Variation in core body temperature indicates fitness in ruminants, and is related to the potential for reproduction
（生殖機能調節に関連した環境適応を示唆する反芻家畜の深部体温変動）

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Chapter 1: General introduction

To achieve food security and health in the world, it is necessary to develop sustainable farming practices. There is a need to produce sufficient food to meet the demands of an increasing population. Animal protein accounts for the one-third of the protein intake, and 17 % of the calorie intake, in the world (OECD -Organization for Economic Cooperation and Development, 2021). Any improvement in the efficiency of the livestock industry would contribute to a sufficient supply of animal protein worldwide. However, the situation for the livestock industries is predicted to become worse, due to climate change and the increased use of intense forms of animal housing. Therefore, a novel approach to solve these problems would be to enhance the capacity for livestock to adapt to the changing environment. For this purpose, it is necessary to understand the fitness in ruminants and to find the new biomarker to assess the fitness in ruminants comprehensively.

This dissertation focuses on small ruminants, such as sheep and goats. They play an important role in human nutrition, and serve as a useful experimental model for larger ruminants, such as cattle. Ruminants meat accounts for 25 % of worldwide meat consumption (Ritchie and Roser, 2017). Therefore, it is worth gaining a better understanding of the physiological responses of ruminants to environmental challenges, and the relationship with reproduction, to develop new strategies for the sustainable production of ruminants.

I have focused on the core body temperature (T_c) as an important physiological response in the maintenance of homeostasis in mammals. The main purpose of this dissertation is to assess the hypothesis that variation in the T_c can be used as a new biomarker of the fitness and reproductive performance of animals, especially in ruminants. I analysed the circadian rhythm of the core body temperature (CRT) and stress-induced hyperthermia (SIH) as well-known forms of heterothermy in response to exogenous challenges.

The first aim of my thesis is to understand the effect of genotypes and phenotypes that have a relationship with temperament on the thermal response to psychological stressors, to better understand temperament and its effect on physiological responses. The adequacy of SIH and CRT as biomarkers of psychological stress was assessed is sheep. The second aim of

my thesis is to explore the effect of environmental stress that livestock can experience in daily management on the parameters of the CRT. The comprehensive analysis combined several stressors (energy intake, movement restriction, and ambient conditions) in goats. The third aim of my thesis is to test the validity of the amplitude of the CRT as a marker of reproductive ability, especially in the context of the endocrine control of reproduction. The relationship of the CRT profile and LH secretion was investigated in goats. The fourth aim of my thesis is to explore the possible neural circuitry that is involved in the interaction between energy homeostasis, reproduction, and core body temperature.

Chapter 2: Stress induced hyperthermia in sheep with temperaments determined by genotype or phenotype

In the first experimental chapter (Chapter 2) of my thesis, I assessed the effect of genotypes and phenotypes that are related to temperament. I compared the T_c response to psychological stress during behavioural assessment tests and during exposure to a potential predator, a sheep dog, in groups of sheep with different genotypes and phenotypes. I selected the sheep based on an SNP in the tryptophan 5-hydroxylase (TPH2) gene that codes for an enzyme in the synthesis of serotonin. The activity of the TPH2 enzyme is impacted by the polymorphism, and therefore affects the level of serotonin in the brain. The SNP has been reported to be associated with temperament, as determined by the responses of sheep to an isolation box test (IBT). The responses of sheep were characterised two ways; genetically using groups defined as “genotype AA” (related to calm temperament) and “genotype GG” (related to nervous temperament), and phenotypically using groups defined as “low responder” (with a low IBT score) and “high responder” (with a high IBT score) that is selected based on the IBT score in the behavioural tests. While genotype had no effect on SIH, phenotype did, with high responders exhibiting larger SIH in response to dog exposure. In addition, the same sheep didn’t show SIH when they walked the same distance in the absence of a dog, and the behavioural test induced larger SIH with much lower activity increase. The results suggest that SIH would be a good biomarker of psychological stress because it can discriminate the level of stressors and individual reactivity. In addition, the amplitude of the CRT was increased after the exposure of the dog. The results suggest that strong psychological stress can induce an increase in the amplitude of the CRT.

Chapter 3: The profile of the circadian rhythm of core body temperature in goats: the effect of ambient temperature, restriction of movement, and modification of energy intake

In the second experimental chapter (chapter 3), I investigated the T_c response of ovariectomized (OVX) goats to several potential stressors that ruminants can experience in farming settings. I used OVX goats to exclude the effect of gonadal hormone on the Tc and eliminate the fluctuation depends on the estrous cycle. I analysed the effect of the ambient temperature, energy intake (EI), and tethering on the characteristics of the CRT. I conducted the experiment twice, in a hot/humid season (summer) and in a cold/dry season (autumn) between the summer solstice and winter solstice in Japan. The amplitude of the CRT was positively correlated with ambient temperature in summer, but in autumn there was no correlation between ambient temperature and the amplitude of the CRT. These results suggest that the animals' homeostasis might be more affected by the heat stress than by cold stress. The data support the possibility that the amplitude of the CRT can be used as a biomarker of fitness to heat stress in goats. Further, I assessed the effect of restricted feeding and tethering on the characteristics of the CRT. In the livestock management, various kinds of restraint are still used in some forms of farm management, such as tie-stalls housing. Animals tethered to the individual stanchions, they can access to food and water, and can sit and stand, but with movement restriction. Neither restricted feeding or tethering had an effect on the amplitude of the CRT, but it did impact on the mesor, cosinor minimum, and cosinor maximum of the CRT decreased in response to the EI restriction in tethered group, but not changed in untethered group. These results suggest that tethering can attenuate the hypothermia that is induced by low energy intake. Data in the present chapter suggest that housing systems, such as tie-stalls or stanchions, might induce disturbances to the adaptive hypothermic heterothermy of livestock, and induce excess energy expenditure during under nutrition.

Chapter 4: The relationship between luteinizing hormone secretion and the circadian rhythm of core body temperature in goats

In the third experimental chapter (Chapter 4), I investigated the relationship between the characteristics of the CRT and the secretion of luteinizing hormone (LH) as an indicator of

the activity of the hypothalamic-pituitary-gonadal (HPG) axis in OVX goats. The characteristics of the CRT were analysed over five consecutive days, and then blood samples were collected one day after and analysed to characterise the parameters of LH secretion, including mean LH, LH pulse frequency, and the inter-pulse interval. The mean and baseline concentrations of plasma LH and the amplitude of the CRT were negatively correlated. These results suggest that the amplitude of the CRT could be used to predict the LH secretion that drives reproductive function in female ruminants. The underlying mechanism connecting the amplitude of the CRT and the LH concentration, and the causal relationship between them, was not investigated in this dissertation. However, variation in the amplitude of the CRT and the LH secretion might be due to variation in the energy status of each goat. If so, it is possible that a common mechanism could control both the T_c and reproduction in response to energy status.

Chapter 5: Central administration of amylin has both facilitatory and inhibitory actions on the gonadotropin-releasing hormone pulse generator in goats

In the fourth experimental chapter (Chapter 5), the role of amylin-calcitonin receptor (CTR) signalling in the control of the activity of the gonadotrophin-releasing hormone (GnRH) pulse generator was investigated. I used a multiple unit activity (MUA) recording system in goats to monitor the activity of the GnRH pulse generator. I administrated amylin, an agonist of the CTR, into the lateral ventricle. I observed a facilitatory effect followed by an inhibitory effect on GnRH pulse generator activity in OVX female goats. I showed that the cells expressing *CALCR* (CTR gene) mRNA were widely distributed around the ventricle. The co-expression of *CALCR* and kisspeptin was quantified using double *in situ* hybridisation for *KISS1* (kisspeptin gene) and *CALCR*, and revealed that only around 1% of the *KISS1* mRNA expressing cells also expressed *CALCR* mRNA in the arcuate nucleus. The results suggest that central amylin-CTR signalling has a biphasic role in the regulation of the GnRH pulse generator by acting on cells other than the kisspeptin neurones of the arcuate nucleus in goats. In addition, amylin-CTR signalling has been reported to be involved in thermoregulation and energy homeostasis. So, the amylin-CTR signalling could be a potential neural circuit that links thermoregulation, energy homeostasis, and reproduction.

Chapter 6: General discussion

My PhD work has added further evidence supporting the concept of the use of the profile of the CRT as a biomarker of an animals' fitness. Variation in T_c is related to the fitness of animals and may reflect the animals' capacity to cope with day-to-day challenges. In the conclusion of my thesis, I suggest a possible mechanism that could integrate the control of energy balance, core body temperature, and reproductive function. I have proposed a new method to estimate an animal's fitness to artificial and natural environments by monitoring the T_c.

OECD (Organization for echonomic cooperation and development) (2021) *Making Better Policies for Food Systems*. OECD iLibrary.

Ritchie H and Roser M (2017) Meat and dairy production. *Our World in Data*.