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ORIGINAL ARTICLE

The effect of continuous darkness and illumination on the function and the morphology of the pineal gland in the domestic pig

I. The effect on plasma melatonin level

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Abstract

OBJECTIVES: Results of the majority of studies have revealed that diurnal changes in circulating melatonin level in the domestic pig differ from the typical patterns observed in other species. The aim of the present investigation was to study the effect of continuous darkness and continuous illumination on plasma melatonin in the pig. MATERIAL AND METHODS: The study was performed on three groups of immature gilts. The first group was kept under 14 hrs light:10 hrs dark cycle (500 lx of fluorescent light during photophase), the second group—under continuous illumination (500 lx of fluorescent light) and the third group—under red light with intensity below 1 lx, which was considered as darkness. The pigs were maintained nine days under above reported conditions and then plasma melatonin was monitored during five consecutive days. RESULTS: The diurnal changes in plasma melatonin concentration with increased levels during scotophases were observed in gilts kept under light:dark cycle, but these changes were characterized by low regularity and repeatability. In pigs kept under continuous darkness the circadian changes in plasma melatonin with the highest levels during natural nights were found. No significant circadian changes in plasma melatonin were noted in gilts exposed to continuous illumination. CONCLUSIONS: The obtained results suggest that the diurnal rhythm of melatonin secretion in the domestic pig is generated endogenously and entrained by environmental light. The present results supported also the previously reported observations showing low regularity of diurnal changes in circulating melatonin in the pig.

Introduction

Diurnal rhythmicity is an important feature of the mammalian endocrine system, clearly visible in secretion of hormonal products of the pituitary and adrenal glands, but most prominent in the function of the pineal gland. In the majority of investigated mammals melatonin secretion by the pineal gland is low during day and increases markedly at night.

It is generally believed that in mammals the rhythm of melatonin synthesis and secretion by the pineal gland is generated endogenously by the circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus [1, 2]. This endogenous rhythm is entrained by environmental light acting via the retina and the neural pathways connecting the retina with the hypothalamus. The inhibitory effect of the light on the activity of the SCN together with the intrinsic rhythmicity of the SCN ensure that melatonin is produced and secreted only during nocturnal darkness. Although the general schema of the endogenous generation and the exogenous synchronization of the rhythm of melatonin synthesis and secretion in mammals is commonly accepted, the precise nature and localization of the processes driving and controlling this endocrine rhythm remain unknown.

From a comparative point of view, the pineal gland of the domestic pig is a very interesting subject of research, because it shows many species specific features of its morphology and physiology. Ultrastructurally, the pinealocytes of the domestic pig are characterized by the presence of numerous electron dense bodies [3, 4], which are probably involved in the secretory processes occurring in the pig pineal gland (for details see part II—this issue). Results of the majority of physiological studies [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16] have revealed that the diurnal changes in plasma or serum melatonin level in the domestic pig do not conform to the typical patterns known from the experiments performed in the other species [17, 18, 19, 20]. The characteristic features of the melatonin secretion in the domestic pig include: (1) the presence of nighttime melatonin rise only under specific light conditions and (2) low regularity and repeatability of plasma melatonin rhythm in individual animals and large variability of melatonin profiles between individuals.

In view of the peculiar morphological and physiological features of the pig pineal gland as well as the significance of the domestic pig as farm animal and as animal-model in biomedical research, we decided to study mechanisms regulating melatonin secretion in this species. The aim of the present work was to investigate the involvement of both endogenous compound and the environmental light in the control of plasma melatonin level in the pig by exposure of animals to continuous illumination and continuous darkness.

Material and methods

The experiment was performed in Poland in April, when the length of natural day was about 14.5 hrs (sunrise: 04:00–04:20, sunset: 18:50–19:00), on 18 female crossbred pigs. The animals, aged 95±4 days, were purchased from a commercial farm, where they were kept under natural photoperiod. The pigs were divided into three equal groups: I—control group kept under 14 hrs light:10 hrs dark conditions, II— group exposed to continuous darkness, III—group exposed to continuous illumination. During the whole experiment the animals were kept in individual coops in three separated rooms. Gilts had free access to water and were fed twice daily (09:00, 14:00) with standard food. The coops were cleaned every day at 08:30. Handling of animals was performed in agreement with "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and Polish law on the protection of animals.

After the one-week-long acclimatization period (under natural photoperiod) in animal-laboratory rooms, the pigs were anesthetized with penthobarbital (Vetbutal-Biowet, Poland) and left jugular veins were cannulated via cephalic veins according to the method described by Kotwica et al. [21]. After surgery, the gilts were kept under conditions of the controlled artificial illumination. The first group (control) was exposed to a cycle of 14 hrs light:10 hrs dark. During photophase (06:15–20:15) intensity of illumination provided by fluorescent tubes was 500 lx on the level of animal eyes. The second group was kept under red light with intensity less than 1 lx and these conditions were referred to as darkness. The third group was exposed to continuous fluorescent illumination with intensity of 500 lx on the level of animal eyes. After nine days, the blood samples were drawn at 2 hr intervals during a period of five days signed as day I, II, III, IV, V. For simplification of the data analysis each day was considered to be started at 06:15. Blood sampling in darkness was performed with the help of local red illumination with light intensity below 2 lx, which was never directed to animal eyes. Plasma samples, prepared with the use of heparin (Polfa-Warsaw, Poland) as anticoagulant, were stored at –20°C until melatonin assay.

Plasma concentrations of melatonin were measured by slightly modified direct RIA of Fraser and co-workers [22] using a sheep anti-melatonin antibody (No. G/S/704-6483, Stockgrand Ltd, University of Surry, Guilford, UK) and ^{3}H-melatonin (specific activity 86Ci/mmol, NEN Du Pont). Detailed proto-

col, characteristic and validation procedures of the assay were previously described [15]. Samples from individual gilts were run within the same assay.

Statistical analyses of the data were conducted after logarithmical transformation. The data from the assays of the samples taken during days I–V were analyzed using the repeated measures analysis of variance with the group as the main factor and the time of sampling as the repeated measure. This analysis showed the significant effect of the time and the lack of the significant effect of the group on plasma melatonin level as well as the significant interaction between them. In view of this: (1) the effect of the sampling time on plasma melatonin concentration was tested separately for each group during each day (including the first sample taken during the next day) using the repeated measures ANOVA and the LSD test as *post hoc* procedure and (2) comparisons between each group at each sampling time-point were made by the one-way ANOVA. Additionally, areas under curves of the individual melatonin profiles (AUC) were computed and compared between groups by the one-way ANOVA. In all statistical analyses, a value of $p \leq 0.05$ was accepted as significant. Values showed on Figures 2–6 are geometric means.

Results

Within-group analysis

Control group—the pigs kept under 14 hrs light:10 hrs dark cycle

Among six pigs kept under condition of 14 hrs light:10 hrs dark, regularly occurring nocturnal rises in plasma melatonin level were observed in four investigated animals during the entire sampling period (Fig. 1—pigs LD 1, LD 2). In one pig the lack of night-time elevation of plasma melatonin level was noted during day II and in the other one—during day IV (Fig. 1—pig LD 3).

In the control group significant night-time rises in the mean plasma melatonin level were observed during days I–V (Figs. 2–6). Generally, when compared to the levels noted during previous photophase, the mean concentration of plasma melatonin was significantly elevated between 22:00 and 06:00 during the entire sampling period (for details see Figs. 2–6). Only during day IV plasma melatonin level remained significantly elevated at 08:00 and was significantly higher at this sampling-point than during day III between 14:00 and 20:00 (Fig. 4).

Group of pigs kept under continuous darkness

In three out of six gilts kept in continuous darkness concentration of plasma melatonin showed relatively regular circadian changes with elevated levels between 18:00 and 08:00 during the entire sampling period (Fig. 1—pig DD 2). In the other three pigs the changes in plasma melatonin level were more irregular, but the tendency to rise during natural nights was visible (Fig. 1—pig DD 1).

In the group of pigs kept under continuous darkness, significant increases during natural nights (when compared to previous "natural days") were noted at days I, II, IV and V (Figs. 2–6). During day I and day II plasma melatonin concentration was significantly higher at 18:00 than at 16:00 as well as between 20:00 and 06:00 than between 12:00 and 16:00. Plasma melatonin level was also significantly higher during day II at 08:00 than during day I between 12:00 and 16:00 as well as during day III at 08:00 than during day II at 16:00. No significant increase in plasma melatonin level was noted during natural night at day III. During day IV plasma melatonin level was significantly higher between 18:00 and 06:00 than between 10:00 and 16:00. Plasma melatonin level was also higher during day V at 08:00 than during day IV at 12:00 and 14:00. During the last day of sampling (day V) concentration of melatonin in plasma was significantly higher between 18:00 and 6:00 than between 10:00 and 14:00 (and at 18:00, 24:00, 02:00, 04:00, 06:00 than at 16:00).

Group of pigs kept under continuous illumination

Among the pigs kept under continuous illumination plasma melatonin level was relatively stable with small fluctuations in three gilts(Fig. 1—pigs LL 1, LL 2). In the other three pigs fluctuations in concentration of plasma melatonin were higher, however they were not related to the shifts between natural days and nights (Fig. 1—pig LL 3). Only in one pig the marked increase in plasma melatonin level was noted during natural night at the third day of sampling.

In the group of pigs kept under continuous illumination the effect of sampling time on plasma melatonin level was not significant (Figs. 2–6).

Between-group analyses

The differences in the mean plasma melatonin between the three investigated groups of pigs were not significant during the entire sampling period (Figs. 2–6). No significant differences between groups were also noted in AUC (Fig. 7).

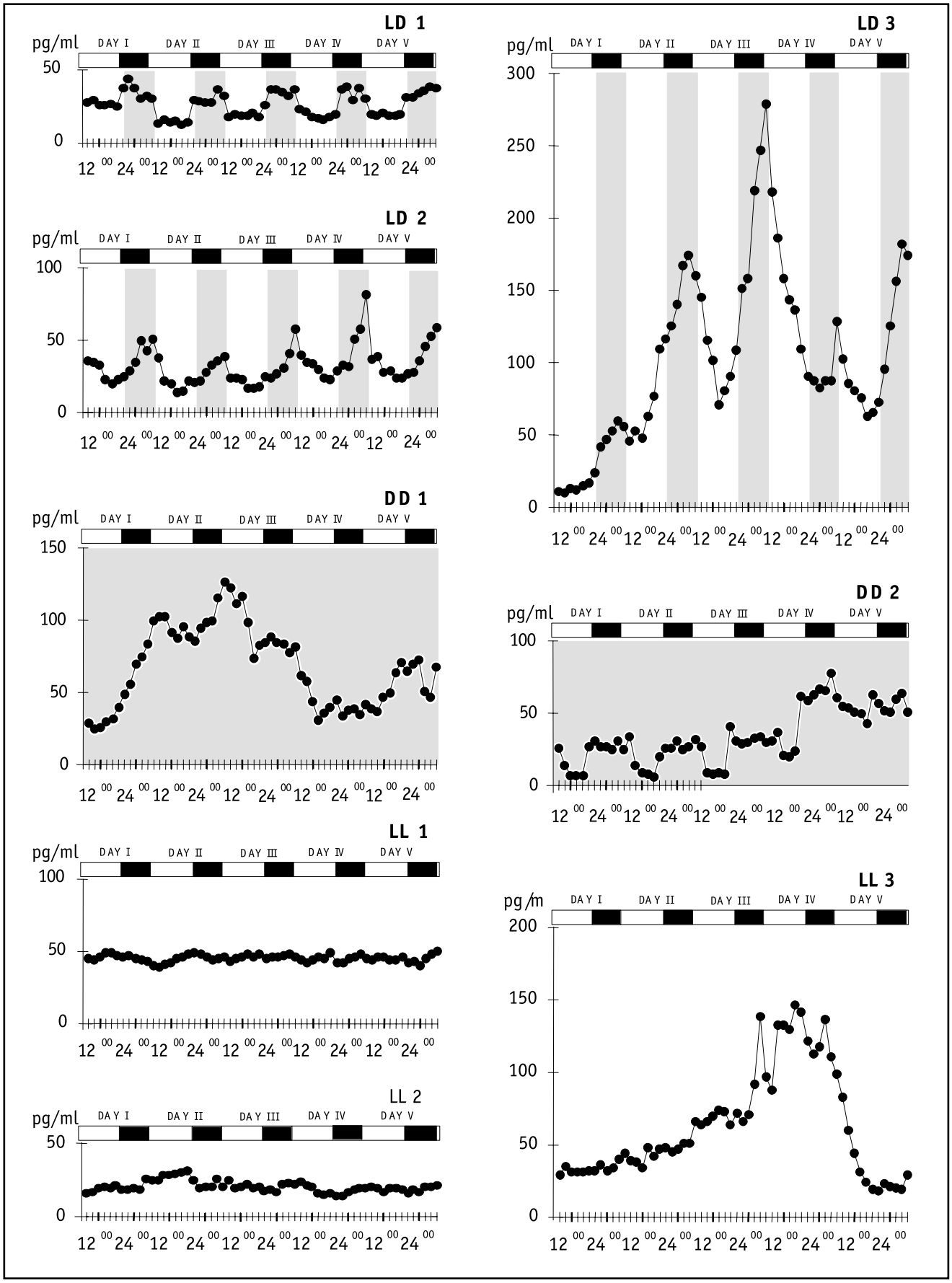

Fig.1. Individual plasma melatonin profiles in the pigs kept under light-dark cycle (LD 1, LD 2, LD 3), continuous darkness (DD 1, DD 2) and continuous illumination (LL 1, LL 2, LL 3). Horizontal white-black bar represents approximate lengths of light and dark phases of natural diurnal cycle at the time the experiment was performed.

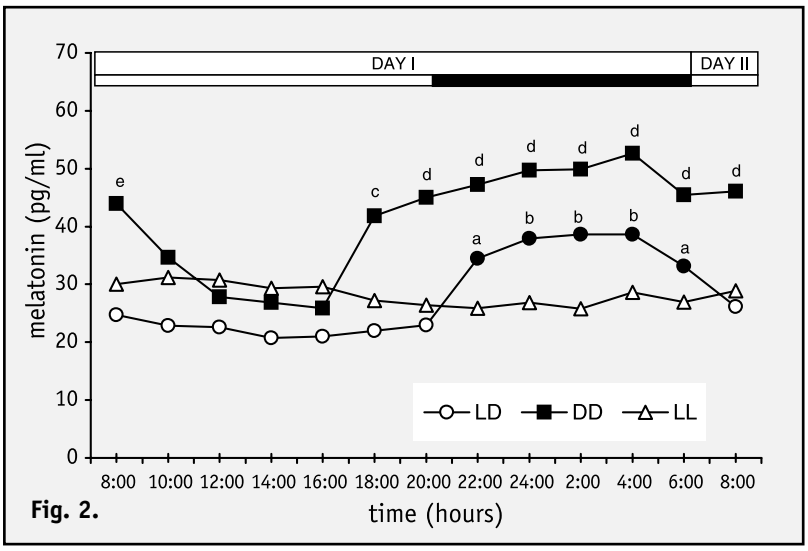

Fig. 2.

Fig. 2. Geometric means of plasma melatonin concentration in the groups of the pigs kept under light-dark cycle (LD), continuous darkness (DD) and continuous illumination (LL) during day I. Significant differences in the group kept under LD: a—vs. 10:00–20:00 during day I, b— vs. 08:00–20:00 during day I. Significant differences in the group kept under DD: c—vs. 16:00 during day I, d—vs. 12:00–16:00 during day I, e—vs. 14:00–16:00 during day I. The differences between groups were not significant at all sampling points. Open circles and triangles— samples taken in light, closed circles and squares—samples taken in darkness. Horizontal white-black bar shows lengths of light and dark phases in the room with the control pigs.

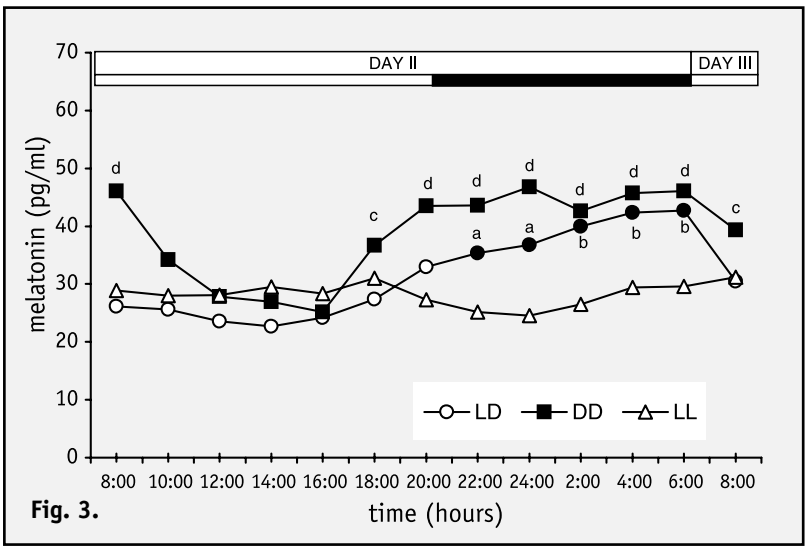

Fig. 3.

Fig. 3. Geometric means of plasma melatonin concentration in the groups of the pigs kept under light-dark cycle (LD), continuous darkness (DD) and continuous illumination (LL) during day II. Significant differences in the group kept under LD: a—vs. 12:00–16:00 during day II, b—vs. 08:00–18:00 during day II. Significant differences in the group kept under DD: c—vs. 16:00 during day II, d—vs. 12:00–16:00 during day II. The differences between groups were not significant at all sampling points. Other explanations see Fig. 2.

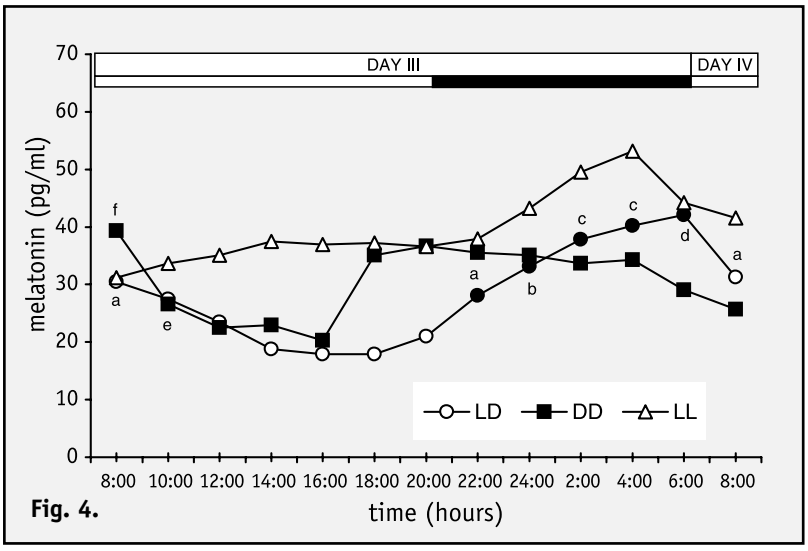

Fig. 4.

Fig. 4. Geometric means of plasma melatonin concentration in the groups of the pigs kept under light-dark cycle (LD), continuous darkness (DD) and continuous illumination (LL) during day III. Significant differences in the group kept under LD: a—vs. 14:00–20:00 during day III, b —vs. 12:00–20:00 during day III, c—vs. 10:00–22:00 during day III, d—vs. 08:00–22:00 during day III, e—vs. 14:00–18:00 during day III. Significant differences in the group kept under DD: f—vs. 16:00 during day III. The differences between groups were not significant at all sampling points. Other explanations see Fig. 2.

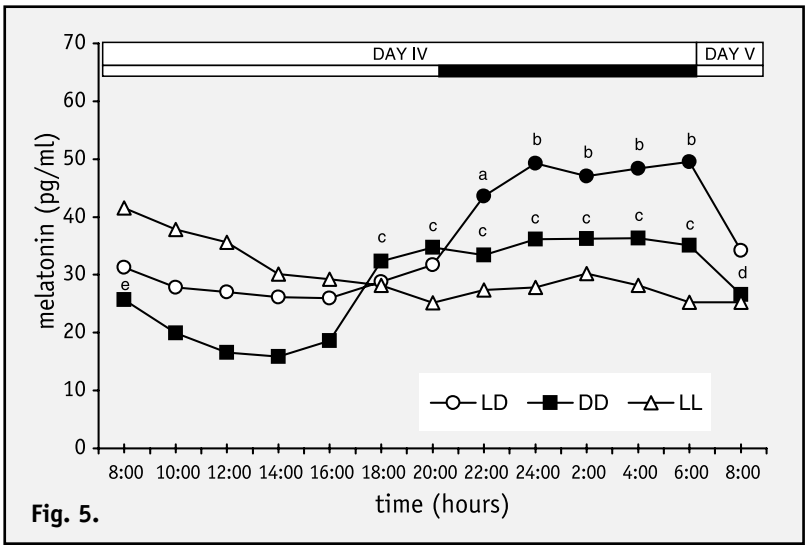

Fig. 5. Geometric means of plasma melatonin concentration in the groups of the pigs kept under light-dark cycle (LD), continuous darkness (DD) and continuous illumination (LL) during day IV. Significant differences in the group kept under LD: a—vs. 10:00–16:00 during day IV, b—vs. 08:00–20:00 during day IV. Significant differences in the group kept under DD: c—vs. 10:00–16:00 during day IV, d—vs. 12:00–14:00 during day IV, e—vs. 14:00 during day IV. The differences between groups were not significant at all sampling points. Other explanations see Fig. 2.

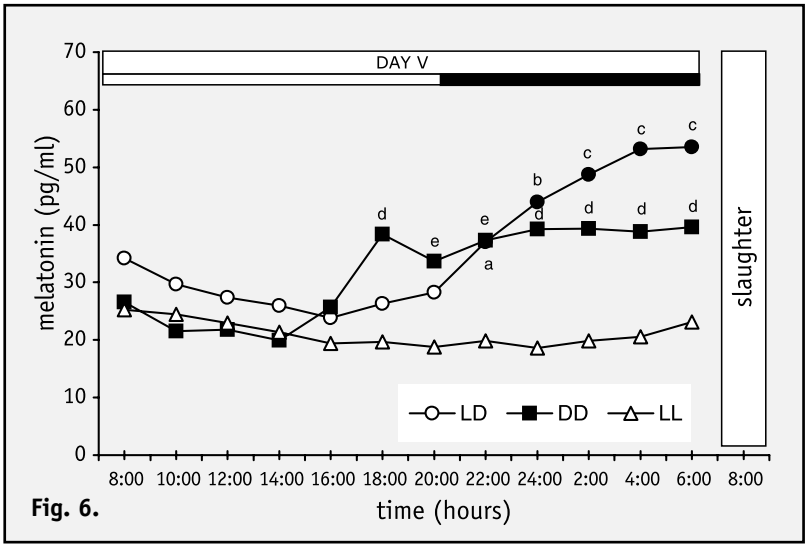

Fig. 6. Geometric means of plasma melatonin concentration in the groups of the pigs kept under light-dark cycle (LD), continuous darkness (DD) and continuous illumination (LL) during day V. Significant differences in the group kept under LD: a —vs. 10:00–20:00 during day V, b—vs. 08:00–20:00 during day V, c—vs. 08:00–22:00 during day V. Significant differences in the group kept under DD: d—vs. 10:00–16:00 during day V, e—vs. 10:00–14:00 during day V. The differences between groups were not significant at all sampling points. Other explanations see Fig. 2.

Discussion

The obtained results demonstrated: (1) the presence of the diurnal changes in plasma melatonin concentration, with increased levels during scotophases, in immature gilts kept under 14 hrs light:10 hrs dark cycle; (2) the low repeatability of the diurnal changes in plasma melatonin level during consecutive days in individual gilts kept under 14 hrs light:10 hrs dark cycle; (3) the presence of the circadian changes with the highest levels during natural nights in pigs kept under continuous darkness; and (4) the lack of the significant changes in plasma melatonin level in gilts kept under continuous illumination.

The most characteristic feature of the melatonin secretion in the domestic pig, observed in the majority of studies, is the presence of the diurnal melatonin rhythm in plasma or serum only under certain light conditions. In the widely cited study of McConnell and Ellendorff [5] night-time rises in plasma melatonin level were observed under 12 hrs light:12 hrs dark conditions and were not noted when the animals were kept under 16 hrs long or 8 hrs long photoperiods.

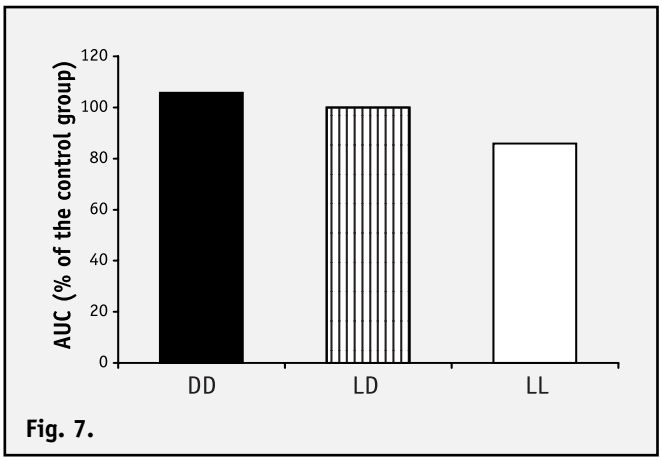

Fig. 7. Means AUC of plasma melatonin profiles in the groups of the pigs kept under light-dark cycle (LD), continuous darkness (DD) and continuous illumination (LL) expressed as percent of the value in the control group.

The hypothesis about the lack of night-time rise in melatonin secretion in pigs kept under both short and long photoperiods was simultaneously supported by the study of Reiter and co-workers [23]. These authors showed no night-time increase in the activity of enzymes associated with melatonin synthesis as well as in melatonin concentration in the pineal gland of pigs killed in winter and summer. Further studies demonstrated the presence of nocturnal rises in serum melatonin in barrows kept under 12 hrs light:12 hrs dark conditions [7] and their lack in gilts kept under short as well as long photoperiods [8, 10]. More recently, the presence of the significant nocturnal elevation in circulating melatonin level was demonstrated by Lewczuk and co-workers in pigs kept under long summer days [16, 24] and by Green et al. [12] in pigs kept under duration of photoperiod varied from 10 to 15 hrs. However, in the other study of Diekman and Green [14] no significant differences in serum melatonin concentration were noted between photophase and scotophase in gilts exposed between 07:30 and 19:00 to artificial illumination with intensity of 700 lx or to direct sunlight. In view of the inconsistent occurrence of the nocturnal rises in the circulating melatonin in the domestic pig observed in different light conditions, the present results showing the significant diurnal rhythm of plasma melatonin in the gilts kept under 14 hrs light:10 hrs dark cycle should be emphasized.

The lack of the night-time rises in circulating melatonin in the domestic pig under certain conditions was analyzed as being related to the insufficient intensity of illumination during daytime [9, 14], the mode of changes in the length of photophase (decrease or increase of photoperiod duration, drastic or stepwise changes in photoperiod) [12] and the sexual maturation of the animals [13, 14]. However, the results obtained until now do not confirm crucial influence of any of these factors on the presence of the nocturnal elevation of circulating melatonin in the domestic pig [12, 13, 14].

The second characteristic feature of melatonin secretion in the domestic pig reported in the majority of studies is high inter-individual variability of the melatonin patterns [5, 7, 10, 12, 15]. Our previous study, in which plasma melatonin level was monitored during 110 hrs in immature gilts kept under 12 hrs light:12 hrs dark cycle, demonstrated large irregularity of the diurnal changes in plasma melatonin during consecutive days [15]. The present results obtained in the control group also confirmed the low consistency in the diurnal patterns of plasma melatonin level in the domestic pig. The low regularity of the diurnal changes in circulating melatonin is the most probable explanation of high pig to pig variability observed in the studies with short-term monitoring of the pineal hormone level. In our opinion, the irregularity of the diurnal changes in plasma melatonin level (which resulted in high variability of both day-time and night-time concentrations of plasma melatonin) together with low amplitude of its nocturnal rise may be responsible, at least in part, for the inability of the detection of nocturnal melatonin rise under some light conditions.

Results opposite to the above presented were obtained by Paterson and co-workers [25], who showed well-entrained plasma melatonin rhythm in pigs kept under both short (8 hrs) and long (16 hrs) photoperiods in Australia. The possible sources of the differences between the results of Paterson's study and the majority of other investigations were widely discussed, but they still remain unknown [12, 26]. In our opinion, the presence and regularity of the diurnal changes in circulating melatonin in the pig may be dependent on genetic factors. In the study performed on gilts obtained from a different herd than in our other experiments, we observed well-entrained and very regular diurnal rhythm of plasma melatonin level during five consecutive days of sampling in five out of six investigated pigs kept under long photoperiod [16]. Such regular diurnal rhythm was not observed by us in other experiments and we suspected that the genotype may influence the function of the circadian timing system in the domestic pig.

In the present study, the significant circadian changes in plasma melatonin concentration with increased levels during natural nights were noted in the pigs kept under continuous darkness. This result may be interpreted as evidence for the endogenous generation of the circadian rhythm of melatonin secretion in the domestic pig. The presence of the circadian rhythm of serum melatonin in pigs kept in conditions of constant light deprivation was previously observed by Griffith and Minton [27].

The present investigation did not address the problem of the participation of non-photic factors in entraining of melatonin secretion [28] in the pigs exposed to continuous darkness. In our study two factors, which may entrain melatonin secretion, should be taken into consideration: animal feeding every day at 09:00 and 14:00 as well as coop cleaning every day at 08:30. However, the studies performed on rodents have shown that the schedule of food availability has a weak influence on circadian rhythm synchronization [29, 30, 31].

It should be noted that there were differences in the duration of "nocturnal" rise in plasma melatonin level between the pigs kept under 14 hrs light:10 hrs dark condition and under continuous darkness. Plasma melatonin level was significantly elevated in the gilts

exposed to 14 hrs light:10 hrs dark cycle between 22:00 to 06:00 and in the pigs kept under continuous darkness between 18:00 and 08:00. The difference in duration of "nocturnal" rise in plasma melatonin may be interpreted as evidence for the role of environmental light in the synchronization of melatonin secretion to light-dark cycle in the domestic pig.

The lack of significant circadian changes in the mean concentration of plasma melatonin in gilts exposed to continuous illumination observed in the present study shows that exposure of pigs to light with intensity 500 lx for nine days abolished the circadian rhythm of the melatonin secretion. Our results obtained in the pigs kept under continuous illumination differ from those reported by Griffith and Minton [27], who showed the presence of the circadian rhythm of serum melatonin in pigs kept in the conditions of constant illumination with intensity of 200 lx. The most likely explanation of these differences is 2.5 times lower intensity of illumination used in the study of Griffith and Minton [27], which may be too low to block melatonin rises during subjective night. According to our data [16], melatonin generation system in the domestic pig is not very sensitive to light during the night, because fluorescent illumination with intensity of 500 lx at animal head, turned on for one night, did not block the nocturnal rise in plasma melatonin.

Griffith and Minton [27] also noted markedly higher serum melatonin level in pigs kept under continuous illumination than in continuous darkness. This result was not confirmed in the present study, which shows no significant differences in the mean concentration of plasma melatonin during the entire sampling period as well as in AUC between the investigated groups of pigs.

Summing up, the presence of the circadian rhythm of plasma melatonin in the gilts kept under continuous darkness and its lack in the animals exposed to continuous illumination suggest that the diurnal rhythm of melatonin secretion in the domestic pig, like in other mammalian species, is generated endogenously and regulated by the environmental light. Low regularity and repeatability of diurnal changes in plasma melatonin levels are specific features of the domestic pig. Further studies are needed to determine which anatomical structure and cellular mechanism of circadian timing system are responsible for this irregularity.

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