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Changes of Interleukin-1β and Testosterone Concentration after Nonsteroidal Anti-Inflammatory Drug Treatment at Different Times Relative to Endotoxin Administration in an Equine Model

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The aim of the study was to determine the effect of a single dose of flunixin meglumine on serum interleukine-1β and serum and seminal plasma testosterone given at various times after the administration of endotoxin in stallions. Four healthy stallions were infused with endotoxin (LPS) from *Escherichia coli* 055:B5 in doses of 0.3 μg/kg b.w. (group ENDO). Eight healthy stallions were treated with flunixin meglumine (1.1 mg/kg b.w., IV) 30 min (group ENDO+FM1, n=4), and 60 min (group ENDO+FM2, n=4) after the administration of LPS. Blood samples were obtained before infusion of LPS (marked as time 0) and 1, 2, 3, 4, 5, 6, 7, 8, 24, 48 and 72 hrs thereafter. In response to endotoxin infusion, the reaction of the stallions included fever (increased rectal temperature), increased heart rate, and leucopenia. Administration of endotoxin had an influence on the level of IL-1β (increase at 3-6 h after LPS administration) in the blood serum and testosterone in the blood serum (decrease at 3-24 h and increase at 48-72 h after LPS administration) and in seminal plasma (decrease at 24 h after LPS administration). Flunixin treatment prevented an endotoxin-induced increase in rectal temperature, heart rate, with no influence on the decrease of white blood cell numbers. Administration of flunixin meglumine (especially 60 min after LPS infusion) decreased the serum IL-1β level. FM (especially 30 minutes after LPS infusion) had a positive effect on the late changes of serum testosterone concentration after addition of endotoxin. Injection of FM (30 and 60 minutes after LPS administration) also had a positive effect on the seminal plasma T level of the stallions.

Key words: Horse, endotoxemia, nonsteroidal anti-inflammatory drug, interleukin-1β, testosterone.

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Endotoxin is a toxic lipopolysaccharide (LPS) component of Gram-negative bacterial cell walls. Lipopolysaccharide is a strong stimulator of many specific and non-specific organism reactions. Many of the deleterious effects of endotoxin are mediated by cytokines, among which most important are tumor necrosis factor-α (TNF-α), IL-6 and interleukin-1 which is referred to as IL-1α and IL-1β and proinflammatory substances (CALKINS *et al*. 2002; CANNON *et al*. 1990; CAVAILLON 1995; DEBOND *et al*. 1996; CONTI *et al*. 2004; MACKAY *et al*. 1991; MACKAY & LESTER 1992; MORRIS *et al*. 1990, 1992; DANEK 2002; SEETHANATHAN *et al.* 1990).

Pathophysiological effects of bacterial endotoxin or lipopolysaccharide are similar and induced endotoxemia with many clinical signs, including haemodynamic and hematologic alterations in adult horses and ponies (BASKETT *et al*. 1997; BOTTOMS *et al*. 1981; DANEK 2006, 2011; KRUMRYCH *et al*. 1997; MORRIS *et al*. 1992; WIŚNIEWSKI *et al*. 1992; TADROS & FRANK 2012).

The effect of endotoxin action on the male reproductive functions has been partly discovered. Administration of endotoxin displays many alterations

in male hormonal properties (ALLEN *et al*. 2004; BOSMANN *et al*. 1996; CAO *et al*. 2010; COLLODEL *et al*. 2012; HALES *et al*. 2000; SOKKAR *et al*. 2003; WALLGREN *et al*. 1989, 1993; WALLGREN 1989). Many changes in serum plasma concentration of testosterone and quality of the semen were observed in stallions after LPS injection (DANEK 2002, 2003).

The reactions to endotoxin in sexually active subjects could be specific. Another study indicated that sexual steroids could be endogenous regulators with relations to cytokine and may modulate their production (CUTOLO *et al*. 1995; GILLIVER *et al*. 2006; LI *et al*. 1993; MORISHITA *et al*. 1999; POLAN *et al*. 1988).

Flunixin meglumine (flunixin, FM) is a nonsteroidal anti-inflammatory drug and prostaglandin cyclooxygenase inhibitor which is used to prevent adverse effects of endotoxin on a number of body systems in horses (BASKETT *et al*. 1997; BOTTOMS *et al*. 1981; LEES & HIGGINS 1984; MCALLISTER 1994; MOORE *et al*. 1986). Preliminary studies indicated that FM injection modulated the negative effects of endotoxin on testicular function in the boar (WALLGREN *et al*. 1995).

In contrast to previous studies (DANEK 2006), the aim of this study was to determine the effect of a nonsteroidal anti-inflammatory drug – flunixin meglumine treatment on the changes of serum interleukin-1β and serum and seminal plasma testosterone concentration in endotoxemic stallions, especially in relation to the differential time injection of FM after endotoxin administration.

Material and Methods

A total of 12 clinically healthy stallions of Polish Konik (Polish primitive horse) were investigated. The examinations were carried out during the mating season (April-July). The stallions were divided into three groups.: ENDO (4 stallions aged 4-12 years and weighing 260-400 kg), ENDO+FM1 (4 stallions aged 6-12 years and weighing 300-420 kg), and ENDO+FM2 (4 stallions aged 4-13 years and weighing 260-380 kg). The experimental groups were given an endotoxin alone (Group ENDO) and endotoxin followed by an injection of flunixin meglumine (group ENDO+FM1-2).

Lipopolysaccharide from *Escherichia coli* serotype 055:B5 (Sigma Chemical Co.) was used. This endotoxin was given intravenously after dissolution in 500 ml apyrogenic physiological saline solution (0.9% NaCl). The stallions were infused with a LPS dose of 0.3 μg/kg b.w. The nonsteroidal anti-inflammatory drug – flunixin meglumine (Finadine vet., ScanVet., Denmark) was administered as a single intravenous injection in the dose of 1.1 mg/kg b.w. Flunixin meglumine (FM) was injected 30 min (Group ENDO+FM1), and 60 min (group ENDO+FM2) after the infusion of endotoxin. The above procedures were carried out before the functioning of the official ethical committee for experiments on animals in Poland and followed strictly the standards of good laboratory practice developed in the National Veterinary Research Institute in Puławy.

Clinical examinations comprised observations of animals, measurement of rectal temperature (Tr), and heart rate (HR) performed immediately before the infusion (marked as time 0) and 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 24 h, 48 h, and 72 h thereafter. Semen was sampled at 24 h, 48h and 72 h before (mean of all marked as time 0) and in the 24 h, 48 h and 72 h after LPS or LPS+FM administration. After the clinical examinations the whole blood was taken (always at the same time for each stallion) from the jugular vein using a catheter (Secalon® Kathy 1, Viggo, Spectramed, Swindon, UK). The blood samples were collected into EDTA-vacutainer tubes and tubes for serum. The blood in plain tubes was allowed to clot at room temperature, then kept at 4°C and serum was collected by centrifugation. The serum samples were stored at -20°C or at -70°C for 30 days until assayed. The white blood cell (WBC) counts were determined using a Sysmex F800 analyser (TOA Medical Electronics Co., Japan). The interleukin-1β concentration was determined in stallions with the use of the immunoassay kit for human interleukin-1β, IL-1β-IRMA (DANEK 2002).

Ejaculate was collected with an artificial vagina (Missouri type AV, Nasco, Fort Atkinson, USA). Semen was sampled at 24 h, 48 h and 72 h before (mean of all marked as time 0) and in 24 h, 48 h and 72 h after LPS or LPS+FM administration. The seminal plasma samples were obtained through centrifugation of gel-free semen volume at 1500 g for 15 min and stored at -20°C for 30 days until assayed. The level of testosterone (T) in blood serum and seminal plasma was measured with the use of the RIA kit (DANEK 2006).

The data were analyzed statistically using the Statistica Stat Soft PL program with ANOVA variance analysis. The mean values were compared using the Tukey test. The differences were statistically significant at $P<0.05$.

Results

In stallions receiving endotoxin (ENDO), a significant increase in rectal temperature was ob-

served within 2 to 7 h after administration of LPS. The maximum increase in T_r was noted at 4 h to 39.1°C, $\Delta_{\%}$ – 5.5%. In the group of stallions with endotoxin and FM (ENDO+FM1, ENDO+FM2), rectal temperature increased between the 4 and 5h, respectively. The maximum increase in T_r was noted also at 4h to 37.6°C, $\Delta_{\%}$-1.8% and to 37.8°C, $\Delta_{\%}$-2.4%, respectively (Fig. 1).

The heart rate of the experimental stallions was markedly increased. A significant increase was noted in the group of ENDO stallions at 2-3 h, in the group ENDO+FM1 and ENDO+FM2 at 2 h, with the maximum increase at 2 h, to 62.7 beats/min, $\Delta_{\%}$ – 65.0% beats/min, to 70.0 beats/min, $\Delta_{\%}$ – 72.8% beats/min and to 57.0 beats/min, $\Delta_{\%}$ – 48.0% beats/min, respectively (Fig. 2).

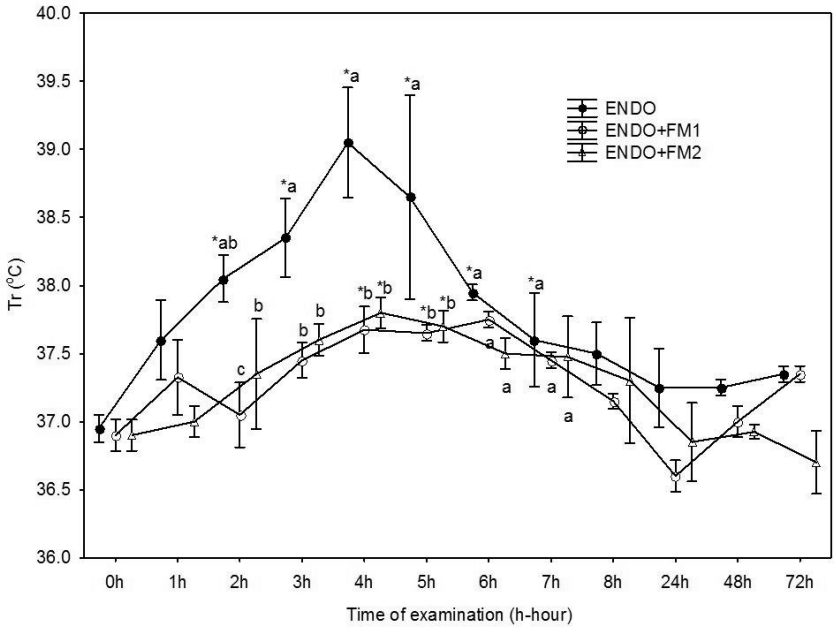

Fig. 1. Rectal temperature in the stallions (mean ± SD).
ENDO – group stallions with endotoxin (0.3 μg/kg b.w., IV), ENDO+FM-1 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 30 min after the infusion of endotoxin), ENDO+FM-2 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 60 min after the infusion of endotoxin), * – significant differences compared to the time 0, a:b:c – significant differences between groups, at P<0.05.

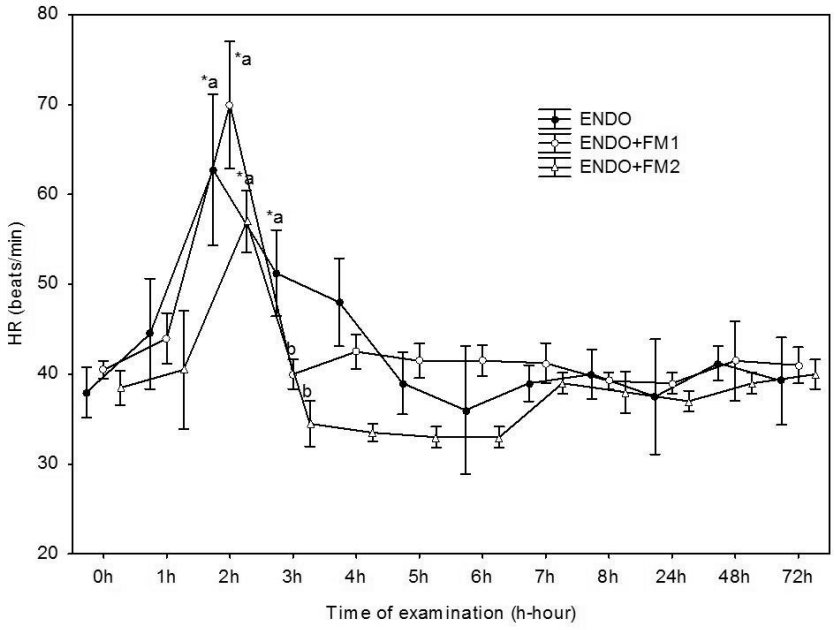

Fig. 2. Heart rate in the stallions (mean ± SD).
ENDO – group stallions with endotoxin (0.3 μg/kg b.w., IV), ENDO+FM-1 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 30 min after the infusion of endotoxin), ENDO+FM-2 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 60 min after the infusion of endotoxin), * – significant differences compared to the time 0, a:b – significant differences between groups, at P<0.05.

Endotoxin-treated stallions had a significantly reduced number of WBC (Fig. 3). The counts of WBC decreased at 1-4 h in the group ENDO and at 1-7 h in the group ENDO+FM1 and ENDO+FM2. The largest decrease in the WBC was at the 2nd h of the experiment and was characterized by a decrease in the number of the WBC to 2.1 x 10^9/l, $\Delta_{\%}$-73.3% (Group ENDO), to 2.0 x 10^9/l, $\Delta_{\%}$-71.8% (ENDO+FM1) and to 1.9 x 10^9/l, $\Delta_{\%}$-72.5% (ENDO+FM2). In the groups of stallions the number of WBC increased again at 48-72h.

The results (Fig. 4) show also that stallions from endotoxin and endotoxin+flunixin meglumine

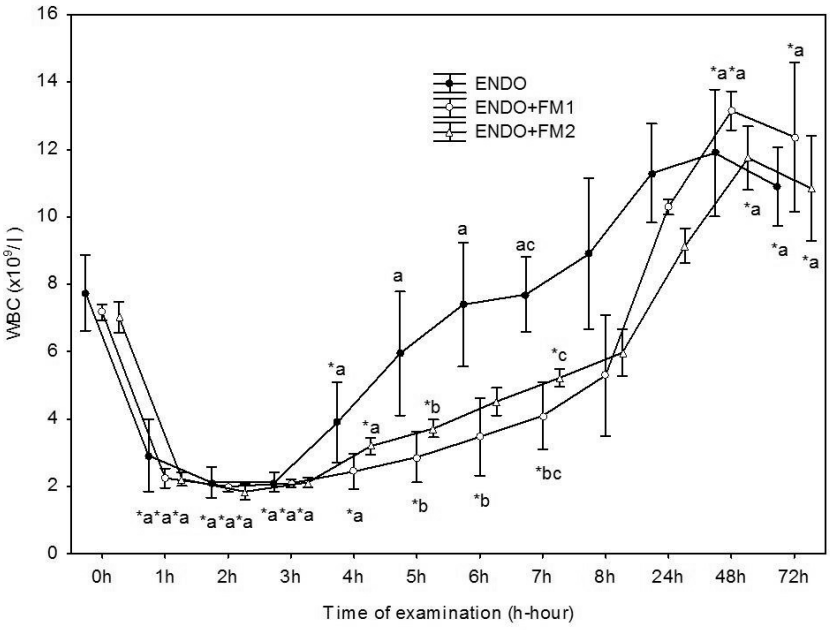

Fig. 3. White blood cell numbers in whole blood of stallions (mean ± SD).
ENDO – group stallions with endotoxin (0.3 µg/kg b.w., IV), ENDO+FM-1 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 30 min after the infusion of endotoxin), ENDO+FM-2 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 60 min after the infusion of endotoxin), * – significant differences compared to the time 0, a:b:c – significant differences between groups, at P<0.05.

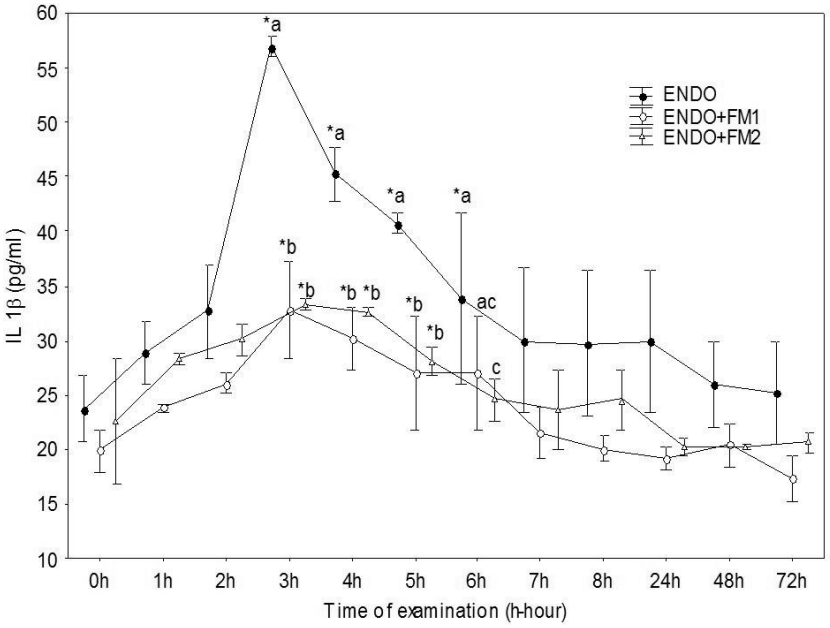

Fig. 4. Changes of serum interleukin-1β level in the stallions (mean ± SD).
ENDO – group stallions with endotoxin (0.3 µg/kg b.w., IV), ENDO+FM-1 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 30 min after the infusion of endotoxin), ENDO+FM-2 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 60 min after the infusion of endotoxin), * – significant differences compared to the time 0, a:b:c – significant differences between groups, at P<0.05.

groups demonstrated a statistically significant increase in serum IL-1β concentration after endotoxin administration at 3-6 h and at 3-5 h, respectively. The maximum increase of IL-1β level took place in hour 3 to 56.7 pg/ml ($\Delta_{\%}$ – 127.7) in the group ENDO, 32.6 pg/ml ($\Delta_{\%}$ – 63.9) in the group ENDO+FM1 and to 33.2 pg/ml ($\Delta_{\%}$ – 47.1) in the group ENDO+FM2.

After endotoxin administration, there was a statistically significant decrease in testosterone T between 3-24 h, after which there was an increase between 48-72 h (Fig. 5). At 6h, there was a maximum decrease in the T concentration to 0.15 ng/ml, $\Delta_{\%}$-89.1%. In stallions ENDO+FM1 and ENDO+FM2, there was a statistically significant decrease in the T concentration between 2-24h and between 2-8h,

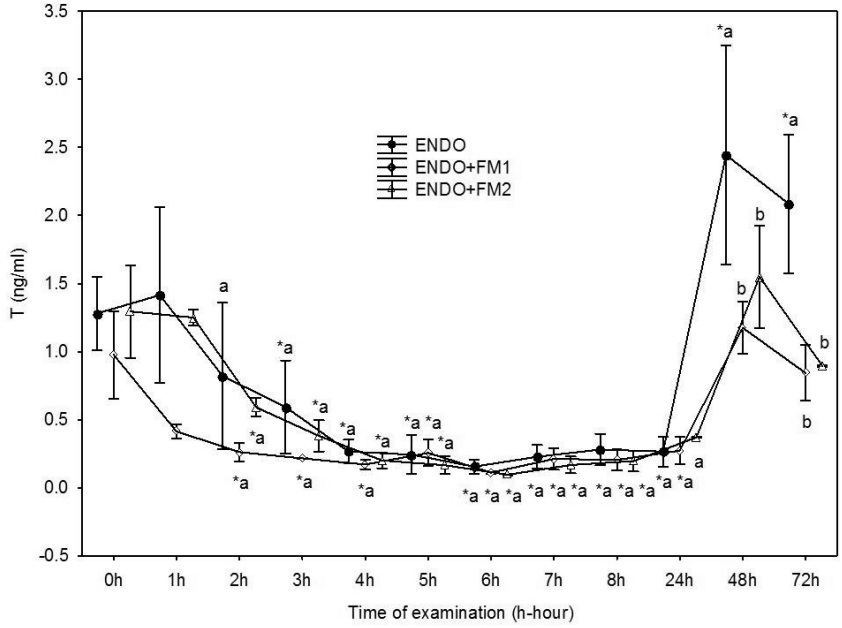

Fig. 5. Concentration of testosterone in blood serum of the stallions (mean ± SD).
ENDO – group stallions with endotoxin (0.3 μg/kg b.w., IV), ENDO+FM-1 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 30 min after the infusion of endotoxin), ENDO+FM-2 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 60 min after the infusion of endotoxin), * – significant differences compared to the time 0, a:b – significant differences between groups, at P<0.05.

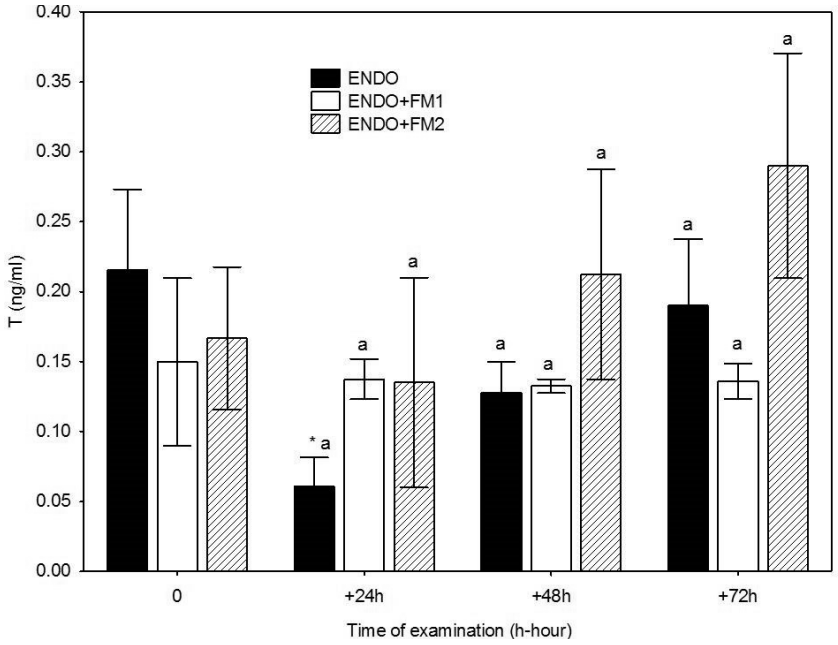

Fig. 6. Concentration of testosterone in seminal plasma of the stallions (mean ± SD).
ENDO – group stallions with endotoxin (0.3 μg/kg b.w., IV), ENDO+FM-1 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 30 min after the infusion of endotoxin), ENDO+FM-2 – group stallions with endotoxin+flunixin (1.1 mg/kg b.w., IV, 60 min after the infusion of endotoxin), * – significant differences compared to the time 0, a:a – no significant differences between groups, at P<0.05.

respectively. After which, there was a non-significant increase in the testosterone concentration between 48-72h after endotoxin+flunixin meglumine administration. At 6h there was a decrease in the T concentration to 0.12 ng/ml, $\Delta_{\%}$-88.0% (group ENDO+FM1) and to 0.10 ng/ml, $\Delta_{\%}$-92.0% (group ENDO+FM2).

Worthy of attention is the fact that only the ENDO group exhibited a statistically significant decrease in the T concentration in seminal plasma to 0.06 ng/ml ($\Delta_{\%}$-72.7%) at 24h after administration of LPS *E. coli* (Fig. 6).

Discussion

Fever, tachycardia and leucopenia are common in adult horses and ponies during experimental endotoxemia (BASKETT *et al*. 1997; BOTTOMS *et al*. 1981; MORRIS *et al*. 1992; WIŚNIEWSKI *et al*. 1992). All LPS-treated stallions developed signs and hematologic changes consistent with endotoxemia. Previous studies also demonstrated that in stallions (DANEK 2002, 2006) the maximum increase in Tr and HR also occurred at 4 hours and 2 hours after administration of 0.3 μgLPS/kg b.w., respectively. In response to endotoxin the stallions reacted with leucopenia and maximum decrease of WBC number in peripheral blood at 2 hours after administration of endotoxin. This has been found in other studies (BASKETT *et al*. 1997; KRUMRYCH *et al*. 1997; MORRIS *et al*. 1992). In stallions the maximum decrease in WBC was also at 2 hours after administration of 0.3 μgLPS/kg b.w. (DANEK 2002, 2006).

In horses, LPS induced proinflammatory cytokine production, including TNFα, interleukin-6, interleukin-1β (BASKETT *et al*. 1997; DANEK 2002; MACKAY *et al.* 1991; MACKAY & LESTER 1992; VEENMAN 2002). The results of this study confirm induced increase in serum interleukin-1β level in all groups after administration of endotoxin to stallions. The maximum increase in the concentration of IL-1β took place in hour 3 after infusion of LPS *Escherichia coli*. These results are consistent with those of a previous study in stallions which documented that administration of LPS (0.3 μg/kg b.w.*)* to stallions results in significant changes in IL-1β concentration (DANEK 2002). The equine endotoxemic shock model also indicated that IL-α+β in the blood samples increased differently at 30 to 270 min (maximum at 240 min) after a slow infusion of LPS (2 μg/kg b.w.) to male Shetland ponies under general anesthesia (VEENMAN *et al.* 2002). However, *in vitro* tests demonstrate that lipopolysaccharide caused a significant increase in IL-1 production, as measured by ^{3}H – thymidine incorporation at 3.0 and 20.0 hours of incubation (SEETHANATHAN *et al*. 1990). Studies in humans (CANNON *et al*. 1990) also demonstrated that in septic patients the levels of IL-1β were higher and in subjects after *Escherichia coli* endotoxin infusion the concentration of interleukin increased from a baseline to a maximum at 180 min. In dogs (MYIAMOTO *et al*. 1996) with experimentally induced endotoxic shock (after injection of 500 μg/kg b.w. of LPS *Escherichia coli*) it was found that IL-1-like increased between 30 and 60 min and was hardly detectable from 6-24 h after an LPS injection. Studies in laboratory animals also indicated LPS-induced increases of serum IL-1β (YAZAR *et al*. 2007, 2010) and also seminal plasma IL-1β (COLLODEL *et al*. 2015).

Administration of endotoxin to the stallions caused changes in the levels of blood serum testosterone and resulted in a statistically significant decrease in the T concentration maximum at 6 h and again an increase between 48-72 h. In previous investigations (DANEK 2003, 2006), similar changes of testosterone concentration were demonstrated in blood serum after infusion of 0.3 μg/kg b.w. LPS *Escherichia coli* in the stallions. It should be added that studies with hCG (group ENDO+hCG) indicated that the inhibition of steroidogenesis (decrease of serum T concentration) by endotoxin observed at 5-24 h and the subsequent peak (at 48 h) of the serum testosterone was significantly higher than in the group of stallions receiving only LPS (DANEK 2011).

In comparison, in rams a decrease of serum T concentration occurs for about 12-24 hour, while in boars an increase in the level of this hormone takes place especially in the 1-2 hour after endotoxin *S. typhimurium* administration with a later tendency for a decrease in its concentration (WALLGREN *et al*. 1989; WALLGREN 1989). Serum testosterone decreased 90% 2 hours after endotoxin injection in mice and this decrease was sustained for 9 days (BOSSMAN *et al*. 1996). Inflammation-induced oxidative stress as the acute effects of LPS impairs Leydig cell mitochondrial steroidogenesis (ALLEN *et al*. 2004).

The infusion of endotoxin *Escherichia coli* also has a negative influence on the semen hormonal status of the stallions. In the presented study, in stallions from the group ENDO, a statistically significant decrease in the testosterone concentration occurred in the seminal plasma. The previous work (DANEK 2003) also showed that 24 hours after administration of *Escherichia coli* LPS in the dose of 0.3 μg/kg b.w. there was a significant decrease of T concentration up to 0.08 ng/ml. These results evidenced that the administration of endotoxin to the stallions causes changes in the levels of seminal plasma testosterone which can result in disturbances of biosynthesis in the testis and accu-

mulation impairment of this hormone in the semen.

The use of 1.1 mg/kg b.w. of flunixin meglumine in a single dose 30 min and 60 min after LPS administration in stallions did not cause rectal temperature or heart rate increase as high as in the group receiving only the endotoxin. The injection of FM did not reduce white blood cell counts which were similar to those noted in the group of stallions with LPS alone.

Flunixin meglumine is a non-narcotic analgesic agent with anti-inflammatory and antipyretic activity which is used for the suppression of signs of endotoxemia in horses (BASKETT *et al*. 1997; LEES & HIGGINS 1984; MOORE *et al*. 1986; TEMPLETON *et al*. 1987; TUREK *et al*. 1985). Previous studies indicated that FM in the dose of 1.1 mg/kg given 5 min after the injection of *Escherichia coli* LPS had, as now, a positive influence on the clinical and hemodynamic (outside WBC in whole blood) changes occurring during endotoxemia in stallions (DANEK 2006).

Nonsteroidal anti-inflammatory drugs (NSAIDs) differentially regulate cytokine production. Some NSAIDs decreased IL-1β protein, but not mRNA for IL-1β, indicating a post-translational effect on the production of IL-1β (D'HELLENCOURT 1996; MASCAGNI *et al*. 2000; TSUBOI *et al*. 1995; SHAHRIARI *et al*. 2011; SULLIVAN *et al*. 2002). In the present study it was shown that flunixin (in the dose of 1.1 mg/kg b.w., injected 30 min and 60 min after the infusion of endotoxin) had a positive effect on serum IL-1β concentration in the endotoxemic stallion. Studies in mice also suggest that flunixin meglumine (FM was administered 2.5 mg/kg b.w. dose subcutaneously) significantly inhibited the level of serum interleukin-1β after endotoxin administration (YAZAR *et al*. 2007).

Injection of flunixin had modulated effects on the concentration of serum testosterone after LPS administration in the stallions. This effect of the FM (added 5 min after LPS infusion) was also observed in DANEK 2006. However, the injection of FM (especially in the 30 min after LPS infusion) influenced differently later (at 24-72 hour) changes of serum T level induced by endotoxin administration. The treatment with FM (in the 30 min and 60 min after LPS infusion) had a positive effect on endotoxin- induced changes of testosterone in seminal plasma of the stallions (no decrease at 24 h after LPS administration).

Studies in boar (WALLGREN *et al*. 1995) indicated that flunixin (as a single dose 10 min prior to an endotoxin injection) reduced the endotoxin-induced infiltration of PMN into the testicular interstitium and morphological changes of Leydig cells and caused an initial marked decrease in blood plasma testosterone (during a 2 hour delay to the increase of concentration of T).

In conclusion, it should be stated that *Escherichia coli* endotoxin administration results in pathological responses of serum interleukin-1β and testosterone levels in the stallions. Injection of flunixin meglumine (1.1 mg/kg.b.w., at 30 min and especially at 60 min) after endotoxin infusion to stallions significantly reduced serum IL-1β levels compared with results in stallions receiving LPS alone. In turn, the treatment of these stallions with the FM (especially at 30 min after LPS infusion) did not affect early changes in serum T level and had a significant effect on the latter changes of serum testosterone concentration after addition of endotoxin. However, a positive influence of flunixin meglumine injection was noticed in seminal plasma T concentration of endotoxemic stallions. It is believed that flunixin meglumine had (injection time-dependent and blood time-disposition of FM administered IV, at a dosage of 1.1 mg/kg) a positive effect, through anti-inflammatory activity (also in the stallion reproductive system), on endotoxin-induced disturbances of testosterone biosynthesis and concentration of this hormone in the serum and seminal plasma. These changes should be taken into consideration in the evaluation of *Enterobacteriaceae* (*Escherichia coli*) toxemia states and effects of nonsteroidal anti-inflammatory drug (flunixin meglumine) treatment in males during the reproductive season.

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References

ALLEN J.A., DIEMER T., JANUS P., HALES K.H., HALES D.B. 2004. Bacterial endotoxin lipopolysaccharide and reactive oxygen species inhibit Leydig cell steroidogenesis *via* perturbation of mitochondria. Endocrine **25**: 265-275.

BASKETT A., BARTON M.H., NORTON N., ANDERS B., MOORE J.N. 1997. Effect of pentoxifylline, flunixin meglumine, and their combination on a model of endotoxemia in horses. Am. J. Vet. Res. **58**:1291-1299.

BOSMANN H.B., HALES K.H, LI X., LIU Z., STOCCO D.M., HALES D.B. 1996. Acute *in vivo* inhibition of testosterone by endotoxin parallels loss of steroidogenic acute regulatory (StAR) protein in Leydig cells. Endocrinology **137**: 4522-4525.

BOTTOMS G.D., FESSLER J.F., ROESEL O.F., MOORE A.B., FRAUENFELDER H.C. 1981. Endotoxin-induced hemodynamic changes in ponies: effects of flunixin meglumine. Am. J. Vet. Res. **42**: 1514-1518.

CALKINS C.M., D.D. BENSARD B.D., SHAMES E.J., PULIDO E., ABRAHAM N., FERNANDEZ X., MENG C.A., DINARELLO C.A., MCINNTYRE R.C.JR. 2002. IL-1 regu-

lates *in vivo* CXC chemokineinduction and neutrophil sequestration following endotoxemia. J. Endotoxin. Res. **8**: 59-67.

CANNON J.G., TOMPKINS R.G., GELFAND J.A., MICHIE H.R., STANFORD G.S., MEER J.W.M., ENDRES S., LONNEMANN G., CORSETTI J., CHERNOW B., WILMORE D.W., WOLFF S.M., BURKE J.F., DINARELLO C.A. 1990. Circulating Interleukine-1 and Tumor Necrosis Factor in septic schock and experimental endotoxin fever. J. Infect. Dis. **161**: 79-84.

CAO D., LI Y., YANG R., WANG Y., ZHOU Y., DIAO H., ZHAO Y., ZHANG Y., LU J. 2010. Lipopolysaccharide-induced epididymitis disrupts epididymal beta-defensin expression and inhibits sperm motility in rats. Biol. Reprod. **83**:1064-1070.

CAVAILLON J.M. 1995. Cytokines in inflammation. CR Seances Soc. Biol. Fil. **189**: 531-544.

COLLODEL G., CASTELLINI C., DEL VECCHIO M.T., CARDINALI R., GEMINIANI M., ROSSI B., SPREAFICO A., MORETTI E. 2012. Effect of a bacterial lipopolysaccharide treatment on rabbit testis and ejaculated sperm. Reprod. Domest. Anim. **47**: 372-378.

COLLODEL G., MORETTI E., BRECCHIA G., KUŽELOVÁ L., ARRUDA J., MOURVAKI E., CASTELLINI C. 2015. Cytokines release and oxidative status in semen samples from rabbits treated with bacterial lipopolysaccharide. Theriogenology **83**:1233-1240.

CONTI B., TABAREAN I., ANDREI C., BARTFAI T. 2004. Cytokines and fever. Front Biosci. **9**: 1433-1449.

CUTOLO M., ACCARDO S., VILLAGGIO B., BARONE A., SULLI A., BALLEARI E., BASON C., FELLI L., GRANATA O.M., AMODIO R., CASTAGNETTA L. 1995. Androgen metabolism and inhibition of interleukin-1 synthesis in primary cultured human synovial macrophages. Mediators Inflamm. **4**: 138-143.

DANEK J. 2002. Level of Interleukin-1β and clinical signs after endotoxin injection in stallions. Bull. Vet. Inst. Pulawy **46**: 179-185.

DANEK J. 2003. Effect of *Escherichia coli* endotoxin on the levels of testosterone and estradiol-17 in blood serum and seminal plasma and on the semen characteristics in the stallion. Bull. Vet. Inst. Pulawy **47**: 191-209.

DANEK J. 2006. Effects of flunixin meglumine on selected clinicopathologic variables, and serum testosterone concentration in stallions after endotoxin administration. J. Vet. Med. A Physiol. Pathol. Clin. Med. **53**: 357-363.

DANEK J. 2011. Effect of human chorionic gonadotropin on endotoxaemic stallions. Bull. Vet. Inst. Pulawy **55**: 223-229.

DEBOND E.S.J.M., NIEMARKT A.E., TAMMINGA R.Y.J., KIMPEN J.L.L, KAMPS W.A., DELEIJ L.H.M.F. 1996. Lipopolisaccharide-induced cytokine production in peripheral blood mononuclear cells: intracellular localization of tumor necrosis factor α and interleukin 1β detected with a three-color immunofluorescence technique. Histochem. Cell Biol. **106**: 593-598.

D'HELLENCOURT C.L., DIAW L., CORNILLET P., GUENOUNOU M. 1996. Differential regulation of TNF alpha, IL-1 beta, IL-6, IL-8, TNF beta, and IL-10 by pentoxifylline. Int. J. Immunopharmacol. **18**: 739-48.

GILLIVER S.C., ASHWORTH J.J., MILLS S.J., HARDMAN M.J., ASHCROFT G.S. 2006. Androgens modulate the inflammatory response during acute wound healing. J. Cell. Sci. **119**: 722-733.

HALES K.H., DIEMER T., GINDE S., SHANKAR B.K., ROBERTS M., BOSMANN H.B., HALES D.B. 2000. Diametric effects of bacterial endotoxin lipopolysaccharide on adrenal and Leydig cell steroidogenic acute regulatory protein. Endocrinology **141**: 4000-4012.

KRUMRYCH W., WIŚNIEWSKI E., DĄBROWSKA J., DANEK J. 1997. Activation of blood neutrophils in horses with the use of different doses of endotoxin. Bull. Vet. Inst. Pulawy **41**: 41-47.

LEES P., HIGGINS A.J. 1984. Flunixin inhibits prostaglandin E_2 production in equine inflammation. Res. Vet. Sci. **37**: 347-349.

LI Z.G., DANIS V.A., BROOKS P.M. 1993. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells *in vitro*. Clin. Exp. Rheumatol. **11**: 157-162.

MCALLISTER C.G. 1994. Nonsteroidal anti-inflammatory drugs: Their mechanism of action and clinical uses in horses. Vet. Med. **89**: 237-240.

MACKAY R.J., LESTER G.D. 1992. Induction of the acute-phase cytokine, hepatocyte-stimulating factor/interleukin 6, in the circulation of horse treated with endotoxin. Am. J. Vet. Res. **53**: 1285-1289.

MACKAY R.J., MERRIT A.M., ZERTUCHE J.M., WHITTINGTON M., SKELLEY L.A. 1991. Tumor necrosis factor activity in the circulation of horses given endotoxin. Am. J. Vet. Res. **52**: 533-538.

MASCAGNI P., SABBATINI V., BIORDI L., MARTINOTTI S., ALLEGRETTI M., MARULLO A., CASELLI G., BERTINI R. 2000. R- and S-isomers of nonsteroidal anti-inflammatory drugs differentially regulate cytokine production. Eur. Cytokine Netw. **11**: 185-192.

MOORE J.N., HARDEE M.M., HARDEE G.E. 1986. Modulation of arachidonic acid metabolism in endotoxic horses: comparison of flunixin meglumine, phenylbutazone, and a selective thromboxane synthethase inhibitor. Am. J. Vet. Res. **47**: 110-113.

MORRIS D.D., CROWE N., MOORE J.N. 1990. Serum tumor necrosis factor activity in horses during experimental endotoxemia: correlation with clinical and laboratory signs. Am. J. Vet. Res., **51**: 1935-1940.

MORRIS D.D., MOORE J.N., CROWE N., MOLDAWER L.L. 1992. Effect of experimentally induced endotoxemia on serum interleukin-6 activity in horses. Am. J. Vet. Res. **53**: 753-756.

MORISHITA M., MIYAGI M., IWAMOTO Y. 1999. Effects of sex hormones on production of interleukin-1 by human peripheral monocytes. J. Periodontol. **70**: 757-60.

MYIAMOTO T., FUJINAGA T., YAMASHITA K., HAGIO M. 1996. Changes of serum cytokine activities and other parameters in dogs with experimentally induced endotoxin shock. Jpn. J. Vet. Res. **44**:107-118.

POLAN M.L., DANIELE A., KUO A. 1988. Gonadal steroid modulate human monocyte interleukin (IL-1) activity. Fertil. Steril. **49**: 964-968.

SHAHRIARI S., REZAEI A., JALALZADEH S.M., MANI K., ZAMANI A. 2011. Effect of Ibuprofen on IL-1β, TNF-α and PGE2 Levels in Periapical Exudates: A Double Blinded Clinical Trial. Iran. J. Immunol. **8**: 176-182.

SEETHANATHAN P., BOTTOMS G.D., SCHAFER K. 1990. Characterization of release of tumor necrosis factor, interleukin-1, and superoxide anion from equine white blood cells in response to endotoxin. Am. J. Vet. Res. **51**: 1211-1225.

SOKKAR S.M., DARWIESH G., MADBOOLY A. 2003. Study of the pathological effect of *Escherichia coli* endotoxin in rams. J. Vet. Med. B **50**: 226-230.

SULLIVAN M.H.F., ALVI S.A., BROWN N.L., ELDER M.G., BENNETT P.R. 2002. The effects of a cytokine suppressive anti-inflammatory drug on the output of prostaglandin E_2 and interleukin-1β from human fetal membranes. Mol. Hum. Reprod. **8**: 281-285.

TADROS E.M., FRANK N. 2012. Effects of continuous or intermittent lipopolysaccharide administration for 48 hours on the systemic inflammatory response in horses. Am. J. Vet. Res. **73**: 1394-1402.

TEMPLETON C.B., BOTTOMS G.D., FESSLER J.F. 1987. Endotoxin – induced hemodynamic and prostaglandin changes in ponies: effects of flunixin meglumine, dexamethasone, and prednisolone. Circ. Shock **23**: 231-240.

TSUBOI I., TANAKA H., NAKAO M., SHICHIJO S., ITOH K. 1995. Nonsteroidal anti-inflammatory drugs differentially

regulate cytokine production in human lymphocytes: up-regulation of TNF, IFN-gamma and IL-2, in contrast to down-regulation of IL-6 production. Cytokine **7**: 372-379.

TUREK J.J., TEMPLETON C.B., BOTTOMS G.D. 1985. Flunixin meglumine attenuation of endotoxin-induced damage to the cardiopulmonary vascular endotelium in pony. Am. J. Vet. Res. **46**: 591-596.

VEENMAN J.N., DUJARDIN C.L.L., HOEK A., GROOTENDORST A., KLEIN W.R., RUTTEN V.P.M.G. 2002. High volume continuous venovenous haemofiltration (HV-CVVH) in an equine endotoxaemic shock model. Equine Vet. J. **34**: 516-522.

WALLGREN. M., KINDAHL H., LARSSON K. 1989. Clinical, endocrinological and spermatological studies after endotoxin in the ram. Zentralbl. Veterinarmed. A **36**: 90-103.

WALLGREN M. 1989. Clinical, endocrinological and spermatological studies after endotoxin injection in the boar. Zentralbl. Veterinarmed. A **36**: 664-675.

WALLGREN, M. KINDAHL H., RODRIGUEZ-MARTINEZ H. 1993. Alterations in testicular function after endotoxin injection in the boar. Int. J. Androl. **16**: 235-43.

WALLGREN M., KINDAHL H., RODRIGUEZ-MARTINEZ H. 1995. Modulation of endotoxin-induced andrological alterations by flunixin meglumine in the boar. Zentralbl. Veterinarmed. A **42**: 357-369.

WIŚNIEWSKI E., KRUMRYCH W., DANEK J. 1992. Latency period and developmental phases of fever in horses. Bull. Vet. Inst. Pulawy **36**: 31-38.

YAZAR E., ER A., UNEY K., ALTUNOK V., ELMAS M. 2007. Effect of flunixin meglumine on cytokine levels in experimental endotoxemia in mice. J. Vet. Med. A Physiol. Pathol. Clin. Med. 54: 352.

YAZAR E., BULBUL A., AVCI G.E., AYSE E.R., UNEY K., ELMAS M., TRAS B. 2010. Effects of enrofloxacin, flunixin meglumine and dexamethasone on disseminated intravascular coagulation, cytokine levels and adenosine deaminase activity in endotoxaemia in rats. Acta Vet. Hung. **58**: 357-367.