Praca oryginalna *Original paper*

Changes of internal temperature and locomotor activity under the conditions of endotoxin fever, pyrogenic tolerance and its suppression in pigeons

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Summary

The aim of the study was to investigate changes of internal temperature and locomotor activity of birds in conditions of endotoxin fever, pyrogenic tolerance and its suppression. The experiment was performed in pigeons (n = 12). On the first day of the experiment a state of endotoxin fever was evoked. The pigeons were categorized into two groups: experimental and control. The first group of the animals (n = 8) received Escherichia coli LPS intravenously once at the dose of 10 µg/kg b.w. whereas the control pigeons (n = 4) were administered in the apyrogenic saline intravenously once at the dose of 1 ml/kg b.w. On the second and third day of the experiment the state of pyrogenic tolerance was induced in the pigeons and their internal temperature and locomotor activity were investigated. To this end the experimental group of birds received E. coli LPS intravenously twice at 24 h intervals at a dose of 10 µg/kg b.w. Conversely, the control pigeons were twice treated with saline (1 ml/kg b.w.). On the fourth day of the study an attempt of pyrogenic tolerance suppression in the pigeons was carried out. The experimental birds with the stable state of pyrogenic tolerance were then categorized into two subgroups. The first subgroup (n = 4) received Salmonella Abortusequi LPS intravenously at the dose of 10 µg/kg b.w., whereas the second one (n = 4) – E. coli LPS at a double amount of the pyrogen (20 µg/kg b.w.). The control pigeons were administered S. Abortusequi LPS in an analogical dose as the first experimental subgroup.

Results of the study indicated the occurrence of endotoxin fever and depression of locomotor activity of the pigeons in response to the first injection of E. coli LPS. The third administration of the pyrogen stabilized the state of pyrogenic tolerance, manifested by the reduction of the increased internal temperature and the stimulation of pigeon locomotor activity. Whereas in the state of stable pyrogenic tolerance in pigeons the intravenous injection of the other exogenous pyrogen, i.e. S. Abortusequi LPS, and also the double dose of E. coli pyrogen caused the suppression of the tolerance and the restoration of endotoxin fever in the birds.

Keywords: pigeons, endotoxin fever, pyrogenic tolerance

Fever is an immunological (32) and homeostatic (21) reaction of an organism. It is characterized by a rise of internal temperature that is a consequence of temperature control setting (26). Exogenous pyrogens take part in an induction of internal temperature rise (4). Lipopolysaccharide (LPS) is the best known bacterial exogenous pyrogen (16) and it is isolated from various kinds of Gram-negative bacteria genus such as: *Escherichia*, *Salmonella*, *Pseudomonas*, *Vibrio* and the others (11). It constitutes an integral component (70-75%) of the peripheral part of the exterior membrane of Gram-negative bacteria (16). LPS consists of three basic components: lipid A, oligosaccharide of core and antigen O, from which the most important is the first one determining the biological activity and immunostimulating properties of LPS (7). Exogenous pyrogens induce a synthesis and release of endogenous pyrogens (EPs) from host cells such as polymorphnuclear leukocytes (3, 31), monocytes (5, 26), tissue macrophages (1, 10), phagocytic cells of reticulo-

endothelial system (1) and others. EPs belong to different groups of cytokines. The most important are interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (19). However, the pyrogenic function of TNF-α is questionable because it is actually rather treated as an endogenous anti-pyretic (8, 24) or cryogen (6) than pyrogenic cytokine (17).

LPS is responsible for an induction of both thermoregulatory (9, 13, 15, 20, 23, 25, 27-29) and behavioral changes (2, 13) in birds depending on various factors such as pyrogen origin (species of Gram-negative bacteria) (9, 15), dose (13, 20, 23, 27), route (13, 25, 29) and time of pyrogen injection (28, 29), age (9, 15, 20) and genetic type of birds (23).

Tolerance is in exact relation with exogenous pyrogens. Tolerance for these substances is called the pyrogenic tolerance, which depends on the interplay between immunological and neuroendocrinal system (34). The tolerance for LPS is the best known and consists of two phases. The first phase of pyrogenic tolerance (early phase of pyrogenic tolerance) is characterized by a reduction of synthesis and releasing proinflammatory cytokines resulting from changes in an infected cell (7). A stimulation of both cellular (34) and humoral (12) defence of an organism accompanies the early stage of tolerance. However, the second phase of pyrogenic tolerance depends solely on an activation of mechanisms of the humoral defense of an organism (34).

It was confirmed that repeated injections of LPS in some species of birds caused the induction of pyrogenic tolerance manifested with a reduction of raised internal temperature and the stimulation of locomotor activity. The values of those parameters returned to the febrile levels after the suppression of that state. However, it is a little-known problem concerning both thermoregulatory and behavioral changes in the conditions of pyrogenic tolerance and its suppression in pigeons and other species of birds, therefore it requires further complex studies, and a detailed explanation.

Material and methods

The study was performed in adult pigeons (n = 12) with an average body weight of about 300 g, maintained in a stable climatic room (room temperature = 22 ± 1°C, air relative humidity = 60%), and 12 h day/night cycle (the light on at 06:00 a.m. and off at 06:00 p.m.). The birds were kept in individual plastic cages and fed with standard fodder recommended for pigeons with water *ad libitum*. The experiment was approved by the Local Ethics Committee on Animal Experimentation of the Agricultural University of Lublin, Poland.

On the first day of study the state of endotoxin fever was evoked in pigeons and their internal temperature and locomotor activity were recorded. The birds were categorised into two groups: experimental and control. The first group of the animals (n = 8) received intravenously once *Escherichia coli* LPS (Serotype O111:B4, Sigma) at the dose of 10 μg/kg b.w. (10 μg LPS suspended in 1 ml saline). Whereas the control pigeons (n = 4) were administered apyrogenic saline like the above-mentioned group of birds at the dose of 1 ml saline/kg b.w. In both cases the final volume of solutions used was comparable and dependent on the body weight of individual pigeons.

In the second and the third day of experiment the state of pyrogenic tolerance in pigeons was induced and their internal temperature and locomotor activity were investigated. The experimental group of birds received *E. coli* LPS (Serotype O111:B4, Sigma) intravenously twice at a dose of 10 μg/kg b.w. (10 μg LPS in 1 ml saline) at 24 h intervals. Conversely, the control pigeons were twice injected with 1 ml of saline per kg b.w.

On the fourth day of the experiment an attempt of pyrogenic tolerance suppression was made in the experimental pigeons and the changes of their internal temperature and locomotor activity were recorded. The experimental birds from previous day of the experiment (the 3rd d) were categorised into two subgroups. The first subgroup (n = 4) received *Salmonella* Abortusequi LPS (Sigma) at the dose of 10 μg/kg b.w. (10 μg LPS in 1 ml saline), while the second one (n = 4) – *E. coli* LPS (Serotype O111:B4, Sigma) in the amount of 20 μg/kg b.w. (20 μg LPS in 1 ml saline). At the same time, the control pigeons were administered *S.* Abortusequi LPS (Sigma) in an analogical dose to the first experimental subgroup.

During the experiment the both kinds of LPS and saline were intravenously injected into the ulnar vein (*vena ulnaris*) in all pigeons between 09:00 and 09:45 a.m.

Statistical analysis. The obtained results were presented as arithmetic means with standard errors (means ± SEM) after their statistical analysis with the use of Stat View 512 (Abacus Concepts, Berkeley, CA, USA) or Statistica 6.0 software. In order to perform a comparison of several groups against each other the following were used: a variance analysis of Tukey's (for different N), LSD Fisher's or Dunnett's test. Value of $p < 0.05$ was taken for a statistically significant threshold.

The registration method of internal temperature and locomotor activity of pigeons. The measurement of internal temperature and locomotor activity was conducted with the biotelemetry method which depended on an intraperitoneal implantation of previously calibrated VM-FH (MiniMitter, Bend OR, USA) sensors. Before the implantation procedure the sensors were covered with surgical wax and immersed in liquid for sterilization and then in a saline solution. The sensor calibration depended on an adjustment of close waves frequency in temperature of 34 and 38°C. The sensor implantation was conducted in the conditions of general anesthesia which was conducted by a simultaneous intramuscular injection of xylazine and ketamine. After the implantation experimental and control pigeons were placed into individual cages at least 7 d before the start of experiment. In these conditions, after the 7 d of acclimatization, the internal temperature and locomotor activity were registered. The implanted sensors emitted the beacons which were collected through radio aerials of a TR-3000 set. Then the beacons were transmitted to a computer which was fitted with a system of VitalView 3000 data analysis (Mini-

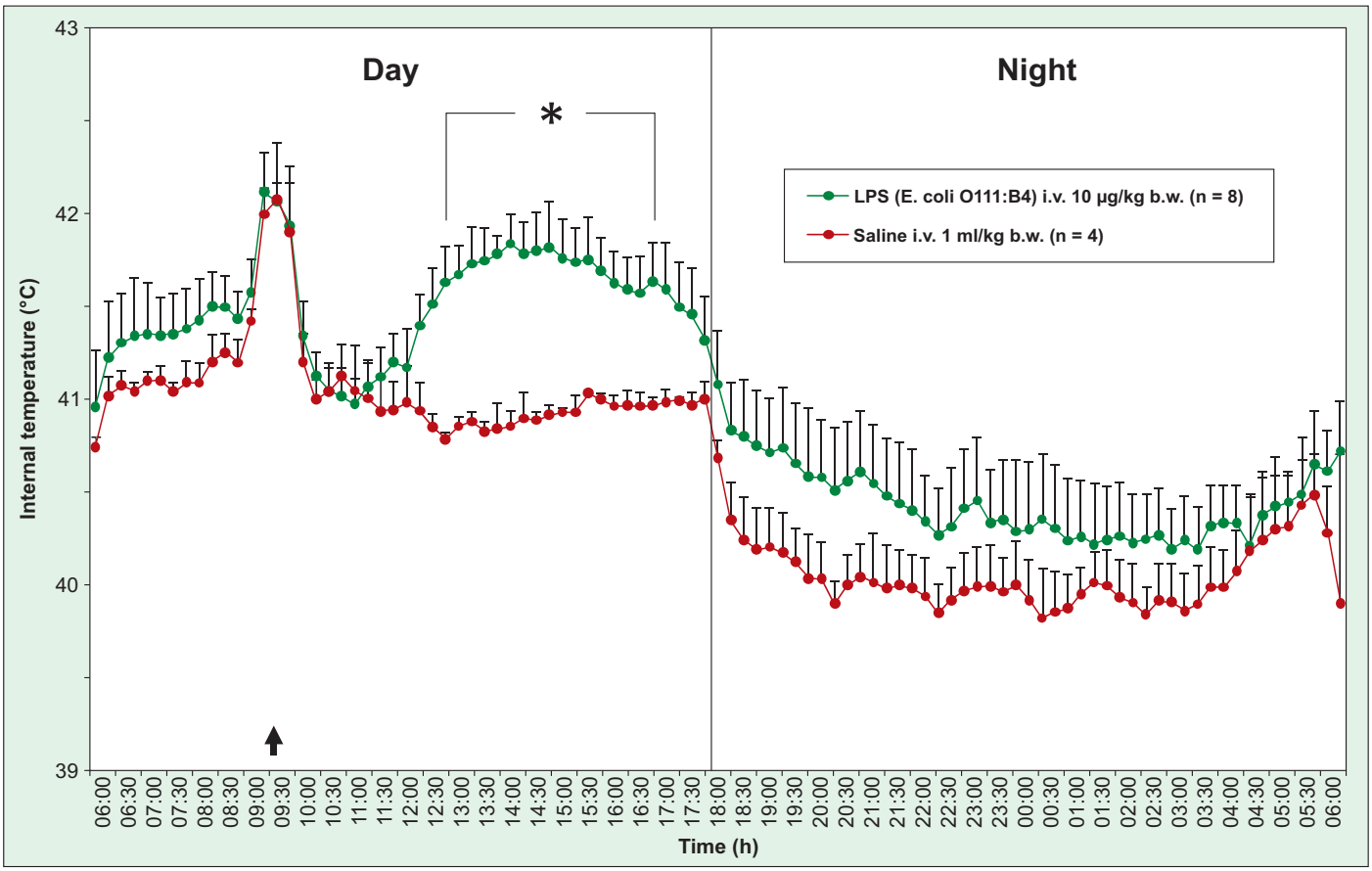

Fig. 1. Changes of internal temperature (means ± SEM) of pigeons in response to the first injection of *E. coli* LPS
Explanations: ↑ – the first injection of LPS or saline; * – statistically significant differences between groups at $p < 0.05$

Mitter, Bend OR, USA). By this computer software the received values of the beacon frequency were converted into the actual internal temperature, which was registered in the form of absolute values accurate up to ± 0.1°C.

The bird locomotor activity measurement was made on the basis of implanted sensor dislocation within three points of radio aerials.

The changes of internal temperature and locomotor activity in pigeons as a result of LPS injection were registered in a 5 min interval during a diurnal cycle (06:00 a.m. – 06:00 a.m.). In order attain better imaging of the received results values of the above-mentioned parameters were averaged over 15, 30 or 60-min.

Results and discussion

The first injection of LPS caused a high fever in pigeons (fig. 1). At the initial period after the injection a small decrease of internal temperature in the experimental birds was noted preceded by a 30-min period of latention, and then its systematic increase was observed. Finally, in comparison with the controls the internal temperature in the pigeons achieved significantly ($p < 0.05$) higher values noted in the afternoon between 0:45 and 4:45 p.m. The peak of the internal temperature was observed at 2:00 p.m. and it reached 41.84 ± 0.16°C. After light-off in the experimental room it rapidly decreased in both groups of examined pigeons. Nevertheless, in the experimental birds at night the internal temperature remained on a higher, but statistically insignificant level in compared with the controls (fig. 1).

When in the experimental birds the locomotor activity examinations were included (fig. 2) other dependences than the above-presented were verified. The first injection of LPS caused its decrease. The locomotor activity in experimental pigeons achieved considerably ($p < 0.05$) lower values in compared with the controls. These differences were recorded between 9:15 and 10:15 a.m., 11:15 and 11:45 a.m., 0:45 and 2:30 p.m., and also at 10:45 a.m. and 4:30 p.m. However, at night higher values of locomotor activity in the experimental birds than in the control one were noted. But these differences were not statistically significant (fig. 2).

The second injection of LPS caused a low intensity fever in pigeons. However, at the beginning of this state (between 10:00 and 11:15 a.m.) immediately after the administration of the injection a short-period fall of internal temperature in the experimental birds was observed in comparison with the controls (fig. 3). Finally, in the pigeons the mean values of the internal temperature were significantly ($p < 0.05$) higher between 0:45 at noon and 2:30 p.m. when compared with the controls. This temperature reached 41.72 ± 0.20°C at 1:30 p.m. Whereas in both groups of birds 45 min before lights-off in the experimental room the decre-

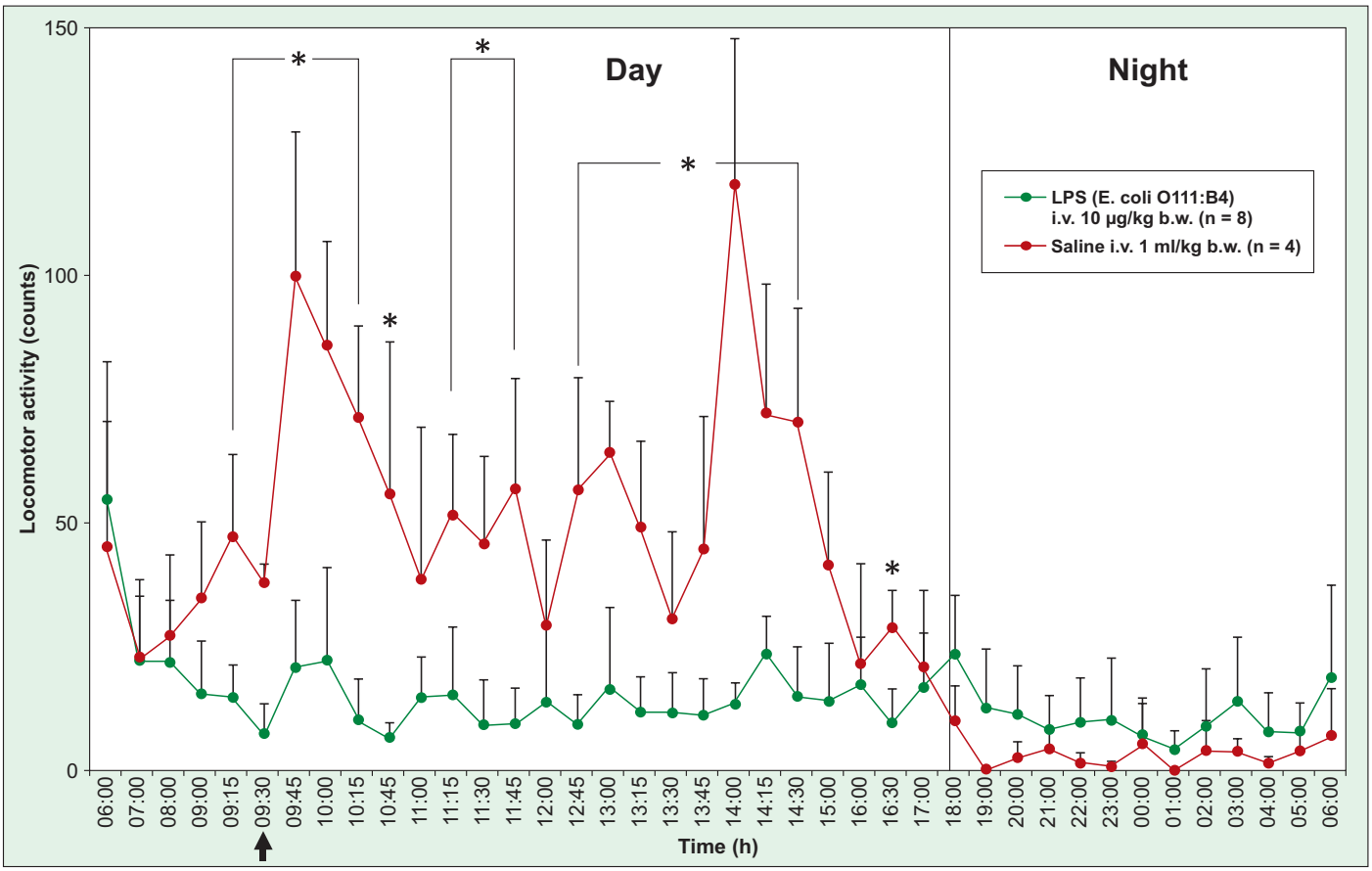

Fig. 2. Changes of locomotor activity (means ± SEM) of pigeons in response to the first injection of *E. coli* LPS
Explanations: as in fig. 1

ase of internal temperature started. However, at night in the experimental pigeons it still had a visibly higher level than in the controls. Moreover, one hour before lights-on in the room a strong rise of the internal temperature in both examined groups of birds was noted (fig. 3).

In response to the second injection of LPS an insignificant fall of locomotor activity in treated pigeons was observed (fig. 4). In spite of this at night it still remained at a higher level in the birds when compared with the control ones.

The third injection of LPS did not cause any fever in pigeons. Immediately after the pyrogen administration a small fall of internal temperature was noted and then its low rise from 41.60 ± 0.23 to 41.87 ± 0.33°C (fig. 5). However, during the day in both groups of birds their internal temperatures maintained a similar level in a range from 40.83 ± 0.22 to 41.61 ± 0.06°C. The observed differences were insignificant. The fall of internal temperature in both examined groups of pigeons preceded and deepened considerably after lights-off in the experimental room. However, in experimental birds the internal temperature stayed on a higher level during the night and reached statistically significant ($p < 0.05$) values at 3:00 and 4:00 a.m. in comparison with the controls. At this time the values averaged respectively: 40.32 ± 0.32 and 40.36 ± 0.31°C. Whereas in the control pigeons their internal temperature achieved 39.57 ± 0.05°C at 3:00 a.m. and 39.73 ± 0.17°C at 4:00 a.m. However, one hour before light-on in the experimental room in both groups of birds a strong rise of internal temperature was noted (fig. 5).

In response to the third injection of LPS there were no significant differences regarding the locomotor activity between both groups of pigeons in the day and at night after the injection (fig. 6). In this period the activity was similar in both groups of animals and oscillated between 0.17 ± 0.25 and 50.08 ± 19.25 IU (fig. 6).

The intravenous injection of *E. coli* LPS in pigeons at the dose of 20 µg/kg b.w. was made at a state of settled pyrogenic tolerance in order to attain its suppression. At the beginning it caused the decrease of internal temperature and then its significant ($p < 0.05$) rise in comparison to the third injection of pyrogen. This administration induced a similar fever in the affected animals as the first injection of LPS (fig. 7). Whereas in response to the injection of *S.* Abortusequi LPS administered for the same purpose at the dose of 10 µg/kg b.w. in pigeons with a settled pyrogenic tolerance, there was a significant ($p < 0.05$) fall of internal temperature observed in comparison with the administration of *E. coli* LPS at the dose of 20 µg/kg b.w. (fig. 7). The *S.* Abortusequi LPS injection subsequently induced a less intense fever and it occurred later reaching 41.61 ± 0.13°C at 3:15 p.m. The charac-

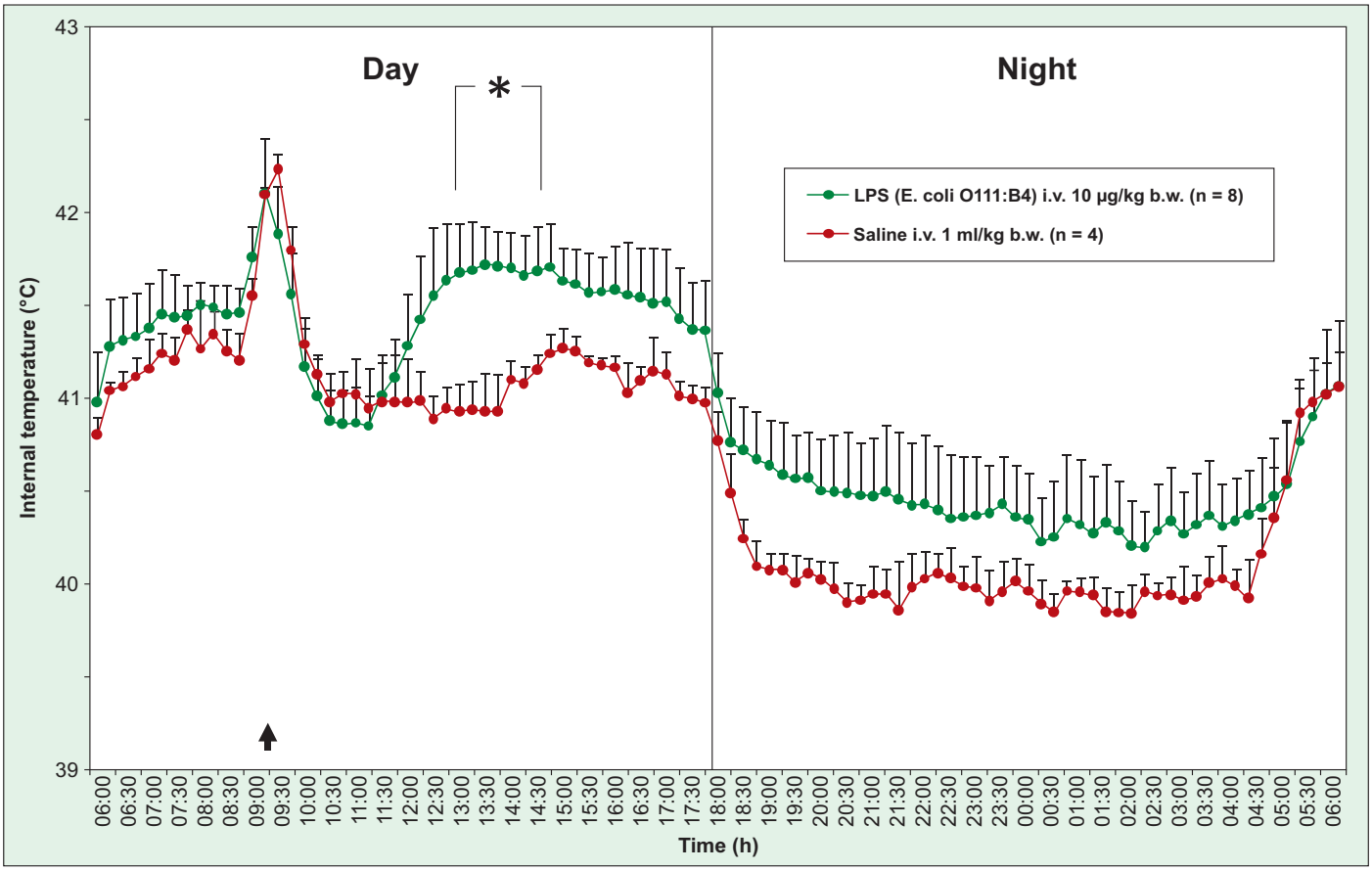

Fig. 3. Changes of internal temperature (means ± SEM) of pigeons in response to the second injection of *E. coli* LPS
Explanations: ↑ – the second injection of LPS or saline; * – statistically significant differences between groups at $p < 0.05$

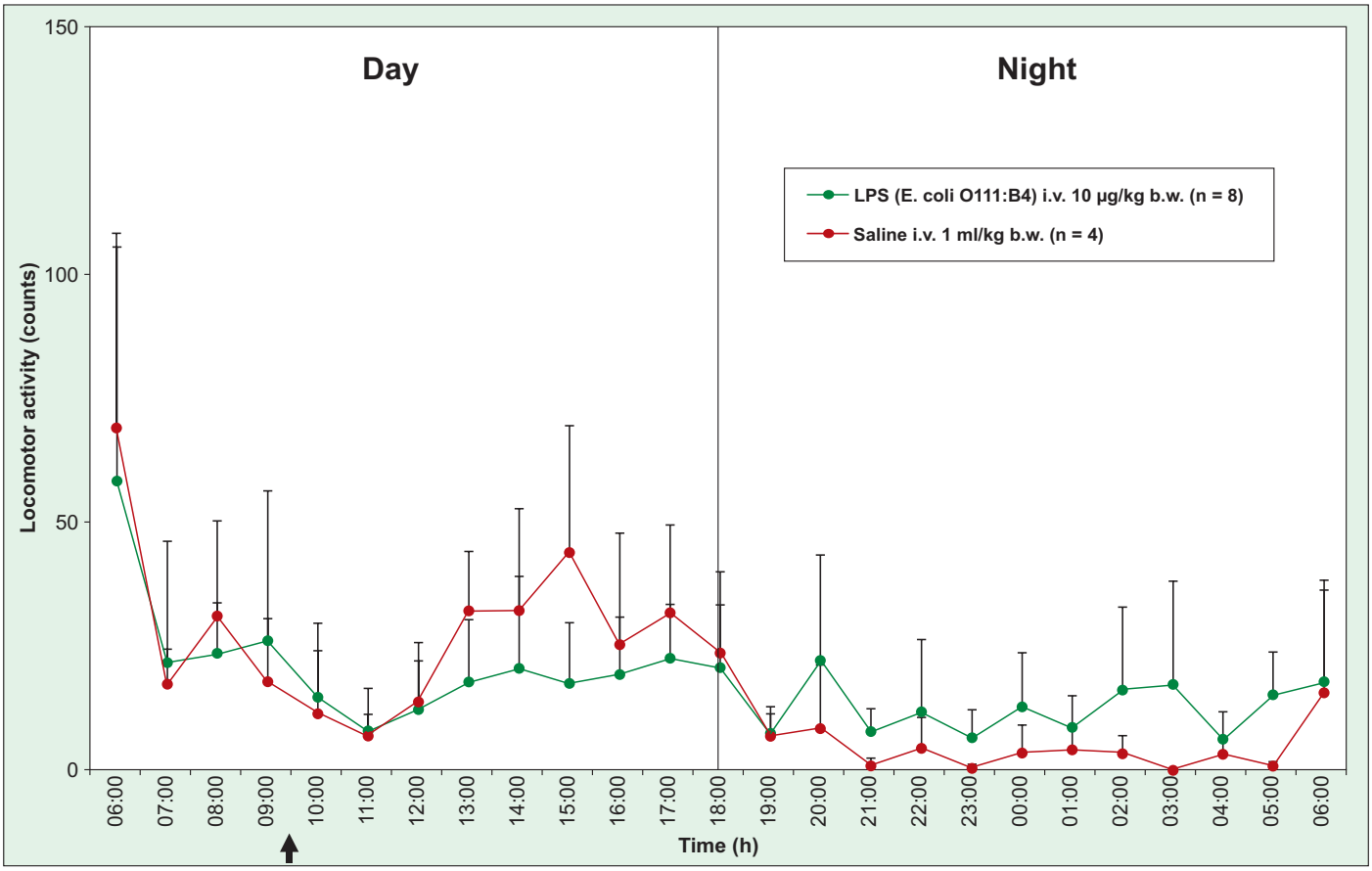

Fig. 4. Changes of locomotor activity (means ± SEM) of pigeons in response to the second injection of *E. coli* LPS
Explanations: ↑ – the second injection of LPS or saline

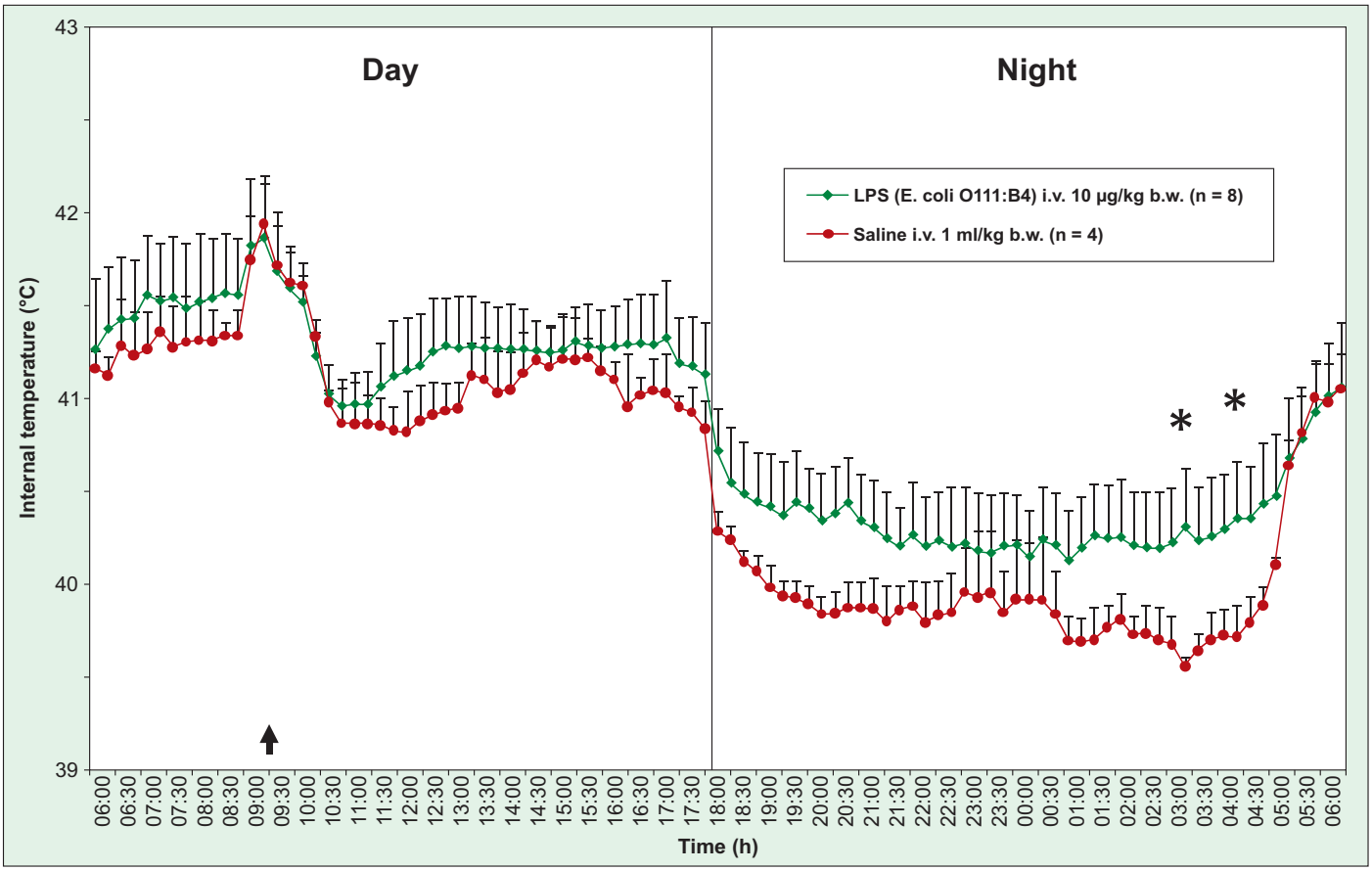

Fig. 5. Changes of internal temperature (means ± SEM) of pigeons in response to the third injection of *E. coli* LPS
Explanations: ↑ – the third injection of LPS or saline; * – statistically significant differences between groups at $p < 0.05$

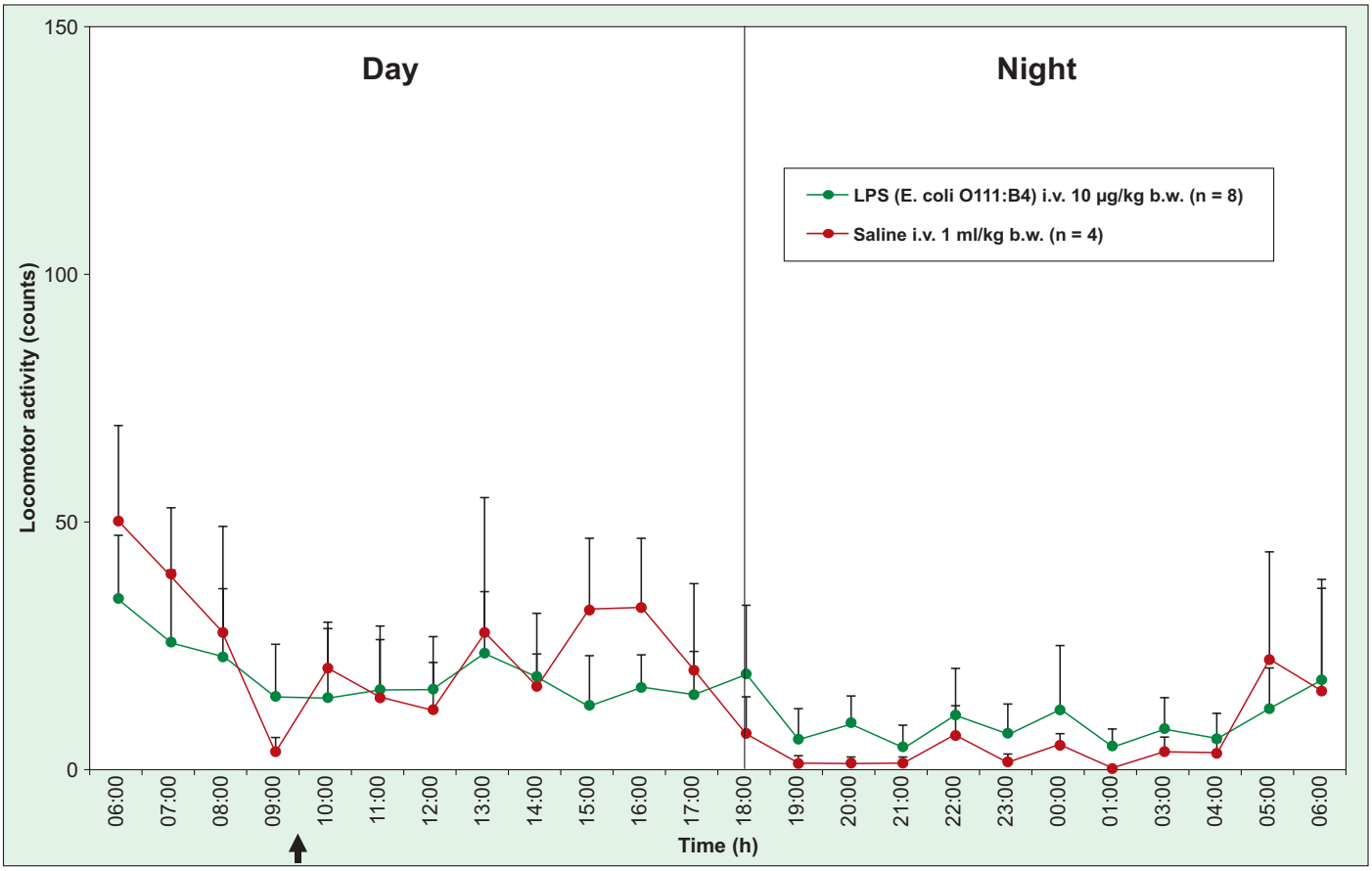

Fig. 6. Changes of locomotor activity (means ± SEM) of pigeons in response to the third injection of *E. coli* LPS
Explanations: ↑ – the third injection of LPS or saline

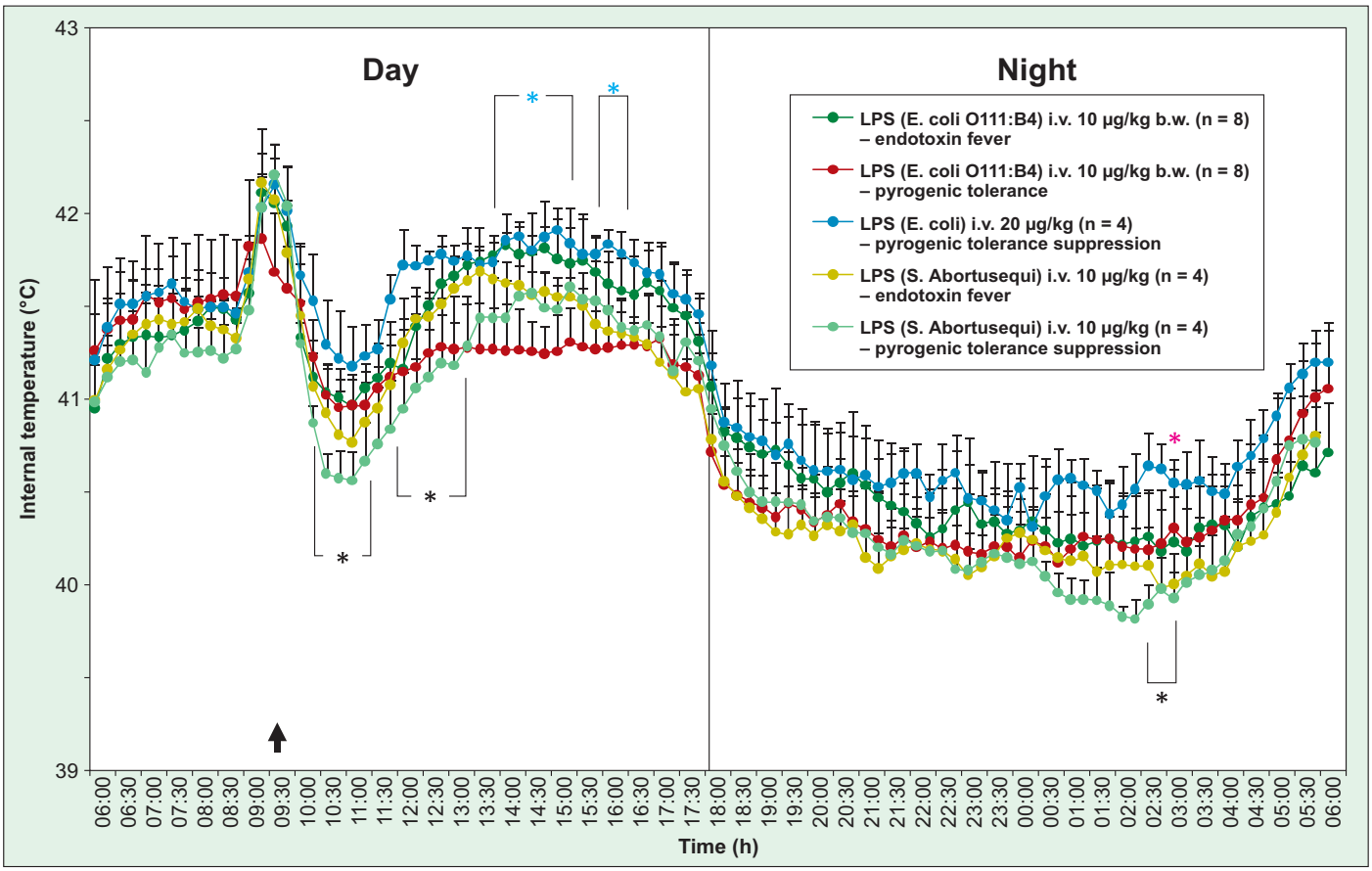

Fig. 7. Changes of internal temperature (means ± SEM) of pigeons after suppression of pyrogenic tolerance
Explanations: ↑ – LPS injections; * – statistically significant differences between *E. coli* LPS (20 µg/kg b.w.) and *S.* Abortusequi LPS group (pyrogenic tolerance suppression) at $p < 0.05$; * – statistically significant differences between *E. coli* LPS (20 µg/kg b.w.) and *S.* Abortusequi LPS group (endotoxin fever) at $p < 0.05$; * – statistically significant differences between *E. coli* LPS (20 µg/kg b.w.) and *E. coli* LPS (pyrogenic tolerance)

ter of the fever was similar to the rise of internal temperature after the pyrogen administration in conditions of endotoxin fever (a maximum value = 41.69 ± 0.13°C at 01:30 p.m.). Whereas the values were visibly higher when compared with those noted after the third administration of *E. coli* LPS. The injections of both kinds of LPS caused for the first 2 hr a fall of internal temperature and then its rise in all the examined birds (fig. 7).

Changes of locomotor activity in pigeons with the suppression of pyrogenic tolerance are presented in fig. 8. It was proved that in the pigeons injected with *S.* Abortusequi LPS for the abolishment of settled pyrogenic tolerance a significant ($p < 0.05$) fall of locomotor activity (after 3 hr) to 5.42 ± 2.71 IU was observed in comparison with the first administration of the pyrogen (36.25 ± 17.54 IU). This state did not change after 4.5 h from the injection when the mean value of locomotor activity was 11.42 ± 5.08 IU and 58.33 ± 25.17 IU as a result of the first administration of *S.* Abortusequi LPS, respectively. However, these differences were statistically significant ($p < 0.05$). Generally, the locomotor activity in birds after the first injection of *S.* Abortusequi LPS remained for a day and night at a higher level when compared with the administration of this LPS for pyrogenic tolerance suppression, and also with the groups of pigeons receiving *E. coli* LPS. During the day statistically significant differences between those groups of pigeons were observed 3 h (36.25 ± 17.54 IU) and 4.5 h (58.33 ± 25.17 IU) after the administration, and at night 10.5 h (23.25 ± 10.88 IU), 11 h (20.92 ± 13.38 IU), 11.5 h (29.08 ± 9.25 IU) and 19.5 h (26.92 ± 14.25 IU) afterward (fig. 8).

Thermoregular mechanisms in birds constitute an interesting investigative subject. Lipopolysaccharide (LPS) belongs to the most often used exogenous pyrogens in animals. In response to its administration a number of changes accompanied with fever and pyrogenic tolerance have been observed in different species of animals. One of the most important is thermoregularity and principles of its function in host organisms during the pyrogen action. Both peripheral and central LPS injections cause fever. Usually it has different courses depending on many factors such as a pyrogen origin (e.g. species of Gram-negative bacteria) (9, 15), age (9, 15, 20) and genetic type of birds (23), dose (13, 20, 23, 27), route (13, 25, 29) and time of pyrogen injection (28, 29). The results of our own studies demonstrated the significant ($p < 0.05$) increase of internal temperature of pigeons in response to intravenous injection of *E. coli* LPS (Serotype O111:B4,

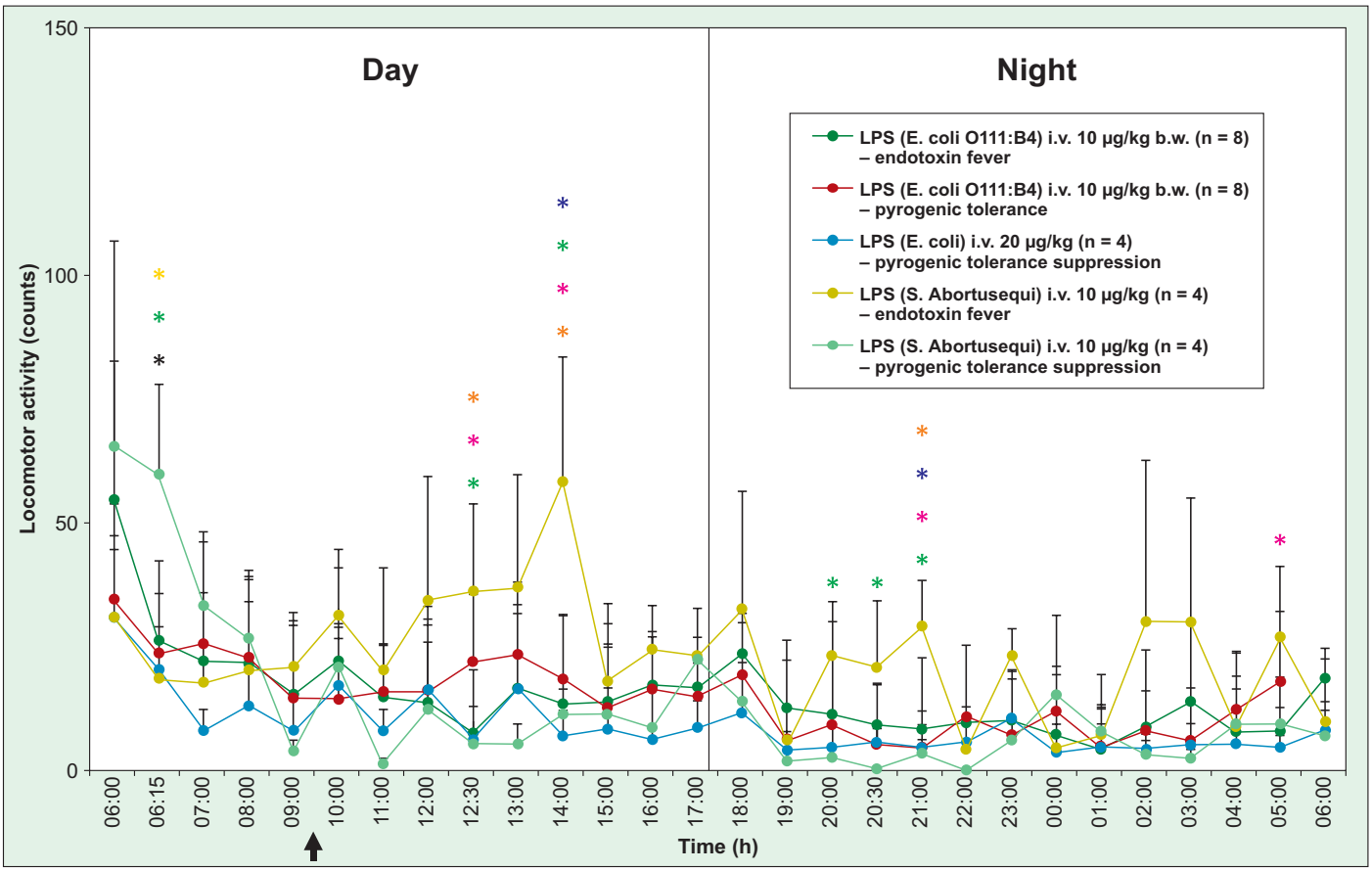

Fig. 8. Changes of locomotor activity (means ± SEM) of pigeons after suppression of pyrogenic tolerance
Explanations: ↑ – LPS injections * – statistically significant differences between *E. coli* LPS (20 µg/kg b.w.) and *S.* Abortusequi LPS group (pyrogenic tolerance suppression) at $p < 0.05$; * – statistically significant differences between *E. coli* LPS (20 µg/kg b.w.) and *S.* Abortusequi LPS group (endotoxin fever) at $p < 0.05$; * – statistically significant differences between *S.* Abortusequi LPS (pyrogenic tolerance suppression) and *E. coli* LPS group (pyrogenic tolerance); * – statistically significant differences between *S.* Abortusequi LPS (endotoxin fever) and *S.* Abortusequi LPS group (pyrogenic tolerance suppression) at $p < 0.05$; * – statistically significant differences between *S.* Abortusequi LPS (endotoxin fever) and *E. coli* LPS group (endotoxin fever); * – statistically significant differences between *S.* Abortusequi LPS (endotoxin fever) and *E. coli* LPS group (pyrogenic tolerance)

Sigma). Our results are analogical to those obtained by Nomoto (28-30) who used the same pyrogen, dose and route of administration in pigeons.

After the injection Nomoto noted the significant increase of the internal temperature of the birds preceded a short period of latention (28-30). In other species of birds, such as chickens (13, 14, 25) and Peking ducks, similar changes were also affirmed (27). However, in the above-presented birds a fall of internal temperature preceded the fever. In this case the fever peak was 270.00 ± 34.15 min after the pyrogen injection and achieved 41.84 ± 0.16°C. Whereas Nomoto's other experiment (28) the peak was noted at 295.00 ± 13.50 min and at that time internal temperature averaged 42.91 ± 0.21°C. Similar results were also obtained Nomoto's second study (30). In this investigation the highest internal temperature was measured 5 h after the administration of *E. coli* LPS and it averaged 42.37 ± 0.12°C. Generally, the higher values of internal temperature noted in Nomoto's experiments probably resulted from time of pyrogen injection because it was made at 1:00 p.m. (28, 30). On the other hand, the maximal fall in temperature was recorded at 6:00 p.m. Whereas in our own study the injection of LPS was performed at 9:30 a.m., and the maximum values of internal temperature were noted at 2:00 p.m. In contrast, at an analogical time there were observed the highest values of internal temperature in the physiological conditions (28) or in control animals (30) which received the placebo. A probable cause of those differences was the time of light on and off in an experimental room because in our own studies they were at 6:00 a.m. and 6:00 p.m. which was three hours earlier than in Nomoto's experiments (09:00 a.m. and 09:00 p.m.) (28, 30). A difference in the control group was the final possible cause. In the case of our own studies it constituted newly-ushered birds which received apyrogenic saline. Whereas in the one of Nomoto's study (28) there were the same pigeons as the experimental ones without the administration of LPS, and internal temperature was registered for two days before pyrogen injection. On the other hand, in the other study of Nomoto (30) in which the control group constituted independent pigeons assigned only for this aim, the peak of internal temperature achieved similar values as in our own studies. Moreover, our

own results also demonstrated that the characteristic fall of internal temperature in conditions of endotoxin fever preceded the night-time. This decrease deepened after the lights off and was in accordance with the physiological curve of internal temperature. Nomoto also obtained similar data (28-30). Nomoto's results and our own study indicated higher values of internal temperature in birds during the life conditions imitated the night. Moreover, the characteristic rise of internal temperature preceded the light on in the experimental room was also demonstrated. The increase was registered as well in the physiological conditions and in control animals both in our own study and other authors too (28-30).

LPS is responsible for behavioral changes in birds. Our own results indicated a significant ($p < 0.05$) depression of locomotor activity in conditions of endotoxin fever of pigeons. The above-mentioned results were in an accordance with those noted in chickens which became recumbent in response to *E. coli* injection (2). Other behavioral changes also accompany endotoxin fever, such as: hypophagia (2, 13), anorexia (13), adipsia or increase of somnolence (2, 13).

Repeated administrations of LPS in birds resulted in a state of tolerance for this pyrogen. Our own studies pointed to a low intensity fever after the second *E. coli* injection in pigeons. Whereas, the third administration of this pyrogen did not evoke the fever in birds. Similar observations were recorded in Japanese quail (*Coturnix coturnix japonica*) at 5 hr after *Salmonella* Typhimurium injection (20). In response to the second administration of this pyrogen there were registered significantly ($p < 0.01$) lower values of internal temperature in comparison with the first injection (20). Whereas after the third LPS administration, those values remained on a similar level as the controls (20). The reduction of internal temperature probably resulted from a depression of pyrogenic cytokine synthesis (7), because in the conditions of LPS tolerance a stimulation of hypothalamo-pituitary-adrenal axis and production of glucocorticosteroids occur (34). This activation caused on the way of nuclear factor kB expression (20) leads to the decrease of cytokine synthesis responsible for febrile increase of internal temperature (7). On the other hand, the depression of febrile reaction also observed during the pyrogenic tolerance depends on an endogenous antipyresis mechanism which „protects an organism from a life threatening temperature rise" (18). In these conditions a cerebral activity of arginine vasopressin is switched on, and it is probably responsible for an endogenous antipyresis (38).

The behavioral changes caused by LPS reflect a state of pyrogenic tolerance. Results of our own studies indicated that the second pyrogen injection evoked a lower decrease of locomotor activity in pigeons than its first administration. Whereas in response to the third injection of LPS at night the locomotor activity was similar to the controls.

In our own study the attempt of *E. coli* LPS tolerance suppression was made using a double dose of the same pyrogen or a comparable amount of LPS derived from another kind of Gram-negative bacteria (*S.* Abortusequi). Our results indicated that birds with settled pyrogenic tolerance had „a normal" reactivity to the next injection of LPS. It was manifested with typical changes of endotoxin fever, i.e. a rise of internal temperature and depression of locomotor activity. In response to the administration of *S.* Abortusequi LPS in pigeons with the pyrogenic tolerance the occurrence of fever was observed, in contrast to the injected animals without previous administrations of LPS. This was probably connected with the time injection of pyrogen (30 min later) in the case of the first discussed group of animals. Whereas at night in these animals there were observed similar and sometimes lower values of internal temperature. They were noted both after the settlement of pyrogenic tolerance, and also in the conditions of fever in response to *S.* Abortusequi injection. It is worth mentioning that the suppression of pyrogenic tolerance for *E. coli* LPS was more intensive in response to the double *E. coli* injection than after *S.* Abortusequi administration, it was especially more visible regarding the changes of internal temperature. In the first group of pigeons the earlier rise and higher values of internal temperature were observed for the whole duration of the experiment if it would be compared with *E. coli* LPS administration at a dose of 10 μg/kg b.w. Those changes occurred the most intensively immediately after the pyrogen injection.

On the other hand, dependences with respect to the pigeon locomotor activity developed rather differently. In response to *S.* Abortusequi injection in animals with the settled pyrogenic tolerance the intense depression of locomotor activity within the first hour after the pyrogen administration was observed, with reference to the third injection of LPS. That depression was still noted during the day in spite of unequivocal rise of locomotor activity in birds. Whereas the *E. coli* injection made for the settlement of pyrogenic tolerance finally caused the reduction of locomotor activity in pigeons in comparison with the third LPS injection. At night both *E. coli* and *S.* Abortusequi LPS injections in pigeons with pyrogenic tolerance caused the distinct reduction of locomotor activity, especially for the second discussed group of animals. In contrast, in the state of endotoxin fever the intense rise of locomotor activity in animals when compared with the third injection of LPS was observed. A lack of the possibility of tolerance transmission between different exogenous pyrogens was known earlier (36, 37). It also was confirmed that the state is possible to transfer only between pyrogens which belong to the same class (a specific character of tolerance). For example in rabbits this possibility has been recorded when *E. coli* LPS was injected in a comparable dose like another pyrogen from Gram-negative bacteria (*S.* Abortusequi) which was administered at a fourfold dose (36).

Different results in our own studies probably resulted from specific changes that depended on the kind of pyrogen and amount of repeations of its injection for the induction of pyrogenic tolerance. Probably it exerted an influence on the level of neuronal signal activation which informs a brain about the actual situation on a periphery (33). It constitutes the basis of the probable explanation for our own observations regarding the more intense febrile response to *E. coli* LPS in animals with settled pyrogenic tolerance. To date it is not known which factor is responsible for the activation of neuronal signals. On the other hand, the occurrence of peripheral tolerance and the possibility of its suppression presented in our own studies demonstrate the constant reactivity of the brain on injections of „new" pyrogen (35). Moreover, it has probably resulted from the lack of pyrogenic tolerance transmission between the brain and a periphery (22).

Changes of internal temperature and locomotor activity associated with endotoxin fever of pigeons constitute one of the visible proofs of an organism's defence against the harmful influence of LPS. On the other hand, the reduction of internal temperature rise and partial return of physiological locomotor activity in conditions of pyrogenic tolerance of the animals indicate the increase of pathogenic factor elimination in the affected organisms. Conversely, the suppression of pyrogenic tolerance in the pigeons effectively restores the state of endotoxin fever. It visibly confirms the lack of tolerance transmission even between pyrogens belonging to the same class.

References

1. *Atkins E., Bodel P., Francis L.*: Release of an endogenous pyrogen in vitro from rabbit mononuclear cells. J. Exp. Med. 1967, 126, 357-384.
2. *Baert K., De Boever S., Duchateau L., De Backer P.*: Sodium salicylate attenuates lipopolysaccharide (LPS)-induced adipsia, but not hypophagia, in broiler chickens. Br. Poult. Sci. 2005, 46, 144-148.
3. *Berlin R. D., Wood W. B. Jr.*: Studies on the pathogenesis of fever XIII. The effect of phagocytosis on the release of endogenous pyrogen by polymorphonuclear leukocytes. J. Exp. Med. 1964, 119, 715-726.
4. *Blatteis C. M., Sehic E.*: Fever: How may circulating pyrogens signal the brain? News Physiol. Sci. 1997, 12, 1-9.
5. *Bodel P.*: Studies on the mechanism of endogenous pyrogen production III. Human blood monocytes. J. Exp. Med. 1974, 140, 954-965.
6. *Derijk R. H., Berkenbosch F.*: Hypothermia to endotoxin involves the cytokine tumor necrosis factor and the neuropeptide vasopressin in rats. Am. J. Physiol. 1994, 266, R9-R14.
7. *Dudek K.*: Wpływ lipopolisacharydu na wystąpienie i przebieg gorączki, kształtowanie się tolerancji pirogenowej oraz wskaźniki immunologiczne i zapalne u gołębi. Praca dokt., Wydział Medycyny Weterynaryjnej AR, Lublin 2007.
8. *Gourine A. V., Leon L. R., Rudolph K., Tesfaigzi J., Kluger M. J.*: Cytokine cascade induced by endotoxin in TNF double receptor knockout mice: evidence supporting a role for IL-10 in mediating antipyretic action of TNF. J. Therm. Biol. 2000, 25, 21-27.
9. *Gregorut F. P., Baptista L. C., Paulim A. S., Guerreiro J. R., Secato E. R., Macari M.*: Influence of age on the febrile response to E. coli and S. typhimurium endotoxins in growing pullets. Br .Poult. Sci. 1992, 33, 769-774.
10. *Hahn H. H., Char D. C., Postel W. B., Wood W. B. Jr.*: Studies on the pathogenesis of fever XV. The production of endogenous pyrogen by peritoneal macrophages. J. Exp. Med. 1967, 126, 385-394.
11. *Henderson B., Wilson M.*: Cytokines induction by bacteria: beyond lipopolysaccharide. Cytokine 1996, 8, 269-282.
12. *Jenkin C. R., Rowley D.*: The role of opsonins in the clearance of living and inert particles by cells of the reticuloendothelial system. J. Exp. Med. 1961, 114, 1223-1236.
13. *Johnson R. W., Curtis S. E., Dantzer R., Bahr J. M., Kelley K. W.*: Sickness behavior in birds caused by peripheral or central injection of endotoxin. Physiol. Behav. 1993, 53, 343-348.
14. *Johnson R. W., Curtis S. E., Dantzer R., Kelley K. W.*: Central and peripheral prostaglandins are involved in sickness behavior in birds. Physiol. Behav. 1993, 53, 127-131.
15. *Jones C. A., Edens F. W., Denbow D. M.*: Influence of age on the temperature response of chickens to Escherichia coli and Salmonella typhimurium endotoxins. Poult. Sci. 1983, 62, 1553-1558.
16. *Kaszowska M.*: Chemical stucture and biosynthesis of lipopolysaccharide – important component of the cell envelope of Gram-negative bacteria. Post. Hig. Med. Dosw. 2004, 58, 333-342.
17. *Kawasaki H., Moriyama M., Ohtani Y., Naitoh M., Tanaka A., Nariuchi H.*: Analysis of endotoxin fever in rabbits by using a monoclonal antibody to tumor necrosis factor (cachectin). Infect. Immun. 1989, 57, 3131-3135.
18. *Kluger M. J.*: Fever: role of pyrogens and cryogens. Physiol. Rev. 1991, 71, 93-127.
19. *Kluger M. J., Kozak W., Leon L. R., Soszynski D., Conn C. A.*: Cytokines and fever. Neuroimmunomodulation 1995, 2, 216-223.
20. *Koutsos E. A., Klasing K. C.*: The acute phase response in Japanese quail (Coturnix coturnix japonica). Comp. Biochem. Physiol. C Toxicol. Pharmacol. 2001, 128, 255-263.
21. *Kozak W.*: Fever as a homeostatic process accompanying infection. Post. Hig. Med. Dosw. 1992, 46, 67-91.
22. *Kozak W., Soszynski D., Szewczenko M., Bodurka M.*: Lack of pyrogenic tolerance transmission between brain and periphery in the rabbit. Experientia 1990, 46, 1010-1011.
23. *Leshchinsky T. V., Klasing K. C.*: Divergence of the inflammatory response in two types of chickens. Dev. Comp. Immunol. 2001, 25, 629-638.
24. *Long N. C., Otterness I., Kunkel S. L., Vander A. J., Kluger M. J.*: Roles of interleukin 1 beta and tumor necrosis factor in lipopolysaccharide fever in rats. Am. J. Physiol. 1990, 259, R724-R728.
25. *Macari M., Furlan R. L., Gregorut F. P., Secato E. R., Guerreiro J. R.*: Effects of endotoxin, interleukin-1 beta and prostaglandin injections on fever response in broilers. Br. Poult. Sci. 1993, 34, 1035-1042.
26. *Mackowiak P. A., Bartlett J. G., Borden E. C., Goldblum S. E., Hasday J. D., Munford R. S., Nasraway S. A., Stolley P. D., Woodward T. E.*: Concepts of fever: recent advances and lingering dogma. Clin. Infect. Dis. 1997, 25, 119-138.
27. *Maloney S. K., Gray D. A.*: Characteristics of the febrile response in Pekin ducks. J. Comp. Physiol. B 1998, 168, 177-182.
28. *Nomoto S.*: Diurnal variations in fever induced by intravenous LPS injection in pigeons. Pflügers Arch. – Eur. J. Physiol. 1996, 431, 987-989.
29. *Nomoto S.*: LPS fever in pigeons. Ann. N. Y. Acad. Sci. 1997, 813, 508-511.
30. *Nomoto S.*: Role of prostaglandin E2 and indomethacin in the febrile response of pigeons. Jpn. J. Physiol. 2003, 53, 253-258.
31. *Nordlund J. J., Root R. K., Wolff S. M.*: Studies on the origin of human leukocytic pyrogen. J. Exp. Med. 1970, 131, 727-743.
32. *Roberts N. J. Jr.*: Fever: Basic Mechanisms and Management. Raven Press. Ltd., New York 1991, pp. 125-142.
33. *Roth J., deSouza G. E. P.*: Fever induction pathways: evidence from responses to systemic or local cytokine formation. Braz. J. Med. Biol. Res. 2001, 34, 301-314.
34. *Soszyński D.*: Mechanisms for development of tolerance to repeated injections of exogenous pyrogens. Post. Hig. Med. Dośw. 2000, 54, 537-551.
35. *Soszyński D.*: Mechanizmy indukowania gorączki w stanach pobudzenia emocjonalnego. Praca hab., Wydział Lekarski AM, Bydgoszcz 2002.
36. *Soszyński D., Kozak W., Szewczenko M.*: Course of fever response to repeated administration of sublethal doses of lipopolysaccharides, polyinosinic:polycytidylic acid and muramyl dipeptide to rabbits. Experientia 1991, 47, 43-47.
37. *Soszyński D., Krajewska M.*: Lack of cross tolerance between pyrogenic effects of LPS and turpentine in rats. J. Therm. Biol. 2002, 27, 229-237.
38. *Wilkinson M. F., Kasting N. W.*: Centrally acting vasopressin contributes to endotoxin tolerance. Am. J. Physiol. 1990, 258, R443-R449.

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