SUMMARY

Avian pineal gland, together with retina and suprachiasmatic nuclei, composes the biological clock system generating the diurnal rhythms of many physiological processes and synchronizing them with the external lighting conditions. Besides that, pineal gland rhythmically (with a nocturnal peak) synthesizes melatonin (MEL) - a hormone which, among others, modulates the development and activity of the immune system. To clarify the role of MEL in the previously shown seasonality of the peritonitis development in chicken, a new experimental protocol was introduced, in which the chickens were reared from the day of hatch under the natural photoperiod-like lighting conditions (LD 16:8 in summer and 8:16 in winter) or in continuous illumination (LL) considered as a functional pinealectomy. Additionally, half of animals from LL group were supplemented with exogenous MEL in drinking water (LL MEL) during subjective night, mimicking its natural availability in the respective LD conditions to check whether it will antagonize the effects of LL. In the preliminary part of the study it was shown that the diurnal rhythm of selected clock gene transcription in the pineal gland *(Cry1, Bmal1, Per3* and *E4bp4*) was in both seasons maintained under LD but not in LL conditions. Most probably this effect was not dependent on MEL availability because its supplementation restored rhythmic transcription of *Per3* gene only. MEL synthesized in the pineal gland, however, seemed to influence biological clock functioning in this organ by the autocrine mechanism involving, among others, *Mel1c* receptors [Chapter 3.1].

In the main part of experiments a hypothesis that seasonality of inflammation is related to the MEL presence in the bloodstream and its ability to inhibit migration of the immune cells was verified. The above described experimental protocol was extended by introduction the additional groups of chicken injected ip with thioglycollate (TG) to induce inflammation in all lighting conditions. Peritonitis was evoked at the beginning or towards the end of light phase and the effects were measured 4 hrs later, during the (subjective) day or at (subjective) night. Inflammation intensity was evaluated by leukocyte influx to the peritoneal cavity, transcription of genes encoding cytokines IL-6 and IL-18 in leukocytes from various compartments, and serum lysozyme content, considered as the avian acute phase protein. The obtained results confirmed the

seasonality of chicken peritonitis development, which was more intense in summer than in winter and during the day than at night. These changes were probably not dependent on MEL alone. Additionally, *IL-6* and *IL-18* cytokine gene transcription in circulating leukocytes was not affected by peritonitis, contrary to that in splenocytes where it was elevated and modulated by light and exogenous MEL. The daily differences in the cytokine gene transcription in both types of leukocytes were also demonstrated indicating that under inflammatory conditions *IL-6* gene was transcribed mainly during the daytime while that of *IL-18* at night. These results suggest an involvement of the biological clock in the control of the daily rhythm of chicken immune parameters (leukocytes and cytokines) [Chapters 3.2 and 3.3]. The hypothesis that cytokines released during inflammation may affect the transcription of the clock genes in the chicken pineal gland was also confirmed, and the effect (stimulatory or inhibitory) depends on the season of hatch. In addition, the transcription of the *Per3* gene in the pineal gland can be considered as one of the "contact points" between the molecular clock, MEL and inflammation [Chapter 3.3].

The results obtained in the previous experiments allowed to reject the hypothesis on the major role of MEL in the seasonality of peritonitis in the chickens suggesting, however, an involvement of the biological clock in this phenomenon. The next step of research was therefore, based on the new hypothesis claiming that the seasonal and daily modulation of the chicken immunity may be related to a peripheral molecular clock located directly in the immune organs. A new experimental model was used to examine the circadian rhythms of *Per3* and *E4bp4* clock genes in the thymus of the control and inflamed chickens (injected with TG). Previously described seasonality of the inflammatory response has been confirmed once more. The diurnal rhythm of clock-regulatory element *E4bp4* gene transcription was up-regulated in chickens with inflammation while that of negative element *Per3* appeared independent of the inflammation. These results indicated that peritonitis does not affect the transcription of at least one clock gene in the thymus and observed increase in the mRNA level of the *E4bp4* gene is probably related to its immune function [Chapter 3.4].

In conclusion, seasonal differences in the daily profile of tested inflammatory parameters are unlikely to be dependent entirely on MEL, but may be related to the

functioning of peripheral oscillators in the immune tissues, receiving feedback information about the inflammatory processes through circulating leukocytes and cytokines.