Circadian Physiological Actions in Pigs

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Circadian rhythms exist in almost all types of cells in mammals. Thousands of genes exhibit approximately 24-h oscillations in their expression levels, making the circadian clock a crucial regulator of their normal functioning. Identification of environmental and physiological inputs that affect circadian gene expressions will help development of novel targets and the corresponding approaches to optimize production efficiency in pigs.

Circadian physiology Oscillators Pigs Lipid metabolism Clock gene

1. Introduction

Circadian rhythms are endogenous autonomous oscillators of physiological activities. They are controlled by the circadian clock directly or indirectly in a 24-h cycle[1]. It is a deeply implemented system during evolution in living organisms for adaptation to a cyclic natural environment[2]. For instance, the metabolic processes synchronizing to the biological clock allows animals to ramp up the necessary functions for the upcoming feeding and adapt to changes in the length of the day and night. However, the effects on swine health and ecology are often overlooked. The circadian clock is based on an array of transcriptional regulators interacting with one another in negative feedback loops[2]. The interlocked transcription–translation feedback loops act as a circadian pacemaker[3]. The basic helix–loop–helix transcription factors circadian locomotor output cycles kaput (CLOCK) and arnt-like protein-1 (BMAL1) make up the core component, directly control period (*PER*) and cryptochrome (*CRY*) transcription. Notably, PER and CRY proteins can inversely repress *CLOCK* and *BMAL1*-stimulated transcription, allowing the cycle to begin anew when *PER* and *CRY* actions are turned-over. Importantly, REV-ERBs (REV-ERBα and REV-ERBβ encoded by *NR1D1* and *NR1D2*, respectively) and retinoic acid receptor-related orphan receptors (RORs) drive the transcription of BMAL1, which are the primary players during the interlocked loop. A better understanding of the circadian physiology will help to show how rhythms could sustain livestock health and improve production.

2. The Circadian Physiological Functions in Pigs

A wide range of physiological and metabolic variations depends on the circadian rhythm[4].The lipid metabolism interconnecting with the circadian clock is essential for growth performance, health, and meat quality in pig production. Interestingly, Zhou and co-workers[5] have uncovered diurnal variations in polyunsaturated fatty acid (FA) metabolism in young pigs under physiological conditions. In circulation, the free FA is seen at its lowest level at 7 pm, and peaks at 3 am and 7 am the next day. While the plasma arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid (DHA) levels followed a similar trend. In contrast, the hepatic linoleic acid (LA) and α-linolenic acid are observed at higher concentrations in the daytime and lower at night,

indicating an immediate absorption after meals during the day[5]. Surprisingly, the total triglycerides (TG) content in the liver of these young pigs did not show daily rhythm in the 24-h study, contrasting the previous observations in rodents[6]. As complex as this daily rhythm of lipid metabolism is reported to be in young animals, it can be reshaped by feeding patterns and nutrient supply. Compared to the conventional administration of three-meals, a dieting strategy of low to high protein contents as breakfast, lunch and dinner, respectively, results in the increased liver weight but decreased hepatic crude fat and reduced plasma TG levels in pigs. This is linked to a down-regulation of lipid metabolism genes, a reduced expression of *PER1*, *PER2* and *CRY2*, and an upregulation of BMAL1 protein expression in the liver[7]. More importantly, feeding according to the circadian system has been shown to improve the growth performance[8] and muscle quality of pigs[8][9], as well as yield better milk lipid profile and production in sows [10]. It appears that energy intake or feeding acts as a robust zeitgeber which synchronizes metabolism to animal peripheral clocks, especially for intensive pig farming. A group of circadian clock genes, i.e., *ARNTL*, *BHLHE*, *CRY2*, *NPAS2*, *NR1D1*, *PER1*, *PER2*, and *SIK1*[11] are shown to express in key metabolic tissues of pigs, including liver, small intestine, dorsal fat and muscle. Their expression levels differ significantly in pigs under fed states, compared to fasting[12]. In contrast, these genes are less affected in the central clock (hypothalamus). The feeding differences also influence the blood glucose and TG levels, which are shown to peak 2 h after eating and 4 h post-meal, respectively, and are accompanied by a significant decrease in free FA in blood[9]. We[13] have demonstrated previously that time-restricted feeding of limited nutrient supply reduces the cholesterol biosynthesis gene program in porcine liver organoids, a regulation that is controlled by RORγ[13][14][15].

As aforementioned, the timing of feed administration plays an important role in cooperating the metabolic responses to energy supply in animals. The nutritional outcomes can be very different, despite providing the same type of food/feed and the same amount of calories[16][17]. It is a true “time giver” to be manipulated in energy management of livestock and human beings, which is linked to the onset of obesity[18][19]. It is reported that a high-fat diet given during 12-h light causes more weight gain in mice than during 12-h darkness [20]. It should be noted that the mice are nocturnal, differing from pigs and humans that are diurnal[21]. More importantly, the light-phase feeding is indeed a common measure in the pig and poultry industries, and therefore is of high relevance. It requires more attention in regard to animal health, and studies of the obesity pathogenesis. Although many aspects of the circadian physiology are controlled by rhythmic input signals from the central clock, they also exhibit cell-autonomous regulation from the peripheral clocks[22]. A two-meals of low-high calcium supplementation dietary regimen is reported to influence the lipid metabolism of a pregnant sow during the third trimester[23]. In this study, the placenta lipid profile is also altered. i.e., the placental LA and DHA concentrations are increased by the high-low calcium feeding. In contrast, the heptadecanoic acid, oleic acid and monounsaturated fatty acid (MUFA) levels are decreased, compared to the low–high group, indicating a transfer of maternal effects[23]. This is mediated by changes of gene expression related to fetal lipid metabolism including FA de novo synthesis, transport, and glycolipid metabolism, as well as key clock genes *PER1*, *PER2* and *CLOCK* in placenta[23]. Collectively, it is suggested that feeding as a whole plays an essential role in circadian physiology of pigs. It is therefore important to regulate nutrition according to the peripheral circadian clocks.

For livestock, reproductive and growth performances are important meters. Wang et al. [24] reveal that the clock gene *BMAL1* plays a critical role in hormone secretion and apoptosis in porcine granulosa cells through the

PI3K/AKT/mTOR pathway. This is closely related to oocyte maturation and follicular development[24]. Furthermore, it is reported that the carbon monoxide (CO) infusion in ophthalmic venous blood alters the clock gene expression (*PER1*, *PER2*, *CRY1*, *CRY2*, REV-ERBs/(*NR1D1/2*)) and their transcriptional factors (*BMAL1*, *NPAS2*, *CLOCK*, *RORβ*) in pigs. In parallel, CO treatment modifies the protein expression of the melatonin synthesis pathway[25]. Melatonin is known to be involved in synchronizing the circadian rhythm, and is especially linked to seasonal reproduction; it is therefore is a potent mediator of the timing and the associated physiological responses[26]. Indeed, melatonin addition has been demonstrated to prevent oocyte senescence after ovulation in pigs[27]. It upregulates the gene expression related to FA oxidation and mitochondrial biogenesis, and increases FA contents, ATP and mitochondria in porcine oocytes, which thereby provides an energy source for oocyte maturation and subsequent embryonic development[28]. This evidence of rhythmic clock gene expressions in the reproductive tissues suggests that they may contribute to fertility optimization[29]. In addition, biological rhythm acting on hormones such as catecholamines and glucocorticoids is shown to be associated with changes of the sympathetic stress. As observed in the sows, urinary catecholamine concentrations are lower in the dark, compared to during the daytime. Chronic activation of the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis is known to be associated solely with the increased catecholamines and glucocorticoids levels[30]. Their concentration in urine is thus a potential biomarker that helps to better understand hormone rhythmic release in circulation. As a non-invasive practice in pig husbandry[30], it could be applied to monitor the physiological changes of sows during pregnancy. The circadian physiology has been intensively studied in mice; less is known about the molecular components of these clocks and the mechanism by which they respond to external stimuli in domestic species[12]. Given the close physiological similarity between pigs and humans, data presented here could be also of interest for mechanistic studies in human physiology.

3. Conclusion

The interconnection between circadian rhythmicity and livestock physiology is becoming one of the major focuses of the field of animal science[31]. Indeed, the temporal organization of a 24-h cycle physiology, metabolism, and behavior is driven by a complex network of multiple cellular circadian oscillations. The rhythmical pattern is synchronized through neuronal and hormonal signals by a core center-clock located in the suprachiasmatic nuclei zone of the hypothalamus. Studies on the regulations of circadian clock gene expression have so far been conducted almost exclusively using commercially available cell lines along with small experimental animals. In contrast, studies using livestock species (either domestic or wild-cross-breeding ones) for the assessment of circadian clock modulation are still rare and with limitations. Specific feeding of circadian time or chrono-treatment is important to animal science, and additional advances should be made in large animal chronobiology.

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