Seasonal alterations in circadian melatonin rhythms of the European wild boar and domestic gilt,

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The pattern of melatonin secretion is rhythmic in the domestic pig and responds rapidly to changes in daylength


Abstract: The aim of the study was to investigate the capability of pigs to respond to abrupt changes in lighting conditions by means of alterations in circadian melatonin profiles. Sixteen pre-pubertal crossbred male pigs weighing 40–45 kg were housed in individual pens in four temperature- and lighting-controlled climate rooms (four pigs per room). In two rooms there was a light–dark cycle of 16 L:8 D (Group A) and in two other rooms 8 L:16 D (Group B). Under both lighting regimens light intensity at pig eye-level was 220–240 lx during the light phase and less than 7 lx (red light) during the dark phase. The lighting regimens were changed after 2 wks to the opposite regimen and the change was repeated after a further 2 wks, so that animals ended up with the same light cycle with which they started. Blood was sampled at 2-hr intervals for 48 hr spanning each time of change in lighting. A further 24-hr sampling was performed at the end of the experiment (2 wks after the last change) in both groups and 1 wk after the change from short to long day lighting in Group A. On 83/86 occasions, pigs exhibited a clear circadian rhythm in plasma melatonin under both lighting regimens. Pigs responded immediately to the change from long to short day lighting by advancing melatonin secretion to the earlier lights-off time and some pigs were able to extend secretion to the delayed lights-on time. For short to long day changeover there was a small immediate response, with secretion pattern following the previously entrained endogenous rhythm to within 3 hr of the previous lights-on time. After 1 wk commencement of secretion was delayed by up to 2 hr, while after 2 wks some pigs were able to delay secretion until lights-off or to cease at lights-on. It is concluded that the domestic pig is able to commence adjustment to abrupt changes in photoperiod within a 1-wk acclimatization by altering circadian melatonin secretion. The present study suggests that it may be possible to use simplified lighting regimens instead of stepwise changing lighting programs in commercial pigeries to reduce the influence of season on production.

Introduction

The domestic pig is considered to be a non-seasonal breeder and capable of producing progeny throughout the year. However, a clear reduction in reproductive performance of sows and gilts during late summer and early autumn has been revealed by a number of studies [for a review see Love et al., 1993; Xue et al., 1994; Peltoniemi et al., 1999]. Season has also been shown to affect sperm quality and testosterone levels of boars in a way that implies reduced fertility during late summer early autumn [Claus and Weiler, 1985]. This impairment of reproduction corresponds to the time of the year when the European wild boar (Sus scrofa scrofa), an ancestor of western domestic pig breeds, exhibits seasonal anoestrus [Mauget, 1982]. The phenomenon is recognized worldwide and is known as “summer” or more accurately “seasonal” infertility [Love, 1978; Peltoniemi et al., 1999]. Photoperiodic information is converted into an endocrine signal in the form of melatonin...
secretion from the pineal gland and in non-tropical seasonal breeding mammals it synchronizes a number of circadian and circannual rhythms with the dark-light cycle and season [for review see Reiter, 1991]. In mammals, the endogenous circadian melatonin secretion, controlled by the suprachiasmatic nuclei (SCN), is entrained to an ambient photoperiodism with release occurring during the scotophase. In humans (and other species studied), illumination acutely decreases nocturnal serum melatonin levels if the light intensity or duration is sufficient [Aoki et al., 1998]. There is a dual effect of light, which has a suppressing effect immediately after exposure to a light stimulus, and a phase-shifting effect, which is detectable usually during the next night after exposure [Hashimoto et al., 1996].

In distinct seasonally breeding mammals such as sheep (short day breeder) and Syrian hamster (long day breeder) the role of melatonin in entraining the seasonal breeding is well established and is related to the duration of melatonin secretion [for review see Ebling and Hastings, 1992]. The existence of circadian melatonin profiles of the domestic pig has remained controversial until now, although changing photoperiod is thought to be the most important factor influencing the hypothalamo–gonadal axis and leading to reduced fertility in late summer–early autumn [for review see Love et al., 1993]. Most of the studies have concluded that the domestic pig does not exhibit a circadian melatonin pattern typical of other mammalian species [Minton et al., 1989; Diekman et al., 1992; Diekman and Green, 1997]. Some studies have concluded that circadian melatonin patterns are evident only under certain lighting regimes or only in some individuals [McConnell and Ellendorff, 1987; Griffith and Minton, 1992; Green et al., 1996; Bollinger et al., 1997]. However, there are two studies that have demonstrated clear and repeatable melatonin profiles in pigs under different lighting conditions [Paterson et al., 1992; Tast et al., 2001]. Studies by Klupiec et al. [1997] and Andersson et al. [2000] have suggested that the controversy resulted, at least to some extent, from inadequacies in the assays used. Paterson et al. [1992] used a stepwise change in lighting regimens in their study mimicking changes in the natural photoperiod. They suggested the failure to demonstrate a nocturnal rise in melatonin under variable lighting programs might be that the domestic pig is unable to respond to the abrupt changes in lighting conditions used in many of these studies.

The aim of the present study was to determine the capability of the domestic pig to respond to abrupt changes in photoperiod. This was investigated by monitoring the changes in circadian melatonin profiles following changes in duration of the scotophase.

Materials and methods

Animals and housing

The experimental animals (n = 16) were selected randomly from crossbred male grower pigs using a body weight of 40–45 kg as the inclusion criterion. During the experiment, pigs were housed in individual pens (1 × 2 m) in four climate rooms (four pigs per room) where temperature (23 ± 1 °C) and lighting were strictly controlled. All the pigs had free access to water and feed throughout the experiment. The feed was a pelleted commercial grower pig diet.

Before the experiment commenced, lighting in each room was adjusted to between 220 and 240 lx during the photophase and less than 7 lx during the scotophase (dim red lights) measured at pig eye-level with a digital light meter (Topcon® IM-2D, Japan). The long day lighting of a 16 hr photophase and an 8 hr scotophase (16 L:8 D) was provided in two rooms (Group A) and the short day lighting of 8 L:16 D in the other two rooms (Group B). In the long day regimen, lights were switched on at 05:00 hr and off at 21:00 hr. In the short day regimen the photophase lasted from 09:00 to 17:00 hr. After 2 wks under these lighting regimens, the lighting conditions were changed to the opposite in each room. The changeover was repeated after a further 2 wks, so that the groups ended up with the same lighting regimen as at the beginning (Fig. 1).

Blood sampling

The eight pigs from each lighting regimen were sampled at 2-hr intervals for 24 hr before and 24 hr after the changeover at 12:00 hr (i.e. two sampling days), except at the end of the experiment, when only one 24-hr sampling was carried out. In addition, a 24-hr sampling was performed in Group A, 1 wk after the changeover from short to long day lighting (Fig. 1). The blood samples were collected via ear-vein catheters. The sample (approximately 8 mL) was drawn into a 10 mL syringe and immediately emptied into a 10-mL plastic tube containing 150 μL EDTA solution (250 mg/mL) and 0.25 g spinning granules (Kwik Spin®, Disposable Products, Ridleyton, S.A.). The samples were refrigerated immediately and centrifuged within 4 hr. For each sample the separated plasma was poured out into a 10-mL plastic
tube and stored at −20°C until the day of analysis.

Melatonin assay

Melatonin concentrations of plasma samples were determined by a commercially available double-antibody radioimmunoassay (Bühlmann Laboratories AG, Switzerland) based on the Kennaway G280 anti-melatonin antibody and using 125I-2-iodomelatonin [Kennaway et al., 1982]. Klupiec et al. [1997] used the same antibody and 3H-melatonin in an assay based on solvent extraction of porcine plasma. Standards and samples were extracted using C18 columns. The sensitivity of the assay was 0.3 pg/mL. Inter- and intra-assay CV for high and low (lot-specific concentrations) quality controls were 14.3 and 10.5%, and 13.9 and 10.0%, respectively.

Statistical methods

Average scotophase and photophase melatonin concentrations. Mean melatonin concentrations during each scotophase and photophase interval were determined for all pigs. A mid-interval mean of the levels at 22:00, 24:00, 02:00 and 04:00 hr was calculated for each scotophase for each pig, while the basal concentration during each photophase was calculated as the mean of the levels at 10:00, 12:00, 14:00 and 16:00 hr, or subsets of those hours. For each scotophase interval an average of the pre- and post-interval basal concentrations was determined for each pig. Three variables were derived for analysis: the logarithms of average basal means and of mid-interval means and the difference between the logarithms, which provided transformed basal-adjusted mid-interval means.

For each of the log-transformed variables the method of linear mixed models was used to assess the fixed effects of lighting regimen (long day or short day), day of regimen (first or fourteenth) and sampling time (first or second changeover or final sampling) and their interactions, together with the random effects of rooms and of pigs. In each analysis, there were large differences among the effects of pigs but the room effects were negligible and were removed from the model. Alternative models, which included effects of groups (A or B) rather than sampling time, were tested but no significant group effects were detected.

Changeovers from long day to short day lighting regimes and vice versa

For each pig, logarithms were taken of the melatonin concentrations at 18:00, 20:00, 06:00 and 08:00 hr before and after each changeover and at the final sampling, and also 1 wk before the final sampling for the pigs in Group A. Basal-adjusted values were calculated by subtracting the logarithms of corresponding average basal concentrations and differences from the logarithms of corresponding mid-interval means were also calculated. The log-transformed data were assigned to two sets, either long day lighting regimens (six sampling days) or short day regimens (five sampling days). Basal-adjusted concentrations at 18:00 hr for the pigs in Groups A and B were analysed separately for each regimen, as were the data at 20:00, 06:00 and 08:00 hr, and differences from the mid-interval means for the four times were analysed similarly.

For each data set the method of linear mixed models was used to estimate as fixed effects and compare the means at the relevant hour for each sampling day for Groups A and B, with pig effects included as random terms in the model. As with the previous analyses, rooms were initially included as random effects but were found to be negligible and so were removed.

Results

On all but a few occasions (83/86), pigs exhibited a clear circadian rhythm in plasma melatonin concentrations under both lighting regimens. On two of the 88 sampling occasions missing samples
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Fig. 2. Mean ± S.E.M. plasma melatonin concentrations of young boars (Group A, n = 8). Pigs were allowed to adjust to the lighting (16 L:8 D) for 2 wks before the lighting was changed to the opposite (8 L:16 D). A 48-hr sampling spanning the changeover with samples collected at 2-hr intervals was performed at the time the lighting was changed. The change and sampling were repeated after a further 2 wks. A 24-hr sampling was performed 1 wk after the change from short to long day lighting and at the end of experiment 2 wks after the last changeover. Black bar under axis illustrates the scotophase.

Fig. 3. Mean ± S.E.M. plasma melatonin concentrations of young boars (Group B, n = 8). Pigs were allowed to adjust to the lighting (8 L:16 D) for 2 wks before the lighting was changed to the opposite (16 L:8 D). A 48-hr sampling spanning the changeover with samples collected at 2-hr intervals was performed at the time the lighting was changed. The change and sampling were repeated after a further 2 wks. A 24-hr sampling was performed at the end of experiment 2 wks after the last changeover. Black bar under axis illustrates the scotophase.

prevented reliable determination of the profile. The typical basal values during the photophase varied between <0.3 (the sensitivity of assay) and 2 pg/mL, and increased during scotophase generally up to values between 5 and 15 pg/mL. During the scotophase interval, there was some variation in melatonin concentrations in both groups, perhaps representing the pulsatile release of melatonin, but no consistent peaks were observed under either lighting regimen (Figs 2 and 3). Melatonin concentrations among individual pigs varied markedly during both the scotophase and photophase intervals (P < 0.001).
There were no significant effects of lighting regimen, day of regime or sampling time on scotophase mid-interval mean melatonin concentrations. However, mean basal melatonin increased significantly between the first and second changeovers (0.78–1.06 pg/mL, P < 0.01) and between the second changeover and final sampling (to 1.55 pg/mL, P < 0.05). For the short day regimen, first day mean basal level (1.32 pg/mL) was significantly greater (P < 0.001) than for the fourteenth day (0.63 pg/mL), but the difference between days for the long day regimen was negligible. When the mid-interval mean concentrations were adjusted for average basal means, the adjustments induced significant effects corresponding to those for basal levels, but in the reverse direction.

When the lighting regimen was changed from long day to short day, there was an immediate change in the pattern of melatonin profile toward alignment with the new lighting conditions (Figs 2 and 3). Mean melatonin levels at 18:00 and 20:00 hr following the change significantly exceeded the basal level (both P < 0.001) and, although the small difference in mean level between 18:00 hr and the scotophase mid-interval was weakly significant (P < 0.10), there was no detectable difference after 20:00 hr (P > 0.50). During the extended scotophase, the mean melatonin levels at 06:00 and 08:00 hr were significantly lower than the mid-interval mean (both P < 0.05) and were not significantly above the basal level (P > 0.20), although some pigs maintained high melatonin levels to 08:00 hr. After 2 wks acclimatization mean melatonin at 18:00, 20:00 and 06:00 hr did not differ from the scotophase mid-interval mean. However, at 08:00 hr, mean melatonin for the pigs in Group B was significantly above basal level (P < 0.001) and only weakly significantly below the mid-interval mean (P < 0.10), while for the pigs in Group A mean melatonin was significantly below the mid-interval mean (P < 0.001) and not significantly different from basal level (P > 0.20), although melatonin for some pigs remained high.

When the lighting regimen was changed from short day to long day there was only a small change in the melatonin profile (Figs 2 and 3). At 18:00, 20:00 and 06:00 hr when lights were on mean melatonin concentration continued to significantly exceed the basal level (P < 0.001, 0.001, 0.01 respectively) but at 08:00 hr the mean was at basal level. After 1 wk there was further progression towards a long day melatonin pattern for the Group A pigs as their mean melatonin at 18:00 hr was at basal level, but the means at 20:00 and 06:00 hr remained significantly above that level (P < 0.001, 0.01 respectively). After acclimatization of the Group A pigs for another week the difference between the mean and the basal level at 20:00 hr was weakly significant (P < 0.10), but at 06:00 hr the difference remained significant at P < 0.01. For the pigs in Group B, however, after 2 wks acclimatization mean melatonin at 20:00 hr remained significantly above basal level (P < 0.001) whereas at 06:00 hr it was at basal level.

Discussion

The present study confirms circadian melatonin profiles in domestic pigs under both short and long day lighting regimens. Abrupt, extreme changes in the duration of photophase did not abolish the circadian profiles.

In this study pigs exhibited a clear circadian rhythm in melatonin concentrations under both lighting regimens. The three exceptions where the melatonin profiles were not detected were most likely caused by technical problems with extraction of these particular samples. In a few cases plasma fibrin clots clogged the extraction columns resulting in a failure of extraction. The mean melatonin concentration during the middle of the scotophase remained unchanged under different lighting programs. These findings give further evidence that the domestic pig exhibits melatonin profiles similar to other mammalian species, and that the earlier controversy as to the existence of such profiles in pigs probably resulted from inadequacies in the assays used [Klupiec et al., 1997; Andersson et al., 2000]. McConnell and Ellendorff [1987] reported a nocturnal increase in melatonin under 12 L:12 D lighting regimen with abolition of the nocturnal melatonin increase under long or short day lighting regimens. The present results contradict those results, as circadian profiles were evident in all sampling occasions including the 24-hr period immediately following the changeovers. This discrepancy is likely to result from some limitations of earlier RIAs referred to earlier.

It has been suggested that domestic pigs might be unable to respond to abrupt changes in lighting in terms of melatonin secretion [Paterson et al., 1992]. If the pig were able to respond only to stepwise changes mimicking the changes in natural photoperiods it would make it difficult to introduce artificial lighting programs to commercial pigeries to overcome the seasonal reduction in fertility. In this study pigs responded within a relatively short time to an abrupt and extreme change in lighting as evident from entrainment of melatonin profiles within 1 wk under a new lighting regimen.
When lighting was changed from long day to short day, pigs were immediately able to respond to the advanced scotophase. The elevation of melatonin concentrations took place almost immediately after lights out, as evident from samples taken 1 hr later, even if the lights-off time was advanced by 4 hr from the previous lights-off time. Also, at the end of the scotophase some pigs were immediately able to extend melatonin secretion until lights on, even if lights-on time was delayed by 4 hr, although average melatonin levels decreased at the time lights were previously switched on.

The changeover in the direction from short day to long day lighting appeared to be more difficult for pigs to respond to, as evident from almost unchanged melatonin profiles during the first 24 hr after the lighting changed. A noteworthy feature in melatonin profiles immediately after a change from short to long day lighting was that melatonin secretion commenced at the same time before and after the changeover, despite the relatively high light intensity (220–240 lx) used during the photophase, and that melatonin remained high into the advanced photophase. However, similar features were evident in most pigs to some extent even after 2 wks acclimatization and might be a “normal” finding when the scotophase is much shorter than 12 hr.

The ability of pigs to respond immediately to the advanced and extended darkphase by prolonging melatonin secretion demonstrates the combined controlling mechanism of melatonin secretion by the endogenous clock (SCN) and the prevailing photoperiod. The immediate melatonin response during the 4-hr advancement of the darkphase was in accordance with the results reported in sheep [Bittman et al., 1983]. However, in that study the onset of the darkphase was advanced by 8 hr rather than 4 hr as in the present study, and the sheep failed to respond in terms of melatonin secretion during the first 4 hr of the advanced darkphase.

The finding that 220–240 lx was not sufficient light intensity to prevent the beginning of melatonin secretion after the short to long day changeover was expected, as the previously entrained endogenous circadian rhythm, created by SCN, is known to control the commencement of secretion. However, the finding that 220–240 lx light intensity caused little suppression during the 4-hr period when the lights-out time was delayed was surprising, although somehow similar to the results of some human studies. Hashimoto et al. [1996] concluded that the light intensity needed to suppress nocturnal melatonin secretion was some-

where between 200 and 500 lx in humans. In addition, the direct suppressive effect of increased light on nocturnal melatonin levels in humans is known to depend on both light intensity and illumination time [Aoki et al., 1998].

The average mid-scotophase melatonin concentration increased with each successive scotophase indicating that the light intensity used became less inhibitory as the trial progressed. The reason for this increase and its physiological significance are not immediately obvious.

It is well known that the activation of N-acetyltransferase (NAT), the rate limiting enzyme in converting serotonin to melatonin in the pineal gland, takes place only under norepinephrine (NE) stimulus [for review see Reiter, 1991]: it is in the darkness or more accurately when the SCN signals the darkness. The results of the present study imply that the onset of darkness can almost immediately promote the activation of NAT, as evident from increased melatonin levels during the advanced scotophase. The results also suggest that 220–240 lx is not sufficient light intensity to acutely suppress NAT activity in the pig during the secretion phase of an entrained endogenous circadian rhythm. This is in accordance with an earlier observation in pigs (Tast et al., 2001), which suggested that 300 lx was an insufficient light intensity to acutely suppress entrained melatonin secretion.

It is concluded that the domestic pig is able to commence adjustment to an abrupt and extreme change in ambient lighting within a 1-wk acclimatization in terms of melatonin secretion. With a change from long to short day lighting, pigs could more readily advance melatonin secretion than to extend it. When lighting changed from short to long day, 220–240 lx is an insufficient light intensity to suppress acutely a previously established melatonin rhythm. Together these data imply a capability of pigs to adjust to changes in photoperiodism and an ability to entrain the circadian rhythms according to altered lighting conditions. The present study suggests that it may be possible to use simple artificial lighting regimens instead of stepwise changing light programs in commercial piggeries to reduce the influence of season on production.

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Literature cited


