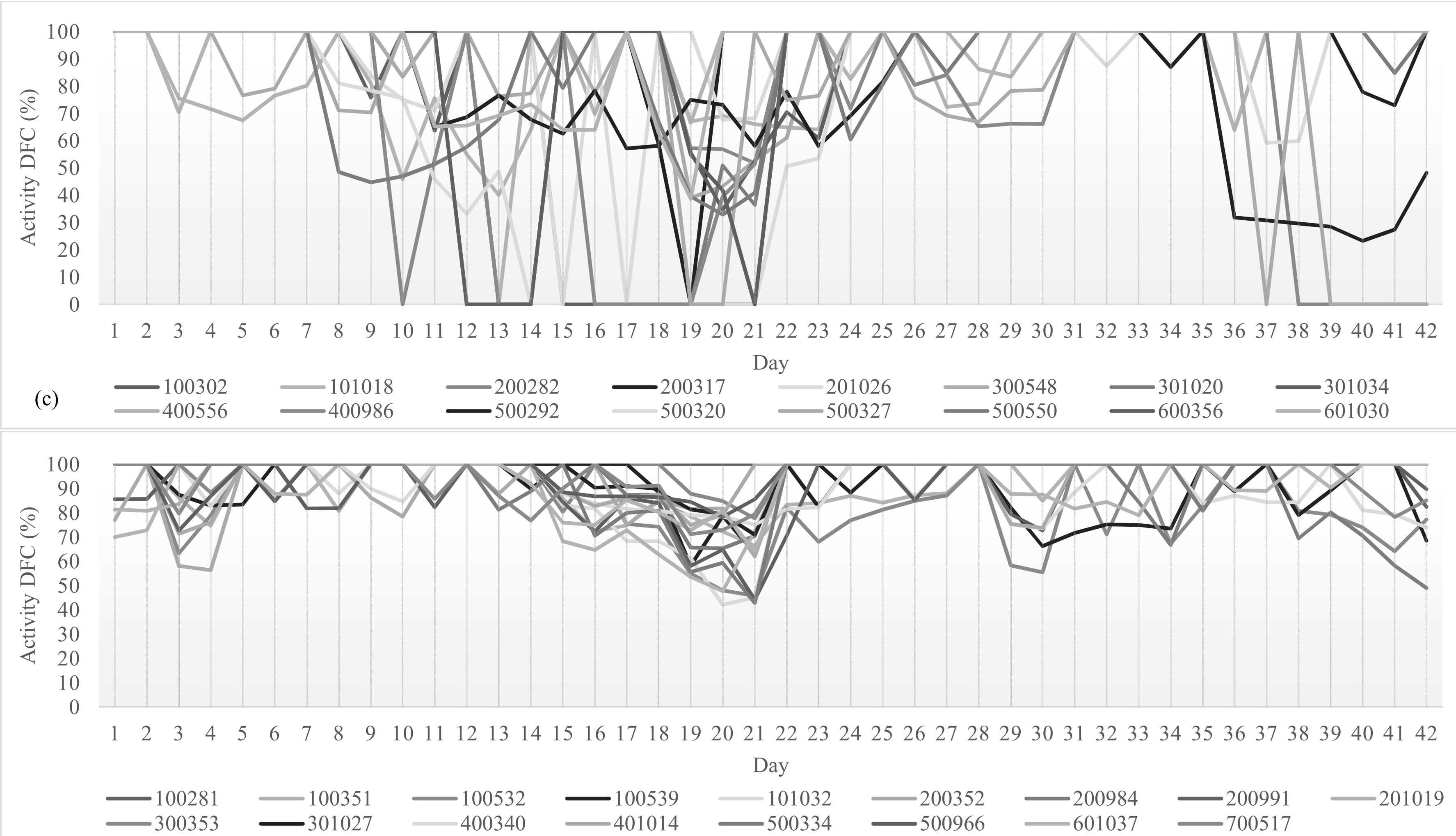
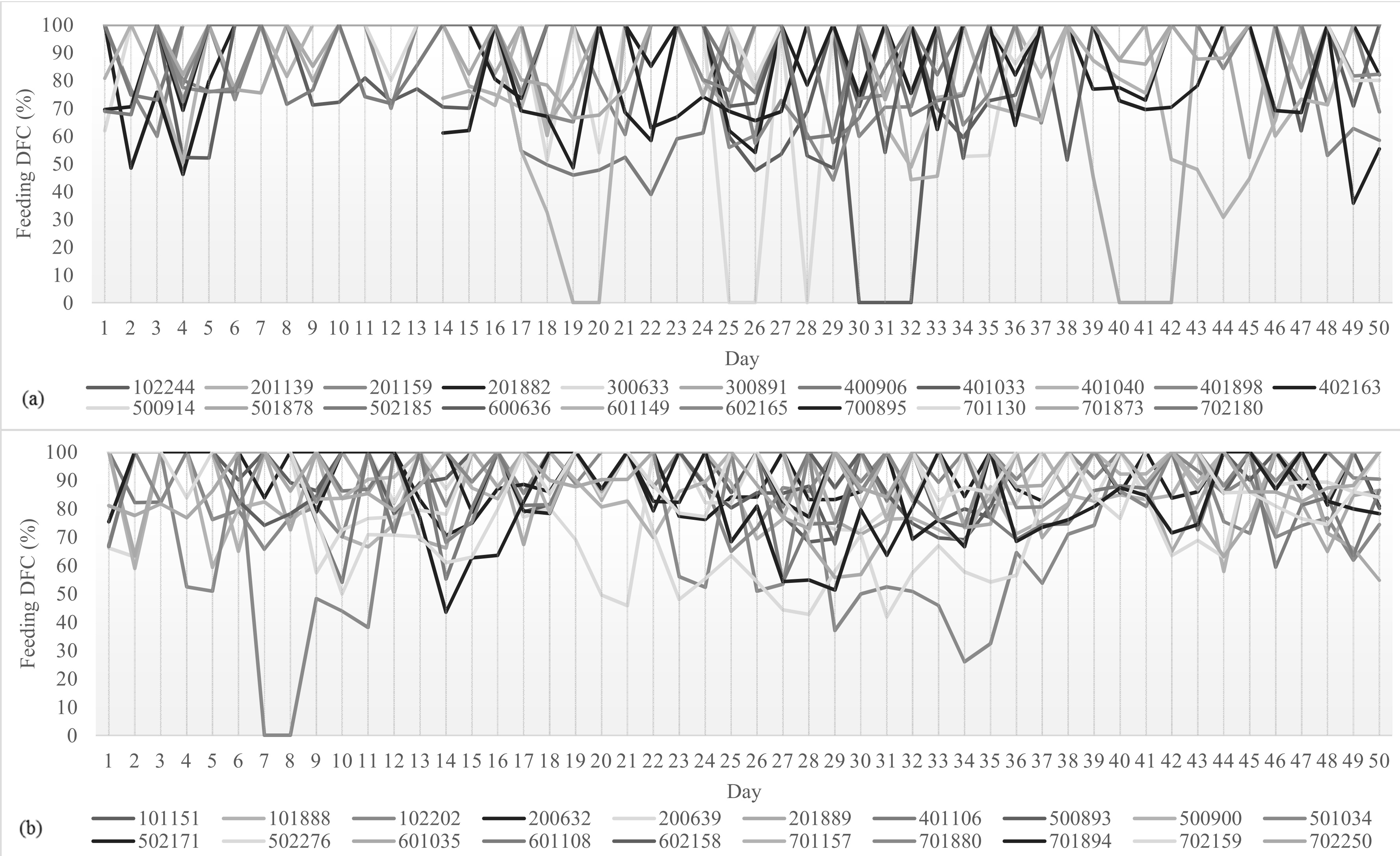


Figure 3 Degrees of functional coupling (DFCs) of activity of steers over the RFI period.

DFCs were calculated for moving seven-day intervals with Day 1 on the y axis covering Day 1 to Day 7 inclusive, and then on. (a) Activity DFCs of Charolais-sired steers fed with a concentrate diet (n = 19); (b) Activity DFCs of Charolais-sired steers fed with a mixed diet (n = 20); (c) Activity DFCs of Luing fed with a concentrate diet (n = 16); (d) Activity DFCs of Luing fed with a mixed diet (n = 17).


(a)
Feeding DFC (%)
Day
102244
201139
201159
201882
300633
300891
400906
401033
401040
401898
402163
500914
501878
502185
600636
601149
602165
700895
701130
701873
702180
(b)
Feeding DFC (%)
Day
101151
101888
102202
200632
200639
201889
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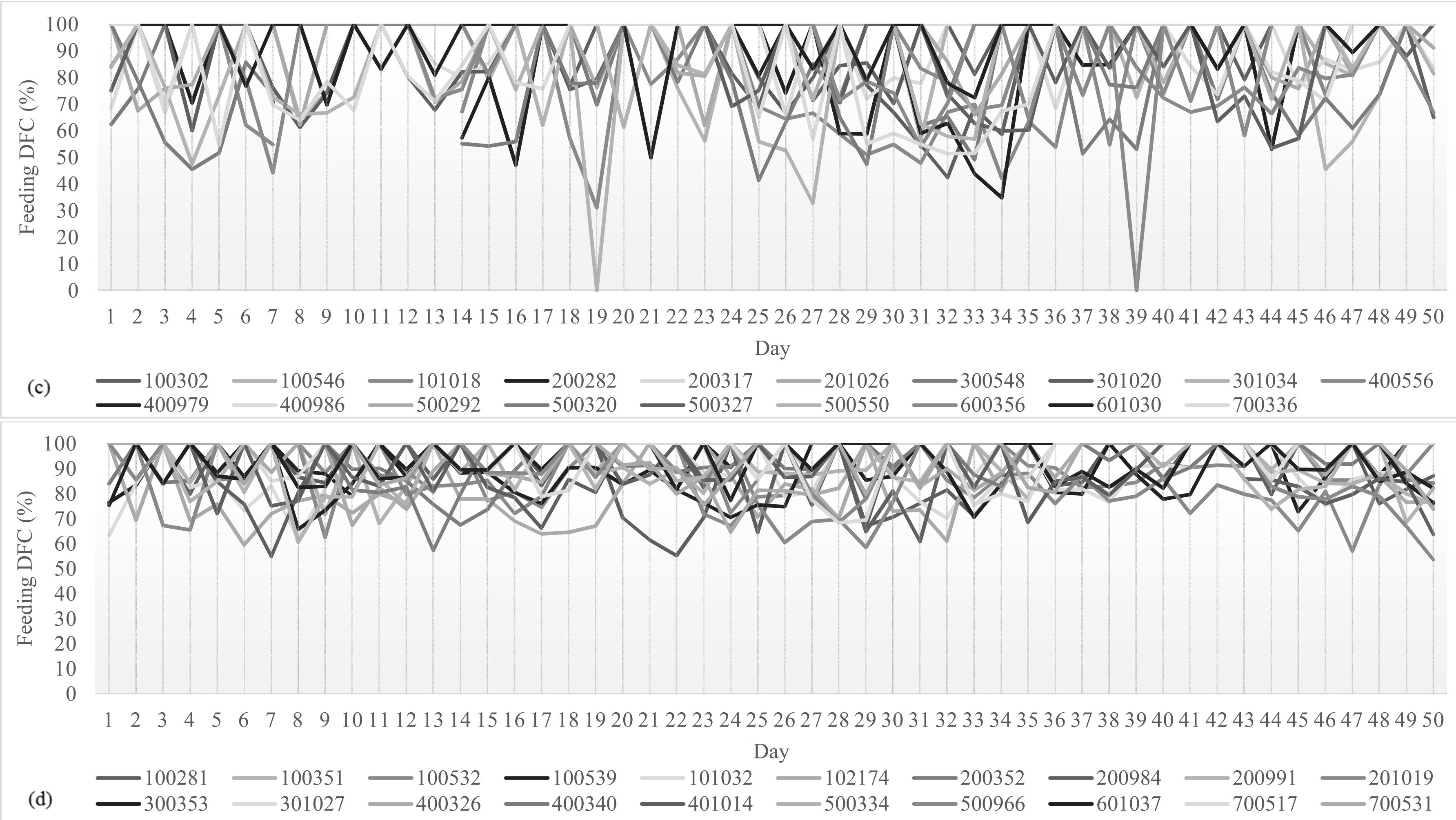

Figure 4 Degrees of functional coupling (DFCs) of feeding of steers over the RFI period.

DFCs were calculated for moving seven-day intervals with Day 1 on the y axis covering Day 1 to Day 7 inclusive, and then on. (a) Feeding DFCs of Charolais-sired steers fed with a concentrate diet (n=20); (b) Feeding DFCs of Charolais-sired steers fed with a mixed diet (n=20); (c) Feeding DFCs of Purebred Luing steers fed with a concentrate diet (n=20); (d) Feeding DFCs of Purebred Luing steers fed with a mixed diet (n=20).

Links between production traits, behavioural variables and circadian rhythms

Considering that behavioural variables were affected by diet and breed and the circadian rhythm data showed effects of diet and interactions between diet and breed, regressions are also displayed within groups, in order to annul data variability caused by these effects (see Table 4). Significant regressions between production traits and behavioural variables for the whole set of experimental animals are shown in Table 4 at the end of this section. Other significant regressions between production traits and circadian rhythm data for the whole set of experimental animals are shown in Table 5 at the end of this section. This study found many links between production traits, behavioural variables and circadian rhythms of activity and feeding, however, just within groups relations are highlighted below.

Charolais-sired steers fed with a mixed diet. Within the group of Charolais fed with a mixed diet, steers with higher number of Bunker Visits showed higher BWG (Regression 1 – Table 4) and there was a relationship between methane emission and Lying Bouts, with steers that had higher number of Lying Bouts also had higher methane emission (Regression 2 – Table 4). For the circadian rhythm of activity, steers with higher activity DFC% and lower variation in the response of activity DFC% (STD) showed higher BWG (Regressions 100 and 101 – Table 5, highlighted in Figure 5). In addition, the BWG was well estimated by a range of factors as shown in the multiple Regression 102 – Table 5. Steers with better FCR (lower FCR) showed higher activity DFC% (Regression 106 – Table 5). Steer feed efficiency was also related with the activity DFC%. The multiple regression 103 (Table 5) showed that steers with higher activity DFC%, lower

DMI and higher methane yield had lower RFI and, thus, were demonstrated to be more efficient. The feeding DFC% was also related with RFI (Regressions 104 and 105 – Table 5). However, steers with higher variation in the response of feeding DFC% and lower DMI showed lower RFI and therefore higher efficiency.

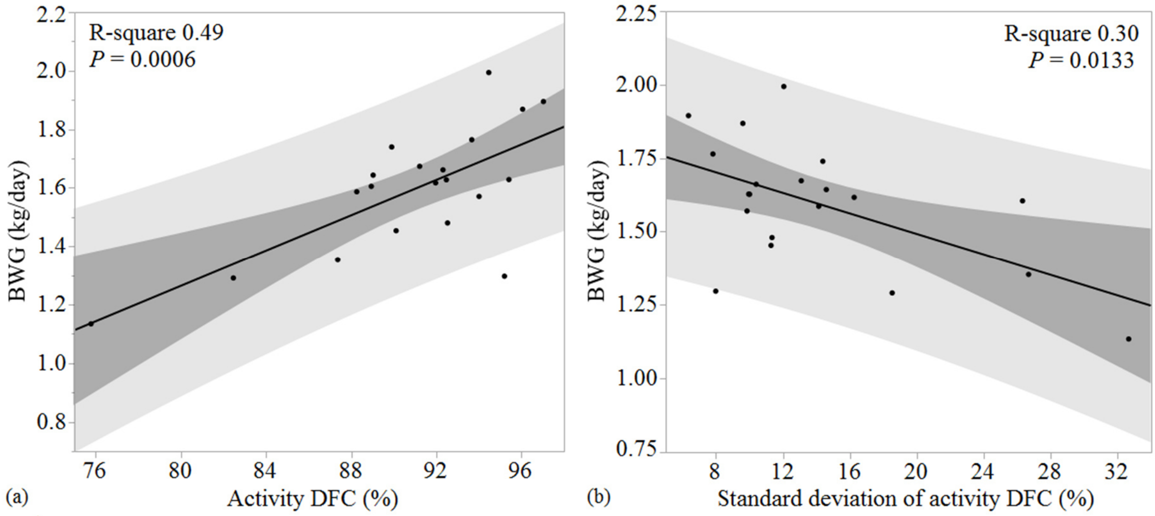

Figure 5 Regressions between BWG and circadian rhythm of activity (DFC) for the group 'Charolais-sired steers fed with mixed diet'.

(a) Linear positive regression between BWG and DFC%; (b) Linear negative regression between BWG and STD of DFC%.

Charolais-sired steers fed with a concentrate diet. Within the group of Charolais fed with a concentrate diet, steers with lower feeding rate were more efficient (Regression 5 – Table 4). There were also relationships between the percentage of time standing and lying with RFI efficiency and FCR. Steers that were standing a lower percentage of time and lying a higher percentage of time were showing to be more efficient (Low RFI) and to have a better FCR (Regressions 6, 7, 8 and 9 – Table 4). In addition, steers that were standing longer had higher subcutaneous fat depth measured over the $12^{th}/13^{th}$ rib (Regressions 10 and 11

– Table 4). Looking at the activity rhythmicity, steers with higher activity DFC% and lower variation on its response (STD) had lower methane emission levels per grams of kg of DMI (methane yield) (Regressions 21 and 22 – Table 5, highlighted in Figure 6). It is important to say that within this group methane yield was neither related with RFI (*P* = 0.81 for linear regression), nor with fat depth measured over the $12^{th}/13^{th}$ rib (*P* = 0.15 for linear regression). The feeding DFC% was positive related with BWG (Regression 15 – Table 5). In addition, steers with higher feeding DFC% had higher subcutaneous fat depth measured over the $12^{th}/13^{th}$ rib (Regressions 19 and 20 – Table 5).

Purebred Luing steers fed with mixed diet. Within the group of Luing fed with a mixed diet, steers with lower feeding rate and higher feeding duration showed lower fat depth measured over the $12^{th}/13^{th}$ rib (Regressions 3 and 4 – Table 4). Simple behavioural traits measured were not related with animal RFI, FCR or BWG (*P* > 0.05). The activity DFC% was related with methane yield. However, for Luing fed with a mixed diet, higher methane yield was linked with higher activity DFC% and lower variation on its response (Regressions 109 and 110 – Table 5, highlighted in Figure 6). The feeding DFC% was related with CH_4:CO_2 molar ratio, and steers with higher variation in the response of feeding DFC% showed lower CH_4:CO_2 molar ratio (Regression 111 – Table 5), implying that steers with higher variation in the response of feeding DFC% had lower methane emissions.

Purebred Luing steers fed with concentrate diet. Steers with lower number of bunker visits and higher feeding rate had a higher BWG (Regressions 12 and 13 – Table 4). However, steers with lower number of bunker visits also showed a lower efficiency with a higher RFI (Regression 14 – Table 4). There was a multiple regression for RFI, and steers which were more efficient had a lower DMI and a higher Feeding rate (Regression 15 – Table 4). In addition, RFI was also related with motion index, with a higher motion index for the more efficient steers (Regression 16 – Table 4). The CH_4:CO_2 molar ratio was lower for steers with higher number of Lying Bouts (Regression 23 – Table 4). There was no significant regression between production traits and activity DFC%. However, the RFI was well explained by a multiple regression with DMI and feeding DFC%. More efficient steers had lower DMI and higher feeding DFC% (Regression 122 – Table 5).

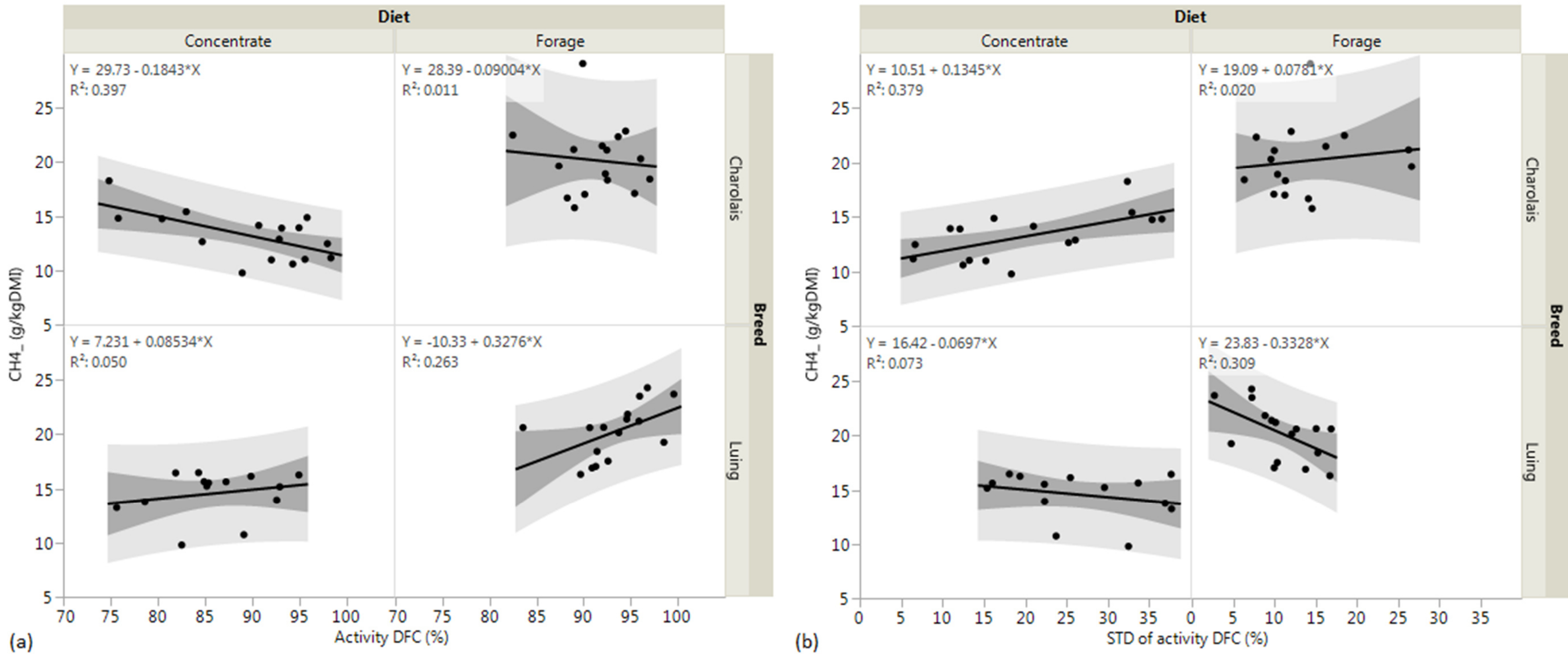

Figure 6 Within group regressions between methane yield and circadian rhythm of activity (DFC).

(a) The regressions between methane yield and activity DFC% were significant for the groups ‘Charolais-sired steers fed with concentrate diet’ and ‘Purebred Luing steers fed with mixed diet’ (*P* < 0.01 and *P* = 0.04, respectively); (b) The regression between methane yield and STD of activity DFC% were significant for the groups ‘Charolais-sired steers fed with concentrate diet’ and ‘Purebred Luing steers fed with mixed diet’ (*P* = 0.01 and *P* = 0.02, respectively).

Table 4 *Overall regression equations between production traits and behavioural variables of Charolais-sired and purebred Luing steers fed either a high concentrate (Concentrate) or mixed forage:concentrate (Mixed) diet*

	Breed		
Diet	Charolais	Luing	All
Mixed	(1) ***BWG = 1.0576 + 0.0102*BV (P=0.03; r^2=0.24);*** -- (2) ***CH_4 g/d = 161.0216 + 0.368*Lying bouts (P=0.03; r^2 =0.30);***	(3) ***FD 12/13th = -1.289 + 0.147*FR (P<0.01; r^2=0.39);*** (4) ***FD 12/13th = 18.254 - 0.0591*FD (P=0.05; r^2=0.21).***	No significant regressions.
Concentrate	(5) ***RFI = -2.169 + 0.0178*FR (P=0.02; r^2=0.24);*** (6) ***RFI = -2.534 + 0.0704*Standing (P=0.02; r^2= 0.31);*** (7) ***RFI = 4.511 - 0.0704*Lying (P=0.02; r^2= 0.31);*** --	(12) ***BWG = 2.260 - 0.0193*BV (P=0.03; r^2=0.23);*** (13) ***BWG = 1.0458 + 0.00507*FR (P=0.05; r^2=0.20);*** -- (14) ***RFI = - 1.649 - 0.0374*BV (P=0.01; r^2=0.30);***	(24) BWG = 1.275 + 0.00328*FR (*P*=0.03; r^2=0.11); -- (25) RFI = - 4.631 + 0.338*DMI + 0.0107*FD (*P*=0.04; r^2=0.52); (26) RFI = -2.185 + 0.0654*Standing

	(8) ***FCR = 3.477 + 0.0906*Standing (P=0.02; r^2= 0.30);*** (9) ***FCR = 12.538 - 0.0906*Lying (P=0.02; r^2= 0.30);*** -- (10) ***FD 12/13th = 1.148 + 0.134*Standing (P=0.02; r^2= 0.29);*** (11) ***FD 12/13th = 14.593 - 0.134*Lying (P=0.02; r^2= 0.29);***	(15) ***RFI = -2.666 + 0.364 DMI - 0.00841 FR (P=0.02; r^2=0.76);*** (16) ***RFI = 1.561 - 0.000268*MI (P=0.03; r^2 = 0.27);*** -- (17) CH_4 g/d = 232.0103 - 2.547*BV (*P*=0.03; r^2=0.27); (18) CH_4 g/d = 176.171 - 0.193*Lying bouts (*P*=0.05; r^2 =0.24); -- (19) CH_4 g/ kg DMI = 21.349 - 0.06656*FD (*P=0.06*; r^2 =0.21); -- (20) CH_4:CO_2 ratio = 0.0787 - 0.000706*BV (*P=0.06*; r^2 =0.22);	(*P*<0.01; r^2=0.20); (27) RFI = 4.358 - 0.0654*Lying (*P*<0.01; r^2=0.20); -- (28) FCR = 3.833 + 0.0818*Standing (*P*=0.04; r^2=0.11); (29) FCR = 12.00955 - 0.0818*Lying (*P*=0.04; r^2=0.11); -- (30) CH_4 g/d = 161.548 - 0.148*Lying_bouts (*P*=0.05; r^2=0.12); -- (31) CH_4:CO_2 ratio = 0.0653 - 0.000358*BV (*P*=0.04; r^2=0.12);

		(21) CH_4:CO_2 ratio = -0.00378 + 0.00161*Standing (*P*=0.04; r^2 =0.25); (22) CH_4:CO_2 ratio = 0.157 - 0.00161*Lying (*P*=0.04; r^2 =0.25); (23) ***CH_4:CO_2 ratio = 0.0679 - 9.0965e^{-5}*Lying bouts (P<0.001; r^2 =0.55).***	(32) CH_4:CO_2 ratio = 0.0585 – 4.842e^{-5} Lying bouts (*P*=0.02; r^2 0.16).
All	(33) RFI = 0.633 - 0.0206*BV (*P*=0.01; r^2=0.15); (34) RFI = 0.926 - 0.00929*FD (*P*<0.01; r^2=0.22); (35) RFI = -1.662 + 0.0140*FR (*P*<.0001; r^2=0.39); --	(49) BWG = 1.860 - 0.00669*BV (*P*=0.01; r^2=0.16); (50) BWG = 1.252 + 0.00346*FR (*P*<0.01; r^2=0.20); (51) BWG = 1.946 - 0.000314*Steps (*P*=0.03; r^2=0.13); -- (52) RFI = 1.0204 - 0.0169*BV	(74) BWG = 1.775 - 0.00371*BV (*P*=0.04; r^2=0.06); (75) BWG = 1.818 - 0.00155*FD (*P*=0.01; r^2=0.08); (76) BWG = 1.357 + 0.00269*FR (*P*<0.001; r^2=0.16); -- (77) RFI = 0.739 - 0.0170*BV (*P*=0.001; r^2=0.13); (78) RFI = 0.605 - 0.00460*FD (*P*=0.02; r^2=0.07);

	(36) FD 12/13th = 4.958 + 0.0106*FD (*P*=0.03; r^2=0.12). -- (37) CH_4 g/d = 104.130 + 1.507*BV (*P*<0.01; r^2=0.26); (38) CH_4 g/d = 101.586 + 0.526*FD (*P*<0.01; r^2=0.24). (39) CH_4 g/d = 220.219 - 0.517*FR (*P*=0.01; r^2=0.18). -- (40) CH_4 g/ kg DMI = 7.689 + 0.211*BV (*P*<0.0001; r^2=0.44). (41) CH_4 g/ kg DMI = 6.773 + 0.0781*FD (*P*<0.0001; r^2=0.46).	(*P*<0.001; r^2=0.27); (53) RFI = -0.244 + 0.00569*FR (*P*=0.02; r^2=0.15); -- (54) FCR = 7.603 - 0.00484*Lying_bouts (*P=0.06*; r^2=0.10); -- (55) CH_4 g/d = 128.757 + 0.881*BV (*P*=0.04; r^2=0.11); (56) CH_4 g/d = 114.817 + 0.380*FD (*P*=0.02; r^2=0.15); (57) CH_4 g/d = -7.224 + 4.4212*Standing (*P*=0.01; r^2 =0.18). (58) CH_4 g/d = 434.896 - 4.4212*Lying (*P*=0.01; r^2 =0.18).	(79) RFI = -0.810 + 0.00848*FR (*P*=0.0001; r^2=0.17); (80) RFI = 0.587 - 0.000498*Steps (*P*=0.04; r^2=0.06); -- (81) FD 12/13th = 5.647 + 0.00883*FD (*P*=0.05; r^2=0.05); (82) FD 12/13th = 3.298 + 0.0929*Standing (*P*=0.03; r^2=0.06); (83) FD 12/13th = 12.588 - 0.0929*Lying (*P*=0.03; r^2=0.06); -- (84) CH_4 g/d = 117.957 + 1.148*BV (*P*<0.001; r^2=0.17); (85) CH_4 g/d = 110.434 + 0.431*FD (*P*<0.001; r^2=0.18);

	(42) CH_4 g/ kg DMI = 25.364 - 0.0862*FR (*P*<0.0001; r^2=0.43). (43) CH_4 g/ kg DMI = 0.891 + 0.418*Standing (*P*=0.02; r^2=0.16). (44) CH_4 g/ kg DMI = 42.706 - 0.418*Lying (*P*=0.02; r^2=0.16). (45) CH_4 g/ kg DMI = 7.926 + 0.00631*Steps (*P*=0.01; r^2=0.19). -- (46) $CH_4:CO_2$ ratio = 0.0429 + 0.000425*BV (*P*<0.01; r^2=0.24). (47) $CH_4:CO_2$ ratio = 0.0362 + 0.000197*FD (*P*=0.0001; r^2=0.38).	(59) (54) CH4 g/d = 197.804 - 0.278*Lying bouts (*P*<0.01; r^2 =0.22). -- (60) CH_4 g/ kg DMI = 11.232 + 0.1483*BV (*P*<0.001; r^2 =0.34). (61) CH_4 g/ kg DMI = 9.529 + 0.0594*FD (*P*<0.0001; r^2=0.40). (62) CH_4 g/ kg DMI = 24.213 - 0.0722*FR (*P*<0.0001; r^2=0.37). (63) CH_4 g/ kg DMI = -0.248 + 0.453*Standing (*P*<0.01; r^2 =0.25). (64) CH_4 g/ kg DMI = 45.110 - 0.453*Lying (*P*<0.01; r^2 =0.25).	(86) CH_4 g/d = 203.345 - 0.375*FR (*P*=0.01; r^2=0.09); (87) CH_4 g/d = 27.630517 + 3.6104901*Standing (*P*<0.01; r^2=0.13); (88) CH_4 g/d = 388.67953 - 3.6104901*Lying (*P*<0.01; r^2=0.13); -- (89) CH_4 g/ kg DMI = 9.476 + 0.178*BV (*P*<.0001; r^2=0.39); (90) CH_4 g/ kg DMI = 8.124 + 0.0685*FD (*P*<.0001; r^2=0.43); (91) CH_4 g/ kg DMI = 24.796377 - 0.0798*FR (*P*<.0001; r^2=0.41);

	(48) CH_4:CO_2 ratio = 0.0849 - 0.000234*FR (P<0.0001; r^2=0.42).	(65) CH_4 g/ kg DMI = 12.361 + 0.00464*Steps (P=0.04; r^2 =0.13). (66) CH_4 g/ kg DMI = 19.653 - 0.0179*Lying_bouts (P=0.05; r^2 =0.11). -- (67) CH_4:CO_2 ratio = 0.0497 + 0.000327*BV (P<0.01; r^2 =0.21). (68) CH_4:CO_2 ratio = 0.0396 + 0.000176*FD (P<0.0001; r^2=0.44). (69) CH_4:CO_2 ratio = 0.0807 - 0.000186*FR (P<0.001; r^2=0.32). (70) CH_4:CO_2 ratio = 0.00786 + 0.00141*Standing (P=0.001; r^2=0.27).	(92) CH_4 g/ kg DMI = 0.122 + 0.441*Standing (P=0.001; r^2=0.21); (93) CH_4 g/ kg DMI = 44.271 - 0.441*Lying (P=0.001; r^2=0.21); (94) CH_4 g/ kg DMI = 11.901 + 0.00418*Steps (P<0.01; r^2=0.10); -- (95) CH_4:CO_2 ratio = 0.0462 + 0.000378*BV (P<.0001; r^2=0.23); (96) CH_4:CO_2 ratio = 0.0378 + 0.000187*FD (P<.0001; r^2=0.42); (97) CH_4:CO_2 ratio = 0.0827 - 0.000212*FR (P<.0001; r^2=0.38);

		(71) $CH_4:CO_2$ ratio = 0.1494 - 0.00141*Lying (*P*=0.001; r^2=0.27). (72) $CH_4:CO_2$ ratio = 0.0488 + 0.0000131*Steps (*P*=0.05; r^2=0.12). (73) $CH_4:CO_2$ ratio = 0.0735 - $8.948e^{-5}$*Lying Bouts (*P*<0.001; r^2=0.32).	(98) $CH_4:CO_2$ ratio = 0.0315 +0.000799*Standing (*P*=0.02; r^2=0.08); (99) $CH_4:CO_2$ ratio = 0.111 - 0.000799*Lying (*P*=0.02; r^2=0.08);

Most important regressions are highlighted in bold and italic. BV = Bunker Visits; FD = Feeding Duration; FD = Feeding Rate; FD 12/13th = Ultrasonic fat depth at the 12th/13th rib; MI = motion index.

Table 5 *Overall regression equations between production traits and circadian rhythms (DFCs) of activity and feeding of Charolais-sired and purebred Luing steers fed either a high concentrate (Concentrate) or a mixed forage:concentrate (Mixed) diet*

Diet	Breed		
	Charolais	Luing	All
Mixed	(100) ***BWG = -1.155 + 0.0302*Activity DFC% (P<0.001; r^2 = 0.49);*** (101) ***BWG = 1.842 - 0.0175*STD of activity DFC% (P<0.01; r^2 = 0.32);*** (102) ***BWG = - 0.0558 + 0.0279*Activity DFC% - 0.168 FCR + 0.0971 RFI + 0.00164 CH_4 g/d (P=0.05; r^2 = 0.89);*** -- (103) ***RFI = 2.663 – 0.0881*Activity DFC% + 0.579*DMI –***	(107) CH_4 g/d = -435.588 + 6.647*Activity DFC% (*P*=0.02; r^2 = 0.31). (108) CH_4 g/d = 240.00553 - 5.136*STD of activity DFC% (*P=0.07*; r^2 = 0.21). (109) ***CH_4 g/kg DMI = -10.327 + 0.327*Activity DFC% (P=0.04; r^2 = 0.26);***	(112) BWG = -0.578 + 0.0232*Activity DFC% (*P*<0.01; r^2 =0.22); (113) BWG = 1.736 - 0.0144*STD of activity DFC% (*P*=0.02; r^2 =0.15).

	0.00757*CH_4 g/d (P=0.04; r^2 = 0.58); (104) ***RFI = 1.118 - 0.256*STD of Feeding DFC% (P<0.01; r^2 = 0.33);*** (105) ***RFI = -2.859 - 0.271*STD of Feeding DFC% + 0.385*DMI (P<0.001; r^2 = 0.72);*** -- (106) ***FCR = 14.249 - 0.0826*Activity DFC% (P=0.04; r^2 = 0.22);***	(110) ***CH_4 g/ kg DMI = 23.832 - 0.333*STD of activity DFC% (P=0.02; r^2 = 0.31).*** (111) ***CH_4:CO_2 ratio = 0.0915 - 0.00280*STD of feeding DFC% (P=0.02; r^2 = 0.29).***	
Concentrate	(114) ***BWG = 3.626 - 0.0205*Feeding DFC% (P=0.01; r^2=0.27);*** (115) BWG = 2.0288 - 0.0505**STD of Feeding DFC%* (*P*=0.03; r^2=0.22); --	(122) ***RFI = 0.932 + 0.294*DMI – 0.0415*Feeding DFC% (P<0.01; r^2=0.81);***	(123) RFI = 0.750 - 0.112**STD of feeding DFC%* (*P*=0.04; r^2 =0.05). -- (124) CH_4 g/kg DMI = 24.362 - 0.119*Activity DFC% (*P*=0.04; r^2 =0.13); --

	(116)*FCR = -1.601 +* *0.0910*Feeding DFC%* *(P=0.04; r^2=0.19);* (117)*FCR = -6.671 +* *0.0776*Feeding DFC% +* *0.539*DMI* *(P=0.02; r^2=0.63);* (118)*FD 12/13^{th} = -6.614 +* *0.136*Feeding DFC%* *(P=0.03; r^2=0.21);* (119)*FD 12/13^{th} = -12.299 +* *0.120*Feeding DFC% + 0.604* *DMI (P=0.03; r^2=0.49);* -- (120)***CH_4 g/kg DMI = 29.726 -*** ***0.184*Activity DFC%*** ***(P<0.01; r^2 =0.40);*** (121)***CH_4 g/kg DMI = 10.515 +*** ***0.134*STD of activity DFC%*** ***(P=0.01; r^2 = 0.38).***	(125)CH_4 g/kg DMI = 11.969 + 0.766**STD of feeding DFC%* (*P*=0.04; r^2 =0.06);

All	(126)BWG = 0.743 + 0.0102*Activity DFC% (*P*=0.04*;* r^2 =0.10); -- (127)RFI = 1.120 - 0.211*STD of *Feeding DFC%* (*P*<0.01; r^2 = 0.16); -- (128)FD 12/13th = 4.423 + 0.289**STD of feeding DFC%* (*P*=0.02; r^2 = 0.12); -- (129)CH_4 g/d = 84.846 + 12.461**STD of feeding DFC%* (*P*=0.01; r^2 = 0.17). -- (130)CH_4 g/kg DMI = 7.000784 + 1.439**STD of feeding DFC%* (*P*<0.01; r^2 = 0.21).	(131) CH_4 g/d = -183.941 + 3.922*Activity DFC% (*P*<0.01; r^2 =0.27); (132) CH_4 g/d = 207.920 - 2.161*STD of activity DFC% (*P*<0.01; r^2=0.24). -- (133) CH_4 g/kg DMI = -19.151 + 0.409*Activity DFC% (*P*<.0001; r^2 =0.43); (134) CH_4 g/kg DMI = 22.555 - 0.274*STD of activity DFC% (*P*<.0001; r^2=0.57). -- (135) CH_4:CO_2 ratio = -0.0271 +	(137) CH_4 g/d = 188.113 - 1.164*STD of activity DFC% (*P*=0.04; r^2 =0.06). -- (138) CH_4 g/ kg DMI = 20.0477 - 0.167*STD of activity DFC% (*P*<0.01; r^2 =0.15). -- (139) CH_4:CO_2 ratio = 0.0700 - 0.000413*STD of activity DFC% (*P*=0.01; r^2=0.10).

	0.00102*Activity DFC% (P<0.01; r^2 =0.29); (136) CH_4:CO_2 ratio = 0.0762 - 0.000633*STD of activity DFC% (P<0.001; r^2=0.33).

Most important regressions are highlighted in bold and italic

Discussion

Behavioural patterns over 24-hour periods

In this study, housed steers started to be active at about 6 o'clock AM, at the same time that animals start to show considerable feeding intake. Activity and feeding behaviour peaked at the same time, at 9 - 12 h, reaching the highest feeding rate of the day. The fresh feed was supplied once per day, approximately at 9 h (*ad libitum*), thus, the activity and feeding rate peak were mainly driven by fresh food availability. In addition, the second activity and feeding peak occurred at same time, about 20 h, strengthening that the activity behaviour of housed steers were mainly associated with feeding. Looking at the 24 h patterns of feeding provides additional information compared to looking only for the overall average of feeding rate, as the study demonstrated the variation of feeding rate across the day. The breed effect in the overall average of feeding rate showed that Charolais demonstrated a higher feeding rate than Luing. However, Luing steers had a superior DMI than Charolais steers over the peak time-interval of 9 - 12 h, when most feed intake occurred. Thus, they had a higher feeding rate within these 180 minutes interval and probably with a high impact on the ruminal parameters.

Behavioural variables and circadian rhythms

Considering the entire day, simple behavioural variables showed that steers fed with a mixed diet (diet effect) and Luing steers (breed effect) spent more time standing, less time lying, and showing a higher feed duration and lower feeding rate. Thus, the high proportion of time spend standing were more likely to be an indirect effect of the feeding behaviour, as more time eating consequently means more time standing.

The circadian rhythms of activity were affected by diet, and steers fed with a concentrate diet showed a decreased activity DFC% and higher variation on its response across experimental days (STD of activity DFC%). The interaction between breed and diet showed that Luing fed with a concentrate diet had even lower values of activity DFC% and higher variation on its response. The circadian rhythm of activity is a variable that has high potential to be a key indicator for the general physiological state of animals, as its rhythmicity is an outcome of a large number of physiological processes (PICCIONE; CAOLA; REFINETTI, 2005). Considering that the rhythmicity of activity of each animal is unique and may vary according to its physiological status (BERGER et al., 2003; SCHEIBE et al., 1999) we plotted the activity DFC% response for each animal. Some steers fed with concentrate started to drop their activity DFC% between the 7th and 13th days in the DFC% figures. This was associated with a change in the barley source (a new batch of barley was introduced to all diets on the 9th experimental day), which had a high impact on the DMI on the day after the change (10th experimental day was included in the DFC% analysis from the 7th until the 13th days, with moving seven-day intervals). Thus, the high variation in the activity DFC% response shown by Charolais steers fed with a concentrate diet was an indicator that these animals may have faced an adaptation process regarding the diet.

Luing steers fed with concentrate showed a higher impact on their rhythmicity with a high number of steers decreasing to values equal or near 0%, indicating a greater difficulty of adaptation linked to the barley change and even a possibility of a metabolic problem. Sub-acute acidosis has no clear symptoms and is the most common metabolic problem that affects feedlot cattle fed with high concentrate diets (ABDELA, 2016; GONZÁLEZ et al., 2012). Steers fed with a mixed diet also showed some effect of the change on barley over the days 14 to 21 in the activity DFC% figures. However, the change had a lower intensity and duration indicating a faster adaptation to the diet. In addition, the variation in the response of the feeding DFC% (STD) was higher for steers fed with concentrate. However, adaptation characteristics to changed diets appeared to be best described by activity DFC% compared to other traits.

Duthie et al. (2017) studied the same two contrasting breeds and diets observing that rumen fluid from concentrate-fed steers had a greater proportions of propionic and valeric acids and lower proportions of acetic and butyric acids, as well as lower abundance of archaeal and protozoa, but more bacteria. Luing is a Scottish breed and is considered more adapted to extensive rangeland systems. This breed is well suited to lower quality diets and thus, it is reasonable to hypothesise that the concentrate diet had higher impact on the activity DFC% in this breed. Differently, Charolais is an important beef breed type in the UK, of large mature weight and typically fast growth rates and are commonly considered suited for intensive systems. The value of undertaking research work with contrasting breed types is underlined by these findings.

Links between production traits, methane emission, behavioural variables and circadian rhythms

In general, steers with lower feeding rate (r^2 = 0.41) and higher feeding duration (r^2 = 0.43) had a higher methane yield. Despite the effect being mainly driven by diet, lower feeding rate may favour a more stable relation between the production, absorption and passage of short-chain fatty acids (SCFA) in the rumen (ASCHENBACH et al., 2011). In addition, the low feeding rate may increase the total time of chewing and increase the amount of saliva produced, influencing bicarbonate secretion and rumen pH. It is well know that the pH of ruminal digesta have a substantial effect on the methanonegic community (ASCHENBACH et al., 2011; BERCHIELLI; PIRES; OLIVEIRA, 2006; KHIAOSA-ARD; ZEBELI, 2014; ROEHE et al., 2016). Thus, feeding rate and duration seems to be the most important simple behavioural variables to help to explain the methane emission levels. The relation between methane yield and the proportion of time standing and lying were weaker (r^2 = 0.21) than the relation between methane yield and feeding behaviour variables, thus, time spend standing was considered more likely to be an indirect effect of feeding behaviour.

An in-depth analysis of the behavioural rhythmicity is important in order to understand better the behavioural relationships with production traits. Activity DFC% showed a relationship with feeding rate. However, activity DFC% not only reflects the most important feeding behaviour, but it is also a reflection of the physiological state of the animals. The feeding rate was closely related with methane yield. However, this relation was mainly influenced by diet, whilst the activity DFC% and its STD showed up differences between individual methane emissions independent of breed or diet. In addition, within groups of the same breed fed with the same diet the activity DFC% was also well related with other production traits. Thus, the closer look within similar groups (same breed and diet) is important in order to annul the breed and diet effects and get a deep understanding about between individual variations.

Within the group Charolais-sired steers fed with mixed diet, the circadian rhythms of activity and feeding explained well the between-individual variations in BWG, RFI and FCR. The most efficient steers within this group showed higher activity rhythmicity, however they also had a higher variation in feeding DFC% (STD). Richardson and Herd (2004) reported that there are many physiological mechanisms contributing to the variation in residual feed intake and that differences in digestion contributed conservatively 10%, and indirect measures of protein turnover suggest that protein turnover, tissue metabolism and stress contributed to at least 37%. The activity DFC%, was firstly developed to measure the welfare and stress of free-ranging animals, and also to detect healthy and adapted organisms (BERGER et al., 1999, 2003; BLOCH et al., 2013; SCHEIBE et al., 1998). Thus, this variable combines many of the physiological mechanisms that contribute to the variation in residual feed intake. The higher variation in feeding DFC% showed by efficient Low RFI steers may be associated with the their capacity on adjust their feeding behaviour, which is itself may be linked to feedback of ruminal parameters.

Within the group purebred Luing steers fed with mixed diet, the circadian rhythms of activity explained well the between-individual variations in methane yield, and within this group a higher activity DFC% was linked with higher methane yield.

However, a higher activity DFC% it is about an adapted animal and consequently an adapted animal may show up better production traits, but the link between activity DFC% and methane emission is a bit more complicate than this and a high activity DFC% will not always means a higher methane yield. As mentioned before, Luing steers are more adapted to extensive rangeland systems, and are well suited to lower quality diets. Considering this, Luing steers with higher activity DFC% were more adapted to a mixed diet. Roehe et al. (2016), observed large phenotypic ranges in methane emission between the extremely low and high emitting animals within breed. These authors found significant differences on methane yield and on archaea:bacteria ratios between sire progeny groups and some of these differences were even larger than the differences between the diets. Thus, they concluded that these results indicate a substantial genetic influence of the host animal in the microbial community composition of the rumen. Thus, the higher methane yield observed in steers with higher activity DFC% might be associated with genetic influence of the host animal (within Luing breed) regarding on archaea:bacteria ratio, and thus, a higher archaea:bacteria ratio may be linked to the measured higher methane yield and better adapted animals.

Charolais-sired steers fed with concentrate diet, the circadian rhythms of activity explained well the between-individual variations in methane yield. Steers with higher activity DFC% and lower variation on its response (STD) had lower methane yield. This may be explained by the same principle above, a higher or lower activity DFC% does not imply simply higher or lower methane yield, but a better or poorly adapted animal. Concentrate diets showed up lower methane yield and the same was observed for Charolais steers more adapted to concentrate diets.

Purebred Luing steers fed with concentrate diet. Although there were no significant regressions between production traits and activity DFC%, this group of steers were less efficient (numerically compared with Luing fed with a mixed diet and significantly compared with Charolais) and as a group they showed lower activity DFC% and higher variation in its response (STD) across the experimental days. Steers in this group appeared to have greater difficulty of adaptation to the barley change with high impact on their rhythmicity. The rumen is closely related with ruminant overall health, as its metabolic efficiency is crucial for digestive efficiency and closely related to cattle efficiency in feed utilization and production (KHIAOSA-ARD; ZEBELI, 2014). Feeding DFC% data explained well the differences between steers within this group, and steers with lower DMI and higher rhythmicity in their feeding patterns were more efficient (Lower RFI).

Conclusions

This study confirmed the great value of an in-depth look at the behavioural rhythmicity. The diurnal patterns of feeding and activity behaviours are strong and highly synchronised. There are a considerable number of relationships between production traits, behaviour and circadian rhythms. Feeding rate and feeding duration were shown to be the most important simple behavioural variables to help explain methane emission levels of cattle. However, the additional analysis of activity circadian rhythms using the DFC mathematical model enables a better understanding of production traits. It is proposed that this is because the activity rhythmicity not only reflects the most important feeding behaviour, but it is also a reflection of the physiological state of animals. Activity rhythmicity is suited to identify differences between individual methane emission independent of breed or diet, and it was also well related with important production traits as feed efficiency and growth of beef steers.

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CHAPTER IV

FINAL CONSIDERATIONS

The study of behavioural rhythmicity showed great value in both contrasting production systems, intensive and extensive systems. The analysis of activity circadian rhythms using the mathematical model of the DFC enables a better understanding of animal responses to environmental influences in extensive systems, as well as enabling a better understanding of the variation between-animal production traits in both systems. The circadian rhythm of activity was shown to be strong and highly synchronised with feeding behaviour in the cattle study. Feeding is crucial to the survival of animals and, thus, may be less susceptible to change than activity rhythmicity. Considering this and the strong synchronisation between feeding and activity, the latter may better explain animal issues that reflect on production traits.

The random regression model method in the sheep study was effective in identifying animals that deviated from population responses. The combination of circadian rhythm analysis and the clustering of individuals into groups based around their regression response to environmental variables provides considerable potential to glean information relevant for group and individual animal management in extensive systems.

This work shows that these approaches may enhance the quality and meaningfulness of data coming from automated sensors. It also provides a potential means of using such data acquisition technology to obtain useful information. Considering the increasing availability of data captured through automated telemetric systems, these findings may have practical applications in precision livestock farming and in the process of the creation of new applications.

In regards to the possibility of creating a new application for livestock management, the activity behaviour (motion index sum for every 15 minute interval) and/or feeding behaviour (feeding rate – dry matter intake for every 15 minute interval) have potential to be used as the continuous output to feed a database using a telemetric system. The DFC analysis has potential to be used as the mathematical model to analyse the response to an input parameter (e.g. weather variables, nutritional inputs) that could provide a benchmark level to link to the random regression model. Target values will support the control of animal processes and would be set according to the production focus; e.g. target could be a DFC average that is considered good for the specific population considering the seasonal changes or an acceptable variation considering the target production trait. The model-based predictive controller could be a range of control methods for the process inputs (e.g. environment), which use continuous feedback of the process output (behavioural rhythmicity, methane emission, etc.). In case of extensive systems, this would make it possible to understand if the lack of rhythmicity primarily is caused by an environmental disturbance or a within-animal problem. Furthermore, it also enables studying the possibility of a model predictive controller for productions traits, such as methane emission, using the DFC mathematical model.

A new technology developed by this concept could assess the animal behavioural circadian rhythm and monitor its response, identifying changes in the behavioural circadian rhythm and, at real time, to detect health or stress problems before it affects animal productivity. Other advantages of the electronic monitoring systems is that data can be continuously collected without the disturbance of human handling and it is less stressful for animals. Moreover, it may provide a pathway to collect data and make decisions and thus optimize monitoring processes, reduce labour and assist other decision-making in the flock or herd management in extensive systems. In addition, it has potential to be used as a tool to select animals that are more adaptable and even animals with more or less methane emission level.

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APPENDICES

APPLIED ANIMAL BEHAVIOUR SCIENCE

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The use of English, punctuation and grammar should be of a sufficient high standard to allow the article to be easily read and understood. Do not quote decimals with naked points (e.g. use 0.08, not .08). Times of day should be in the format 10:00 h. Numbers less than 10 should be text, unless they are followed by a unit of measurement or are used as designators e.g. seven pigs from Group 3 were each trained for 7 days, with three sessions each lasting 3 min. Numbers greater than nine should be written as numerals.

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Present address(es) of author(s) if applicable

Complete correspondence address including e-mail address to which the proofs should be sent

•Abstract

•Keywords (indexing terms), maximum 6 items

•Introduction

•Material studied, area descriptions, methods, techniques and ethical approval

•Results

•Discussion

•Conclusion

•Acknowledgment and any additional information concerning research grants, etc.

•References

•Tables

•Figure captions

•Tables (separate file(s))

•Figures (separate file(s)).

Manuscripts should have numbered lines, with wide margins and double spacing throughout, i.e. also for abstracts, footnotes and references. Every page of the manuscript, including the title page, references, tables, etc., should be numbered. However, in the text no reference should be made to page numbers; if necessary one may refer to sections. Avoid excessive usage of italics to emphasize part of the text. Articles should not normally exceed 25 pages of text (11-point font, aligned left and double spaced) and contain a maximum of six or seven Tables and Figures in total.

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

The introduction "sets the scene" for your work. Do not over-reference statements; two or three key references should suffice unless each adds something specific. The introduction should not normally be more than 750 words (approximately three pages).

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

When locations are given, it should be remembered that this is an international journal and provide the state/county and country, or longitude and longitude for lesser-known locations. Full details of commercial products and technical equipment should be provided, as necessary, including name of the model, manufacturer and location of manufacture, and any Trademarks. As appropriate, a statement should be made that the work has received ethical approval or that the authors have read the policy relating to animal ethics and confirm that their study complies. Data collection and collation: units of all measures need to be specified; the experimental design should be explained together with an explanation of the experimental unit; the ways in which data are derived must be specified (e.g. individual scores were summed for the four, 12-h periods and the mean used for the analysis); the methods used for determining the normality of distribution of the residuals and homogeneity of variances need to be specified; any transformations of data need to be described; statistical analyses need to be reported in full.

Results

This section should include only results that are relevant to the hypotheses outlined in the Introduction and considered in the Discussion. Present results in tabular or graphical form (see following sections) wherever possible. Text should explain why the experiment was carried out, and elaborate on the tabular or graphical data. Sufficient data should be presented so that the reader can interpret the results independently. If data require transformation to be suitable for parametric analyses, then due consideration needs to be given as to which and how data are presented in the manuscript. For example, putting error bars on graphs of the raw or back-transformed data is meaningless if analysis was performed on transformed data. To assist with interpretation of biological meaning, however, back-transformed means (but not errors) could be presented instead of/in addition to transformed be given either in the text, or in the Figures or Tables legends. Include the type of test, the precise data to which it was applied, the value of the relevant statistic, the sample size and/or degrees of freedom, and the probability level. Any assumptions that have been made should be stated. If in doubt, a statistical expert should be consulted.

Discussion

The discussion should interpret the results, and set them in the context of what is already known in the appropriate field. This section should normally start with a brief summary of the main findings. The discussion should be focused and limited to the actual results presented, and should normally not exceed about 1500 words. All results presented in the Results section should be discussed (if they do not warrant discussion, they do not warrant inclusion) and there should be no presentation and discussion of results that have not been presented in the Results section (i.e. no new data presented in the Discussion). Any necessary extensive discussion of the literature should be placed in the Discussion, and not in the Introduction.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

It should provide a brief "take home" message and briefly outline the application/implications of the study's findings.

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A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should

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List funding sources in this standard way to facilitate compliance to funder's requirements:

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General points

• Make sure you use uniform lettering and sizing of your original artwork.

• Embed the used fonts if the application provides that option.

• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.

• Number the illustrations according to their sequence in the text.

• Use a logical naming convention for your artwork files.

• Provide captions to illustrations separately.

• Size the illustrations close to the desired dimensions of the published version.

• Submit each illustration as a separate file.

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You are urged to visit this site; some excerpts from the detailed information are given here.

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• Tables to be sent as editable source files (.doc or .xls) with heading on it.

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Limitations

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Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

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1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;

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Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13.03.03).

Reference to a dataset:

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. http://dx.doi.org/10.17632/xwj98nb39r.1.

References to books

If a book or monograph is cited as a source of specific information, then please give the relevant page(s).

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations.

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• Font Arial 12 should be used for the text, and Arial 11 for tables and references

• The sections should typically be assembled in the following order: Title, Authors,

Authors' full affiliations including department and post/zip codes, Corresponding author, Short title, Abstract, Keywords, Implications, Introduction, Material and methods, Results, Discussion, Acknowledgements, References, Tables, List of figure captions

• The use of small paragraphs with less than 6 to 8 lines must be avoided

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• The manuscript complies with the section specific requirements set out below

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(*a*) arrest the attention of a potential reader scanning a journal or a list of titles;

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(*c*) incorporate keywords or phrases that can be used in indexing and information retrieval, especially **the animal species** on which the experiment has been carried out;

(*d*) avoid inessentials such as 'A detailed study of ...', or 'Contribution to ...';

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Full title directions

• No more than 170 characters including spaces

• Include "Review:", "Invited review:" or "Animal board invited review:" before the full title if required (see above)

• The title of an invited opinion paper should start with "Opinion paper:"

• The title of a short communication should start with "Short communication:"

Authors and affiliations

The names and affiliations of the authors should be presented as follows:

Example

J. Smith1,a, P.E. Jones2, J.M. Garcia1,3 and P.K. Martin Jr2 [initials only for first names]

1*Department of Animal Nutrition, Scottish Agricultural College, West Main Road, Edinburgh EH9 3JG, UK*

2*Animal Science Department, North Carolina State University, Raleigh, NC 27695-7621, USA*

3*Laboratorio de Producción Animal, Facultad de Veterinaria, Universidad de Zaragoza, C. Miguel Servet, 177, 50013, Zaragoza, Spain*

a*Present address: Dairy Science Laboratory, AgResearch, Private Bag 11008, Palmerston North, New Zealand* (for any author of the list whose present address differs from that at which the work was done)

Corresponding author: John Smith. E-mail: John.Smith@univ.co.uk.

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• Five keywords

• Keywords should be short and specific

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Implications must explain the expected impact that the results may have on practice when they will be applied. Impact may be economic, environmental and/or social. Implications should not be limited to presenting the context and objectives, and should not be an "abstract of the abstract". This is written in simple English suitable for non-specialists or even non science readers. The use of non-standard abbreviations is discouraged.

Introduction

The introduction briefly outlines the context of the work, presents the current issues that the authors are addressing and the rationale to support the objectives, and clearly defines the objectives. For hypothesis driven research, the hypothesis under test should be clearly stated. Increasing the knowledge on a subject is not an objective *per se*.

Material and methods

Material and methods should be described in sufficient detail so that it is possible for others to repeat the experiment. Reference to previously published work may be used to give methodological details, provided that said publications are readily accessible and in English.

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Statistics directions

• In the text, the level of significance attained is indicated by the following conventional standard abbreviations (which need not be defined): $P > 0.05$ for non-significance and $P < 0.05$, $P < 0.01$ and $P < 0.001$ for significance at these levels. Exact level of statistical significance (e.g. $P = 0.07$) can also be used

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Separation between Results and Discussion is preferred to highlight the interpretation of results. Presentation of Results and Discussion in a single section is possible but discouraged.

Acknowledgements

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References

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- Knowles TG, Kestin SC, Haslam SM, Brown SN, Green LE, Butterworth A, Pope SJ, Dirk Pfeiffer D and Nicol CJ 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. PLoS ONE 3, e1545.
- Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from ANM September 2016 page 7 microbe to the farm scale. Animal 4, 351-365.
- Pérez-Enciso M, Rincón JC and Legarra A 2015. Sequence- vs. chip-assisted genomic selection: accurate biological information is advised. Genetics Selection Evolution 47, 43. doi:10.1186/s12711-015-0117-5.
- When the article is online but not yet printed, the right format is: Zamaratskaia G and Squires EJ 2008. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal, doi:10.1017/S1751731108003674, Published online by Cambridge University Press 17 December 2008.

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• Issue numbers are not mentioned

• Use "," (not ";") before page numbers

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Examples

- Association of Official Analytical Chemists (AOAC) 2004. Official methods of analysis, volume 2, 18th edition. AOAC, Arlington, VA, USA.
- Littell RC, Milliken GA, StroupWW and Wolfinger RD 1996. SAS system for mixed models. Statistical Analysis Systems Institute Inc., Cary, NC, USA.
- Martin P and Bateson P 2007. Measuring behaviour. Cambridge University Press, Cambridge, UK.
- National Research Council (NRC) 2012. Nutrient requirements of swine, 11th revised edition. National Academy Press, Washington, DC, USA.

• The list of author or editor name(s) and publication years are written as for journal articles (all authors are provided; commas between authors; "and" before the last author where there are two or more authors; full stops after publication years)

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- AOCS Press, Champaign, IL, USA.
- Cambridge University Press, Cambridge, UK.

- International Organization for Standardization, Geneva, Switzerland.
- FAO, Rome, Italy.

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Author A, Author B, Author CD and Author E Year. Chapter title. In Title of book (ed. A Editor and B Editor), pp. first-last page numbers. Publisher's name, City, State (2-letter abbreviation) for US places, Country.

Example

- Nozière P and Hoch T 2006. Modelling fluxes of volatile fatty acids from rumen to portal blood. In Nutrient digestion and utilization in farm animals (ed. E Kebreab, J ANM September 2016 page 8 Dijkstra, A Bannink, WJJ Gerrits and J France), pp. 40–47. CABI Publishing, Wallingford, UK.

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Please note – If proceedings are published in a journal, the article should be formatted as for a journal article and if they have been published as chapters in a book, the article should be formatted as for a chapter in a book.

Examples

- Bispo E, Franco D, Monserrat L, González L, Pérez N and Moreno T 2007. Economic considerations of cull dairy cows fattened for a special market. In Proceedings of the 53rd International Congress of Meat Science and Technology, 5-10 August 2007, Beijing, China, pp. 581–582.

- Martuzzi F, Summer A, Malacarne M and Mariani P 2001. Main protein fractions and fatty acids composition of mare milk: some nutritional remarks with reference to woman and cow milk. Paper presented at the 52nd Annual Meeting of the European Association for Animal Production, 26-29 August 2001, Budapest, Hungary.

• The list of authors and publication years are written as for journal articles (all authors are provided; commas between authors; "and" before the last author where there are two or more authors; full stops after publication years)

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City, State (2-letter abbreviation) for US places, Country.

Examples

- Champaign, IL, USA.
- Cambridge, UK.
- Versailles, France.
- Geneva, Switzerland.

Website directions

References from websites are formatted as follows:

Author(s)/Institution Year. Document/Page title. Retrieved on DD Month YYYY (i.e. accessed date) from http://www.web-page address (URL).

Examples

- Bryant P 1999. Biodiversity and Conservation. Retrieved on 4 October 1999, from http://darwin.bio.uci.edu/~sustain/bio65/Titlpage.htm

• The list of author name(s) and publication years are written as for journal articles (all authors are provided; commas between authors; "and" before the last author where there are two or more authors; full stops after publication years)

Example

- Author A, Author B, Author CD and Author E Year.

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• No capitals for document/page titles except initial capital of the first word and words that ordinarily take capitals

• Dates when documents were retrieved are included in the format: DD Month YYYY, e.g. 10 August 2014

• Web-page addresses are provided

Thesis directions

References from theses are formatted as follows:

Author AB Year. Thesis title. Type of thesis, University with English name, location of the University (i.e. City, State (2-letter abbreviation) for US places, Country).

Example

- Vlaeminck B 2006. Milk odd- and branched-chain fatty acids: indicators of rumen digestion for optimisation of dairy cattle feeding. PhD thesis, Ghent University, Ghent, Belgium.

• The author's name and publication year are written as for journal articles (no punctuation between surname and initials; full stops after publication years)

Example

- Author AB Year.

• No capitals for thesis titles except initial capital of the first word and words that ordinarily take capitals

• Degree levels are provided, e.g. PhD, MSc, etc.

• University names and locations are given and listed as:

• University name, City, State (2-letter abbreviation) for US places, Country.

Examples:

- Louisiana State University, Baton Rouge, LA, USA.
- Cambridge University, Cambridge, UK.

Tables

Tables should be as simple as possible. The same material should not be presented in tabular and graphical form. An indication is given in the text where the table should be inserted. Please refer to the style sheet available at http://www.animal-journal.eu/documents/Animal_style_template.doc.

Table directions

Each table is on a separate page at the end of the main text (one table per page)

• Tables are typed, preferably in double spacing. Single spacing is possible for long tables

• Tables are numbered consecutively using Arabic numbering. They are referred to as Table 1, Table 2, etc., with capital 'T', no italics

• Each table has its own explanatory caption. The caption is sufficient to permit the table to be understood without reference to the text. The animal species and the experimental treatments or the issue under study are indicated in each caption. The caption does not contain too many details about the protocol or the results

• Tables are created in Word using the table function within the programme (without using tabs). Layout can be portrait or landscape

• Large tables are discouraged in the manuscript but they may be submitted as Supplementary Material

• No vertical lines between columns and no horizontal lines between rows of data

• Generally, variables are in rows and treatments in columns

• Column headings are concise

• Separate columns are included to present the basic statistical results: error terms (preferably residual error terms) and levels of significance

• Row items are organized with main items followed by indented sub-items in order, for instance, to group the criteria which share the same type of measurements or the same unit

• For any (sub-)item, only the first letter of the first word is in capitals

• Units are clearly stated either in the caption (only if a limited number of units are used), or for each (sub-)item. Standard abbreviations for units are used

• Footnotes are referenced using superscript numbers

• All abbreviations used in a table are defined as footnotes (preferred option) or in the caption

• Treatment means are reported with meaningful decimals. For guidance, the last digit corresponds to 1/10 of standard error

• The number of decimals for the indicators of residual variability (RSD, SEM, RMSE etc.) are either identical to that chosen for mean values or have one more decimal. The choice is consistent in all the tables

• See above (Statistics) for the presentation of statistical results in tables

Figures

Figures should be as simple as possible. The same material should not be presented in tabular and graphical form. An indication is given in the text where the figure should be inserted. Specific guidelines are provided for images (see Image Integrity and Standards).

Figure directions

Figure captions are all listed on the same page at the end of the main text

• All figures are numbered consecutively in the text. They are referred to as Figure 1, Figure 2, etc., the word ‘Figure’ being spelled out with capital ‘F’, no italics

• Captions begin as Figure 1, Figure 2, etc. They are sufficiently detailed to allow the figure to be understood without reference to the text ("Figure 1 Effect of fat source and animal breed on carcass composition in pigs" is preferred to "Figure 1 Carcass composition"). The animal species and the experimental treatments or the issue under study are indicated in each caption. Abbreviations used in each figure have to be defined in the caption and kept to a minimum

• Figures are not inserted in the text. Each figure (without caption) is uploaded separately with one separate file per figure and no embedded captions in these files

• Figure size should be readable in a width of approximately 175 mm (i.e. the maximum size of printing over two columns). Easy reading of the figure is required

• Ensure that the font size is large enough to be clearly readable at the final print size (should not be less than 8 point, or 2.8 mm, after reduction). We recommend you use the following fonts: Arial, Courier, Symbol, Times, Times New Roman and ensure that they are consistent throughout the figures. In addition, ensure that any fonts used to create or label figures are embedded if the application provides that option

• Symbols and line types should allow different elements to be easily distinguished (generally, solid symbols are used before open symbols, and continuous lines before dotted or dashed lines)

• Figures are usually supplied as black and white

• Colours can be used in figures if they are essential to understanding the figure. Publication charges are made for colour figures. The cost for reproducing figures in colour within the printed issue is £200.00 / $320.00 per figure

• If figures are to be printed in colour, use CMYK (instead of RGB) colour mode preferably

• The figures should preferably be provided as TIFF or EPS files. Other formats such as MS Word, MS Excel, MS PowerPoint, AI and layered PSD (up to CS3) are permitted, provided that figures have been originally created in these formats and that all the embedded artwork is at a suitable resolution.

• The resolutions for TIFF figures at the estimated publication size must be:

- for line figures (e.g. graphs) – 1200 dpi (6000 px for 1 column, 8400 px for 2 columns)
- for figures with different shadings (e.g. bar charts) – 600 dpi (3000 px for 1 column, 4200 px for 2 columns)
- for half tones (e.g. photographs) – 300 dpi (1500 px for 1 column, 2100 px for 2 columns)

• Images from the internet are unacceptable, as most of them have a resolution of only 72 dpi

• When your drawing/graphics application does not provide suitable 'export' options, please copy/paste or import the graphic into a Word document

• For further information, please refer to the Cambridge Journals Artwork Guide, which can be found online at: http://journals.cambridge.org/artworkguide

Image Integrity and Standards

Any image produced by an instrument (e.g. scanner, microscopy…) with the objective of being used to derive quantitative results is considered as original data, and manuscripts that report images without any quantitative findings are not acceptable. Digitalisation of an image converts the image into numerical values which can be analysed like any other numerical value. The full information may prove important beyond what the author would like to show. Hence images submitted with a manuscript should be minimally processed; some image processing is acceptable (and may be unavoidable), but the final image must accurately represent the original data and exclude any misinterpretation of the information present in the original image. In case original data are being used just to illustrate a point, this should be accompanied by a very clear statement in the manuscript telling the reader this and explaining what is being demonstrated. Please refer to the Office of Research Integrity guidelines on image processing in scientific publication.

Image Integrity and Standards directions

• Image acquisition: Equipment and conditions of image acquisition and processing must be detailed in the Material and Methods section. This includes the make and model of equipment, the acquisition and the image processing software, and the image treatment if any. If you export files from an acquisition device, make sure to use a format with no loss of information and do not file them into a higher resolution than that of acquisition. Authors have the responsibility to archive original images, with their metadata, in their original format without any compression or compressed without loss of information.

• Preparation of images for a manuscript: For guidance, we refer to the Journal of Cell Biology's instructions to authors (http://jcb.rupress.org/site/misc/ifora.xhtml#image_aquisition) which states:

1) No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
2) The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (i.e., using dividing lines) and in the text of the figure legend.
3) Adjustments of brightness, contrast, or color balance are acceptable if they are applied to every pixel in the image and as long as they do not obscure, eliminate, or misrepresent any information present in the original, including backgrounds. Non-linear adjustments (e.g., changes to gamma settings) must be disclosed in the figure legend.

For further information, image examples, and more detailed guidance we advise reading What's in a picture? The temptation of image manipulation (reprinted in the Journal of Cell Biology (2004) 166, 11-15).

• If a cropped image is included in the main text of a paper (e.g. a few lanes of a gel), display the full original image, including the appropriate controls, the molecular size ladder and/or the scale as relevant, as a single figure in a Supplementary Material file to facilitate peer-review and for subsequent on line publication.

• The statistical analysis applied to the quantitative data associated with images must clearly define the statistical unit considered (e.g. the animal, the sample…).

• Image screening prior to acceptance: All digital images from manuscripts nearing acceptance for publication will be screened for any evidence of improper manipulation or quality. If the original images cannot be supplied by authors on request, the journal reserves the right to reject the submission or to withdraw the published paper.

Supplementary material

Authors can include supplementary material in any type of text (research article, review article, short communication, etc.). Supplementary material will appear only in the electronic version. A link to this on-line supplementary material will be included by the Copy Editor at the proof preparation stage. Supplementary material will be peer-reviewed along with the rest of the manuscript. The main text of the article must stand alone without the supplementary material. Supplementary material should be presented according to the instructions for the main text. It will not be copy-edited and authors are entirely responsible for the presentation of the supplementary material.

Supplementary material directions

• In the main text, supplementary material are referred to as:

"Supplementary Table S1", "Supplementary Table S2", etc. for tables;

"Supplementary Figure S1", "Supplementary Figure S2", etc. for figures;

"Supplementary Material S1", "Supplementary Material S2", etc. for other material.

For example: "The list of references used for the meta-analysis is given in Supplementary Material S1 and Supplementary Table S1 reports etc."

• Supplementary material is submitted along with the main manuscript in a separate file and identified at uploading as "Supplementary File – for Online Publication Only"

• The title of the article and the list of authors are included at the top of the supplementary material

• No line numbering

• Single spacing

• Unlike the figures included in the main text, each supplementary figure has its own title embedded below the figure

Typographical conventions

Title and headings

As illustrated and detailed above and in the style sheet (see http://www.animal-journal.eu/documents/Animal_style_template.doc), the *animal* conventions apply to (*a*) *Title* of the paper, Authors' names and addresses; (*b*) *Main section headings* such as Abstract, Implications, Introduction, Material and methods, Results, Discussion, Acknowledgements, References; and (*c*) *Subheadings* which can be used at two levels only.

Title and heading directions

• Title – use bold, with an initial capital for the first word only and for words that ordinarily take capitals

• Authors' names – use lower case with initials in capitals (e.g. J. Doe)

• Authors' addresses – use italics

• Headings are left aligned with an initial capital for the first word only, and not numbered

• Main section headings – use bold with no full stop at the end; text follows on the next line (e.g. Abstract)

• Subheading (level 1) – use italics with no full stop at the end; text follows on the next line (e.g. Experimental design)

• Sub-subheading (level 2) – use italics and end with a full stop; text follows on the same line (e.g. Milk fatty acid composition. The fatty acid…)

Abbreviations

All non-standard abbreviations are defined at first use separately in the abstract and in the main text, they should be written in **bold capitals at first occurrence**. To facilitate the understanding of the manuscript, the number of abbreviations should be kept to a minimum (not more than 10 non-standard abbreviations is advised). Abbreviations in the short title or in (sub)headings are discouraged.

Abbreviation directions

• Define abbreviations at first appearance in the abstract, and in the main text

• Authors should avoid excessive use of non-standard abbreviations (a maximum around 10 is advised)

• No author-defined abbreviation in the (short) titles, nor in (sub)headings

• Abbreviations used in tables/figures have to be defined either as footnotes or in the caption

• Do not start a sentence with an abbreviation

The names of the chemicals do not need to be written out in full; chemical symbols are sufficient. Fatty acids are abbreviated using the following rules: cis-18:1 for the sum of cis octadecenoic acids. When isomers are described, the double bond positions are identified by numbering from the carboxylic acid end: c9,t11-18:2; iso-15:0. The terms "omega 3" and "omega 6" are discouraged ed and replaced by "n-3" and "n-6", e.g. 18:3n-3. Trivial names can be used for the most known fatty acids (myristic, palmitic, oleic, linoleic, linolenic) and abbreviations in some cases: CLA for conjugated linoleic acids, EPA for eicosapentaenoic acid, DHA for docosahexaenoic acid. Chemical names and trivial names cannot be mixed in a same table.

Capitals

Capitals directions

• Initial capitals are used for proper nouns, for adjectives formed from proper names, for generic names and for names of classes, orders and families

• Names of diseases are not normally capitalised

Italics

Use italics for:

Italics directions

• Authors' addresses (see above)

• Subheadings (see above)

• Titles for tables (but not captions for figures)

• Most foreign words, especially Latin words, e.g. ad hoc, ad libitum, et al., in situ, inter alia, inter se, in vitro, per se, post mortem, post partum, m. biceps femoris but no italics for c.f., corpus luteum, e.g., etc., i.e., NB, via

• Mathematical unknowns and constants

• Letters used as symbols for genes or alleles e.g. HbA, Tf D (but not chromosomes or phenotypes of blood groups, transferrins or haemoglobins, e.g. HbAA, TfDD)

Numerals

Numerals directions

• In text, use words for numbers zero to nine and figures for higher numbers. In a series of two or more numbers, use figures throughout irrespective of their magnitude

• Sentences do not, however, begin with figures

• For values less than unity, 0 is inserted before the decimal point

• For large numbers in the text substitute 10n for part of a number (e.g. 1.6 106 for 1 600 000)

• Do not use comma separator for numbers greater than 999 (e.g. 100 864)

• The multiplication sign between numbers should be a cross (x)

• Division of one number by another should be indicated as follows: 136/273.

• Use figures whenever a number is followed by a standard unit of measurement (e.g. 100 g, 6 days, 4th week).

• Use figures for dates, page numbers, class designations, fractions, expressions of time, e.g. 1 January 2007; type 2

• Dates are given with the month written out in full in the text and with the day in figures (i.e. 12 January not 12th January).

• For time use 24-h clock, e.g. 0905 h, 1320 h

Units of measurement

The International System of Units (SI) should be used. A list of units is found at http://physics.nist.gov/cuu/Units/units.html. Recommendations for conversions and nomenclature appeared in *Proceedings of the Nutrition Society* (1972) 31, 239-247. Some frequently used units which are not in the SI system are accepted: l for litre, ha for hectare, eV for electron-volt, Ci for curie. Day, week, month and year are not abbreviated. The international unit for energy (energy value of feeds, etc.) is Joule (or kJ or MJ).

A product of two units should be represented as N·m and a quotient as N/m (e.g. g/kg and not g.kg-1). When there are two quotients, present as follows: g/kg per day (not g/kg/day).

Concentration or composition

Composition is expressed as mass per unit mass or mass per unit volume The term *content* should not be used for concentration or proportion.

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Manuscript submission is made electronically through *Editorial Manager* directly via http://www.editorialmanager.com/animal or at www.animal-journal.eu. Any query to the Editorial Office should be addressed through this site. Authors can check the status of their manuscript using *Editorial Manager*. Authors should ensure that the email address of the corresponding author is correct.

You must submit separate files for the following:

• Manuscript (including full text, tables, figure captions, but excluding figures) in DOC/DOCX or RTF format (PDF is not accepted)

• Each figure (without captions). At submission in Editorial Manager, enter a description of each figure (Figure 1, Figure 2a, etc.) in the appropriate box

• Supplementary online-only materials, if relevant

Authors who submit a manuscript have to also provide in the online submission system:

- the type of article (research article, short communication, review article, special issue paper, opinion paper, etc.).

- the section of the scope which is the most appropriate for their manuscript. (http://www.animal-journal.eu/scope.htm).

- any comment and information that might be helpful to the editors ("letter to the editor", etc.; in "Author's comments").

- The names of at least 3 potential reviewers, and give contact details. Reviewers should have no conflict of interest with the authors or the submission. Authors should not nominate reviewers who are their regular collaborators or who work in the same institution or university, and they should nominate *an international spread of reviewers*. The editorial board will use its discretion when selecting reviewers and the suggested reviewers may not be used.

- The names of maximum 3 opposed reviewers in case of established conflict of interest.

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Evaluation of the manuscript

The Editor-in-Chief or the Section Editor may reject manuscripts which do not comply with the scope or which do not have the required standard, or which present obvious errors or misinterpretation of results, or which do not comply with the recommendations for preparation of articles. The Editor-in-Chief or a member of the Editorial Office may also send back to the authors their manuscript for reformatting in the style of *animal*. During the peer-review process, manuscript revisions should be sent back to the Editorial Office within 60 days; otherwise, the manuscript is withdrawn and the revised version will have to be submitted as a new manuscript. In order to provide the shortest possible delay from submission to acceptance only one revision iteration is expected. Manuscript revisions that do not meet the requirements of the handling Editor will be rejected.

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Publication of the manuscript

A free PDF file will be emailed to the corresponding author. To facilitate earlier dissemination, articles are published online in *FirstView* with their doi number at https://www.cambridge.org/core/journals/animal ahead of being published as part of an issue. It should be stressed that ***no change in the paper, even quite minor, is possible once the paper is in the FirstView list***.

VITA

Bruna Nunes Marsiglio Sarout, filha de Joaquim Fernando Almeida Prado Marsiglio e Christina Bertacco Nunes, nasceu na cidade de Rancharia, São Paulo, no dia 23 de novembro de 1983. Cursou o ensino médio no Colégio Alpha de Rancharia, São Paulo. Em abril de 2004 ingressou no curso de graduação em Zootecnia na Universidade Estadual de Maringá, em Maringá, Paraná. Em dezembro de 2008, concluiu o curso de Zootecnia. Durante o curso de graduação desenvolveu atividades de iniciação científica na área de Produção e Nutrição de Ruminantes atuando em atividades de campo e laboratoriais. No ano de 2009 e primeiro semestre de 2010 trabalhou no Instituto de estudos Pecuários desenvolvendo atividades técnico-científicas e atuou como auxiliar de coordenação no Projeto de Pesquisa “Curso de Especialização Gestão Empresarial e Controle de Qualidade da Produção de Leite” da Fundação de Apoio ao Desenvolvimento Científico - FADEC/Seti-PR. Em março de 2010, iniciou o curso em nível de Mestrado no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá, na área de concentração Produção Animal. Foi bolsista CAPES, em nível de mestrado, a partir do segundo semestre de 2010 e em 2012 obteve o grau de Mestre em Zootecnia. No ano de 2013 ingressou no curso em nível de Doutorado, no Programa de Pós-Graduação em Zootecnia da Universidade Federal do Rio Grande do Sul, na área de concentração Produção Animal, como bolsista CAPES. No período de novembro de 2015 a Outubro de 2016 realizou o Doutorado Sanduíche, como bolsista CAPES, no Scotland’s Rural College, Escócia, Reino Unido.