The study was performed on 10 stallions of Polish Primitive Horses divided into two groups: CONTROL (n=3), and ENDO (n=7) – subjected to Escherichia coli LPS injection in the dose of 0.3 \( \mu \)g/kg b.w. The study was meant to determine the concentration of interleukine-1\( \beta \) (IL-1\( \beta \)) in blood serum. Rectal temperature (Tr), scrotal skin temperature (Tss), and heart (HR) and respiratory (RR) rates were also measured. A statistically significant increase in IL-1\( \beta \) concentration was noticed in 3-4 h (maximum in 3 h, \( \Delta \)IL-1\( \beta \)- 24.1 pg/ml) after LPS administration. In endotoxin-treated stallions alterations in clinical signs of the disease were significant and characteristic of endotoxaemia (increased Tr, HR, RR). An increase in Tss was observed within 2-6 h (maximum in 3 h, \( \Delta \)TSS-1.7\( ^\circ \)C) after infusion of the endotoxin.

Key words: stallions, endotoxin, interleukin-1\( \beta \), clinical signs.

Lipopolysaccharide (LPS) is an active component of Gram-negative bacterial cell walls known as endotoxin. Lipopolysaccharide is a strong stimulator of many specific and non-specific organism reactions. Cytokines, among which the most important are tumour necrosis factor-\( \alpha \) (TNF-\( \alpha \)), IL-6 and interleukin-1 which is referred to as IL-1\( \alpha \) and IL-1\( \beta \), are modulators of LPS activity (6, 7).

Interleukin-1 influences immunological processes and circulatory system and also manifests proinflammatory properties. IL-1 and TNF-\( \alpha \) play a major role in inflammation coordinating mechanisms (5). IL-1, like TNF has the ability to induce haemodynamic and haematological changes typical of septic shock (19, 20). The influence of IL-1 on immunological processes is related mainly to T and B lymphocyte activation. This cytokine (together with IL-8) is also a chemotactic factor influencing granulocytes. IL-1 causes neutrophilia; however, an increase in the number of neutrophils is preceded by a neutropenia phase. Influencing the IL-6 synthesis (just like TNF-\( \alpha \)), causes hepatocytes to produce acute phase proteins (5). IL-1 together with TNF-\( \alpha \) and IL-6 are the best known and understood endogenous pyrogens which induce temperature reaction by affecting the thermoregulatory center (9).

Cytokines play an important role in the intercellular communication. The role of Interleukin-1 system and other cytokines in reproduction physiology and pathology has been described (14, 22, 24, 25). Another study indicated that the sexual steroids could be endogenous regulators with relations to IL-1 (11, 15, 21). It is possible that reactions to endotoxin in sexually active subjects could be specific.
It has been demonstrated that endotoxin administration in horses causes an increase in TNF-α and IL-6 blood concentration as well as many changes in metabolism and cardiopulmonary performance. Clinical signs of febrile reactions can also be noticed (1, 2, 4, 12, 13, 16, 17). However, there are no data concerning the influence of LPS injection on the level of interleukin-1β in the blood of horses.

The purpose of this study was to determine the effects of endotoxin treatment on the level of interleukin-1β in blood serum with reference to clinical signs, especially to scrotal skin temperature in stallions.

**Material and Methods**

**Animals.** Ten clinically healthy Polish Primitive Horse (PPH) stallions aged 4 - 14 years and 280-400 kg b.w. were used in the study that was carried out in the reproductive season (April - July). The stallions were divided into two groups: CONTROL (n=3) and ENDO (n=7).

**Endotoxin.** Freeze-dried lipopolysaccharide from *Escherichia coli* serotype 055:B5 (Sigma) was used. This endotoxin was given intravenously to the experimental stallions (ENDO) after dissolution in 500 mL of pyrogenic physiological saline solution (0.9 % NaCl, Polfa). Horses were infused with the dose of 0.3 µg/kg b.w. of LPS. The control stallions were infused with normal saline solution as the experimental group.

**Clinical examinations.** Clinical examinations comprised observations of animals, rectal (Tr) and scrotal skin temperature (Tss), and heart (HR) and respiratory (RR) rates were also measured. The scrotal skin temperature was measured using electronic thermometer which was kept (1 min) in the middle, between left and right part of the scrotum. These measurements were performed immediately before infusion (marked as time 0) and 1, 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 h thereafter. The study was made in a stable where the mean temperature amounted - 16.8°C, humidity - 69.4% and air pressure - 766.2 mm Hg.

**Blood sampling.** The whole blood was taken from the jugular vein with the use of catheter (Secalon® Kathy 1, Viggo). Blood was collected into evacuated clot tubes. Serum samples were stored at -70°C.

**Interleukin assay.** The interleukin-1β concentration was determined with the use of the immunoassays kit for human interleukin-1β, IL-1β-IRMA (Medgenix, Biosource). Analyses of molecular cloning of equine interleukin-1β cDNAs demonstrated that amino acid sequence of equine IL-1β showed 66.7% similarity with that human IL-1β (8).

**Statistical analyses.** The data were analyzed statistically using the Statistica Stat Soft PL program with ANOVA variance analysis. The mean values were compared using Fisher’s test. The differences were statistically significant at P<0.05.

**Results**

**Interleukin-1β level.** The results presented in Fig. 1 show that only the stallions from ENDO group demonstrated a statistically significant increase in the IL-1β concentration in 3-4 (maximum 3 h, ΔIL-1β < 24.1 pg/ml) after endotoxin administration.
Clinical examinations. In stallions receiving LPS, a statistically significant increase in rectal and scrotal skin temperature was observed within 1 to 7 h and 2-6 h after administration of the endotoxin, respectively. In group ENDO the maximum increase in rectal temperature was up to 39.2°C, $\Delta T_{tr}$=2.1°C (Fig. 2) and in scrotal skin temperature to 34.5°C, $\Delta T_{ss}$=1.7°C (Fig. 3).

Fig. 1. Interleukin-1$\beta$ in blood serum (mean ± S.D).

*-significant differences compared to the time 0, a:b - significant differences between groups, at P<0.05.

Fig. 2. Rectal temperature (mean ± S.D).

*-significant differences compared to the time 0, a:b- significant differences between groups, at P<0.05.
In response to endotoxin infusion, stallions’ reaction was also increased HR in 1-3 h, maximum in 2 h, $\Delta_{HR}$ 23.1 beats/min (Fig. 3) and RR in 1-6 h, maximum in 2 h, $\Delta_{RR}$-13.8 breaths/min (Fig. 4).

**Fig. 3.** Scrotal skin temperature (mean ± S.D).

*-significant differences compared to the time 0, a:b- significant differences between groups, at P<0.05.

**Fig. 4.** Heart rate (mean ± S.D).

*-significant differences compared to the time 0, a:b- significant differences between groups, at P<0.05.
Discussion

The present studies show that after administration of endotoxin there was an increase in the concentration of interleukin-1β in the blood serum of stallions. The maximum increase in the concentration of IL-1β took place at hour 3 after infusion of *Escherichia coli* LPS. These results can not be compared to other *in vivo* tests carried out in horses with endotoxaemia since there are none in the literature. However, the *in vitro* tests demonstrate that lipopolysaccharide causes a significant increase in IL-1 production, as measured by \[^{3}H\]thymidine incorporation at hours 3.0 and 20.0 of incubation (23).

LPS-induced monocyte proinflammatory cytokine production (TNF-α) induces synthesis of IL-1 and IL-6 (6, 7). Studies in man (3) demonstrated that in healthy individuals the levels of IL-1β were <70.0 pg/ml in septic patients they were higher (120.0 pg/ml) and in subjects after *Escherichia coli* endotoxin infusion the concentration of interleukin increased in 180 min from a baseline of 35.0 pg/ml to a maximum of 69.0 pg/ml. In dogs (19) with experimentally induced endotoxic shock (after injection of 500 µg/kg b.w. of *Escherichia coli* LPS) it was found that TNF-like activity increased in the 30th minute (maximum after 2 h), IL-1-like between 30 and 60 min and the activity of IL-6 increased in the 1st h, reaching maximum after 1.5 h. TNF-like and IL-1-like activities were hardly detectable from 6-24 h, activity of IL-6 retained high level up to 24 h after an LPS injection. In horses after endotoxin administration TNF-α and IL-6 concentration increased quickly. The mean peak serum activity of these cytokines was observed in 1.5 h and between 3 and 4 h, respectively (1, 2, 4, 13, 16, 17). Taking the above into consideration it can be stated that in horses after endotoxin injection the peak of IL-1β, similarly to IL-6, occurs after maximal increase of TNF-α and the serum/plasma activity peak of IL-1 depends on the endotoxin dosage.

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Fig. 5. Respiratory rate (mean ± S.D).

* - significant differences compared to the time 0, a:b- significant differences between groups, at P<0.05.
An evidence of the stallions' reaction to the administration of endotoxin was the state of pyrexia. An essential element of it was a rise in rectal temperature. The maximum increase in the temperature was observed at hour 4 after injection of endotoxin. The results of these examinations are close to the observations made by other authors examining a reaction of horses to a single dose of Escherichia coli endotoxin (1, 4, 12, 13, 16, 17, 27). In PPH both sexes (28) the maximum increase in rectal temperature was in 4.5 h after administration of 0.3 µg/kg b.w.

Endotoxin infusion caused also a significant increase in scrotal skin temperature with maximum after 3 h in the ENDO stallions. The influence of endotoxaemia on the scrotal skin temperature in stallions is not well known, which makes it difficult to compare the obtained results with those of other authors. The studies in rams (18) showed that scrotal skin temperature increased after T. congolense infection.

Administration of endotoxin resulted in an increase of heart and respiratory rates with maximum at hour 2. Tachycardia and tachypnea following administration of a single LPS dose were also observed by the authors of other studies (1, 2, 4, 13, 17). Previous studies in PPH mares proved the maximum increase of HR and RR at hour 2 after injection of LPS-1 in a dose of 0.1µg/kg b.w. (10). LPS stimulated haemodynamic changes in pulmonary circulation and in cardiovascular function (26). It caused some alterations in vascular stenosis and blood pressure growth, which in turn produced higher heart and respiratory rates.

References