# *In vivo* lipopolysaccharide inflammation model to study the pharmacodynamics of COX-2 inhibitors celecoxib, mavacoxib and meloxicam in cockatiels (*Nymphicus hollandicus*)

**Running title:** PD of COX-2 inhibitors in cockatiels

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**Abstract**

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used in avian medicine for their antipyretic, analgesic and anti-inflammatory properties both during surgery and diseases related with tissue damage and/or inflammation. NSAIDs inhibit cyclooxygenase (COX) enzymes, which are responsible for the induction of pyresis, pain and inflammation. In the current study, an Lipopolysaccharide-induced (LPS) pyresis model was optimized and validated in cockatiels (*Nymphicus hollandicus*). An intravenous bolus injection of LPS (7.5 mg/KG BW) was administered at T0 and T24 (24 hour following the first LPS injection), followed by the assessment of the pharmacodynamic (PD) parameters of the NSAIDs mavacoxib (4 mg/kg BW), celecoxib (10 mg/kg BW) and meloxicam (1 mg/kg BW). The PD parameters (body temperature, clinical appearances, preference of location in the cage and prostaglandin E2 (PGE2) plasma concentrations) were determined during 10 hours following the second LPS injection. Both mavacoxib and celecoxib were able to reduce the LPS-induced hypothermia, but only mavacoxib had a significant increase in clinical appearance of the birds. In contrast, no influence on hypothermia and clinical appearance was observed in the LPS-challenged cockatiels treated with meloxicam. The three NSAIDs were able to inhibit the increase in LPS-induced PGE2 plasma concentrations, however the effect was most pronounced in the birds treated with meloxicam. Based on the presented results, both celecoxib and mavacoxib are more effective than meloxicam to treat hypothermia. Mavacoxib is preferred, since this NSAID has also a positive effect on the clinical appearance of the cockatiels.

**Keywords**
lipopolysaccharide, pharmacodynamic, mavacoxib, celecoxib, meloxicam, cockatiel

**List of abbreviations**

| | |
|---|---|
| AUC | Area under the plasma concentration-time curve |
| ABV | Avian Bornavirus |
| BW | Body weight |
| COX | Cyclooxygenase |
| IV | Intravenous |
| LPS | Lipopolysaccharide |
| NSAID | Non-steroidal anti-inflammatory drugs |
| PDD | Proventricular dilatation disease |
| PD | Pharmacodynamic |
| PK | Pharmacokinetic |
| $PGE_2$ | Prostaglandin $E_2$ |
| $T_x$ | Time before/after administration |
| UPLC-MS/MS | Ultra-performance liquid chromatography - tandem mass spectrometry |

## 1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used in avian clinical practice for their anti-inflammatory, antipyretic and analgesic properties. Although research determining the efficacy of analgesics in avian patients is still in its infancy, the usefulness of NSAIDs has already been proven during clinical trials[1]. NSAIDs reversibly inhibit the cyclooxygenase (COX) enzyme activity, which catalyzes the formation of prostanoids, such as prostaglandins, prostacyclins and thromboxanes, from arachidonic acid[2,3,4]. Three related COX enzyme isoforms have been distinguished, COX-1, COX-2 and COX-3. COX-1 is constitutively expressed in most tissues and is related to gastric cytoprotection, regulation of homeostasis, renal blood flow maintenance and platelet aggregation[3,4,5]. COX-2, which is expressed less constitutively than COX-1, is primarily induced in response to inflammatory stimuli, such as lipopolysaccharide (LPS), cytokines and injury. COX-2 causes vasodilation, increasing vascular permeability, chemotaxis, hyperalgesia and potentiation of other inflammation mediators (i.e. histamine)[3,6,7,8].

Meloxicam is an enolcarboxamide indicated in avian species for the treatment of various painful and/or inflammatory conditions (i.e. proventricular dilatation disease (PDD)) as well as the treatment of birds suffering from inflammation caused by chronic locomotive diseases[2]. Meloxicam is a preferential COX-2 enzyme inhibitor at low therapeutic doses, but higher doses might also induce inhibition of COX-1 enzyme activity (i.e. in human ratio of 50% inhibitory concentration for COX-2/COX-1 = 0.09 in whole blood assays), potentially causing side-effects such as gastrointestinal toxicity, cardiovascular side-effects, etc.[2,9,10]. The coxibs, such as celecoxib and mavacoxib, are selective COX-2 enzyme inhibitors, which provide inhibition of COX-2 enzyme activity without altering the COX-1 enzyme activity. In birds, celecoxib is frequently prescribed by veterinarians to symptomatically treat PDD. PDD is a progressive avian disease affecting *Psittaciformes* which is caused by an avian Bornavirus

96 (ABV)-induced inflammatory response of the gastrointestinal tract as well as the central and
97 peripheral nervous system[11,12]. Mavacoxib is considered as one of the standard therapies to
98 treat dogs suffering from osteoarthritis, but its use in avian clinical practice is until now not
99 common[13,14]. To date, only limited studies have been published investigating the anti-
100 inflammatory, antipyretic and analgesic properties of selective COX-2 enzyme inhibitors in
101 birds[15,16,17]. In 2013, Hoppes et al.[16] investigated the efficacy of meloxicam on disease
102 development and mortality in ABV-infected cockatiels (*Nymphicus hollandicus*). The authors
103 demonstrated that the use of meloxicam might enhance the severity of the ABV infection due
104 to changes in gastrointestinal physiology. Recently, Dhondt et al.[17] determined the
105 pharmacokinetics (PK) and absolute oral bioavailability of celecoxib, mavacoxib and
106 meloxicam in cockatiels. Mavacoxib had a prolonged elimination half-life, enabling less
107 frequent dosing compared to celecoxib and meloxicam. Both authors[16,17] concluded that
108 additional pharmacodynamic (PD) and safety studies are necessary to further conclude if
109 NSAIDs are useful in the treatment of PDD and other painful/inflammatory conditions.

110 LPS (endotoxin) can be found in the outer membrane of Gram-negative bacteria and provokes
111 immune responses resulting in i.e. a change in body temperature, increase in cytokine
112 production and sickness[18]. In 2001, *Escherichia coli* LPS models have been accepted by the
113 European Medicine Agency for the evaluation of anti-inflammatory, antipyretic and analgesic
114 properties of different NSAIDs[19]. LPS models have already been developed and validated in
115 both mammals[20,21] and broiler chickens[22], but to the author's knowledge a cockatiel LPS-
116 induced model still needs to be developed.

117 The aim of the present study was to evaluate the antipyretic and anti-inflammatory properties
118 of NSAIDs in cockatiels (*Nymphicus hollandicus*), as model for the *Psittaciformes*. An *in vivo*
119 cockatiel LPS model was developed and validated to study the PD parameters (body

120 temperature, clinical appearance and plasma prostaglandin $E_2$ ($PGE_2$) concentration) of the
121 COX-2 inhibitors celecoxib, mavacoxib and meloxicam.

## 2. MATERIALS AND METHODS

### 2.1 Animals

This study was conducted with consent of the ethical committee of the Faculty of Veterinary Medicine and Bioscience Engineering of Ghent University (EC2015/114). Care and use of animals was in full compliance with the most recent national legislation[23] and European Directive[24]. A group of 45 cockatiels (*Nymphicus hollandicus*) (23♂/22♀, 102 ± 12 g, 6-12 months old) were group-housed in an aviary (16 m³) during a two-week acclimatization period at the start of the study and during the recovery period after the trial. Birds were cage-housed in pairs, 12h prior the start of the experiment until 12h after the end of the experiment. Animals had *ad libitum* access to a commercially available seed mixture (Big Parakeets Prestige, Versele-Laga, Deinze, Belgium) and tap water. The mean room temperature during acclimatization and experiments was 20 ± 3°C and a 12h light/ 12h dark cycle was applied (light provided by artificial lights).

### 2.2 Development and validation intravenous LPS model

#### *2.2.1 LPS dose determination study*

Seven cockatiels (4♂/3♀) were included to determine the correct dosing protocol for the LPS model. One day prior to the experiments, a temperature sensing pet microchip (Temperature sensing radio-frequency identification base plate system, Biomark Inc., Idaho, USA) was placed subcutaneously in the left pectoral muscle region of the cockatiels. Immediately prior to administration, LPS was mixed with physiological saline (5 mg LPS/mL, 0.9% NaCl). At $T_0$ (time of administration), an intravenous (IV) single bolus of LPS (*Escherichia coli* 0127:B8 purified by phenol extraction, ≥ 500,000 EU/mg, Sigma-Aldrich, Bornem, Belgium) was administered in the *vena cutanea ulnaris superfacialis* (wing vein) to five birds. Each bird received a different dosage: 2.5, 5, 7.5, 10 and 25 mg/kg body weight (BW), respectively.

Body temperate of the birds was registered before and every 30 minutes after administration using a microchip reader (Biomark BIO310/303TS-ANT reader platform, Biomark Inc.). An IV dose of 7.5 mg/kg was considered optimal to induce a clear difference in body temperature (hypothermia) without mortality. Since the body temperature of the cockatiels fluctuated strongly after a single LPS administration, the impact of the source of LPS and a second LPS injection (24 hours after the first injection) on the body temperature was evaluated. Therefore, two cockatiels received either a double IV bolus of LPS from *E. coli* or a double IV bolus of LPS from *Salmonella* Enteritidis purified by phenol extraction (≥ 500,000 EU/mg, Sigma-Aldrich), at a dosage of 7.5 mg/kg BW per bolus. No impact of the source of LPS was observed. Fluctuation of body temperature was less when administering a second LPS injection and the hypothermia was more pronounced (data not shown).

### *2.2.2 Model validation study*

Six cockatiels were randomly divided into two groups (LPS or negative control) of three birds (2♂/1♀). At $T_0$ and $T_{24}$ (24 hours after the first LPS administration), a 7.5 mg/kg BW IV LPS bolus (*E. coli* 0127:B8 purified by phenol extraction, ≥ 500,000 EU/mg, Sigma-Aldrich) or an equivalent 0.9% NaCl bolus was administered to LPS or negative control group, respectively. Body temperature was monitored after the second LPS administration as described in *section 2.2.1* to validate the model.

## 2.3 Pharmacodynamic study

### *2.3.1 Products*

The LPS solution was prepared as described in *section 2.2.1*. Commercially available tablets of celecoxib (Celebrex 100 mg®, Pfizer, Brussel, Belgium) and mavacoxib (Troxocil 20 mg®, Zoetis, Zaventem, Belgium) were grinded and lactose was added until a concentration

of 10 mg/g and 4 mg/g was obtained, respectively. Prior to oral administration, the mixture was dissolved in 0.9% NaCl to obtain a solution of 5 mg celecoxib/mL and 2 mg mavacoxib/mL. A commercially available 0.5 mg/mL meloxicam suspension (Metacam 0.5 mg/mL®, Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany) was used.

*2.3.2 Experimental design*

Thirty-two cockatiels were included in the PD study and were randomly divided into four groups of eight animals (4♂/4♀). All birds received an IV LPS bolus injection (7.5 mg/kg, *E. coli* 0127:B8 purified by phenol extraction, ≥ 500,000 EU/mg, Sigma-Aldrich) at $T_0$ and $T_{24}$. At $T_{12}$ and $T_{22}$ (12 hours and 22 hours after the first LPS administration, respectively), the birds received a 2 mL intra-crop feed bolus (Nutribird A19 High Energy (Versele-Laga):tap water, 25:75, v-v) administered with a curved stainless steel ball tipped feeding needle (ø 2.50mm). The negative control group did not receive any treatment. To administer the second LPS injection concurrently at the moment of the maximum plasma concentration of the different NSAIDs, mavacoxib (4 mg/kg BW), celecoxib (10 mg/kg BW) and meloxicam (1 mg/kg BW) were administered orally (intra-crop bolus) at corresponding time points ($T_{12}$, $T_{22}$, $T_{23}$) to the birds of the mavacoxib, celecoxib and meloxicam group, respectively (**Figure 1**)[17]. Body temperature was assessed after the second LPS administration as described in *section 2.1.1*. Besides, the health and position of the birds were recorded from $T_{24}$ until $T_{34}$ using camera-assisted recording. Different clinical parameters were assessed, including state of consciousness (alertness, apathetic, soporose) signs of illness (ruffled feathers and dyspnea), and time spent on feed- and water uptake, grooming behavior, and exercise (climbing or flying). The percentage of time the bird showed a certain state of consciousness, signs of illness, and time spent on a certain activity was calculated based on a snapshot monitoring method with an interval of 5 min. Moreover, the location of the bird in the cage (on the wire

mesh, on one of both perches or on the floor) was monitored and the percentage of time spent on a certain location was calculated.

### *2.3.3 Plasma sampling*

A sparse sampling protocol was applied because of the limited volume of blood that can be drawn from the cockatiels. Therefore, all sampling points were randomly allocated to different birds within one group, with three sampling points per bird. Blood (0.3 mL/ time point) was sampled by venipuncture from the jugular vein (*vena jugularis*) with a 1 mL syringe and 29G needle. Blood samples were collected at $T_0$ (just before administration) and $T_{24}$, $T_{24.5}$, $T_{25}$, $T_{26}$, $T_{28}$ and $T_{30}$ post administration. The samples were transferred into heparinized collection Eppendorf tubes coated with 10 µg/mL indomethacin (Sigma Aldrich, Diegem, Belgium) and 10 IU heparin (Leo Pharma, Lier, Belgium). Samples were immediately placed on ice and centrifuged for 10 min at 2851 g (4°C) within 2h after blood collection. Supernatant was aliquoted, frozen and stored at -70°C until $PE_2$ plasma concentration analysis.

### *2.3.4 Prostaglandin $E_2$ plasma concentration determination*

$PGE_2$ plasma concentrations were determined by UPLC-MS/MS (ultra-performance liquid chromatography - tandem mass spectrometry) analysis that was based on Plessers et al.[25]. The prostaglandins were extracted using a liquid-liquid extraction. In brief, to 50 µl of plasma were added 25 µL of the internal standard working solution (10 ng/mL, $PGE_2$-d4) and 200 µL of water. The sample was vortexed (15 sec) and acidified using 25 µl of a 1N hydrogen chloride solution. After vortex mixing (15 sec) 6 mL of the extraction solvent (hexane/ethyl acetate, 1/1, v/v) were added. Samples were extracted for 25 min on a roller mixer (Stuart Scientific, Surrey, UK) and centrifuged (2851 × g, 10 min, 4°C). Next, the supernatant was

transferred to a glass tube and evaporated using a gentle nitrogen ($N_2$) stream (40 ± 5°C). The dry residue was reconstituted in 125 µL of a methanol/water (1/9, v/v) mixture. After vortex mixing (15 sec), the samples were filtered through a Millex® PVDF syringe filter (0.22 µm) and transferred into an autosampler vial. An aliquot (10 µL) was injected onto the UPLC-MS/MS instrument for quantification of the $PGE_2$ concentration.

For each group, the mean area under the plasma concentration-time curve (AUC) was calculated using Graphpad 5 Software (Prism, La Jolla, CA, USA).

### 2.4 Statistical analysis

The AUC and individual body temperatures were compared between the different treatment groups and the control group using one way-analysis of variance (ANOVA, SPSS 23.0, IBM Corporations, New York, USA). Post-hoc comparisons were performed according to the Dunnett t-test. The level of significance was set at 0.05. A sinus transformation of the percentage of time the birds exhibit the above described behavioral changes and the time spent on a certain location was first performed before conducting the statistical analysis. One-way ANOVA analysis was performed to compare the percentage the cockatiels of the different groups showed a certain state of consciousness, signs of illness, time spent on feed- and water uptake, grooming behavior, exercise, and time spent on a certain location during hypothermia ($T_{24\text{-}28}$), the period after hypothermia ($T_{28\text{-}34}$) and the entire experiment ($T_{24\text{-}34}$). Post-hoc comparisons between the treatment and control groups were performed according to the Dunnett t-test. The level of significance was set at 0.05. The sparse sampling procedure applied, made it not possible to perform any statistical analysis on the difference of AUC of $PGE_2$ of the different treatment groups.

## 3. RESULTS

### 3.1 LPS model validation study

Mean (±SD) body temperatures of both groups during the model validation study (LPS group and negative control group) are depicted in **Figure 2**. In the LPS group, hypothermia was observed at $T_{24\text{-}28}$, with the lowest body temperature measured at $T_{25.5}$ (39.9 ± 1.15°C), followed by a plateau phase corresponding with the normal body temperature of the cockatiels (41.4 ± 0.69°C). An increase in body temperature (41°C to 42°C) was observed at $T_{32\text{-}34}$ due to elimination of LPS, leading to an increase in activity of the birds. In the negative control group, no hypothermia was observed. The mean body temperature in the negative control group was higher at $T_{24\text{-}28}$ (41.6 ± 0.56°C) than at $T_{28\text{-}34}$ (41.2 ± 0.72°C) since the birds got used to the manipulations and the presence of humans in the room.

### 3.2 Pharmacodynamic study

One bird was excluded from the control group, due to prolonged hypothermia and severe clinical signs of illness. One bird was excluded from the mavacoxib group due to regurgitation of the drug, leading to underdosing of mavacoxib. One bird of the meloxicam and celecoxib group died immediately after the second LPS administration ($T_{24}$). All other birds were adopted after the experiments.

#### *3.2.1 Body temperature*

The mean body temperature of the four different groups (control, mavacoxib, celecoxib and meloxicam) is depicted in **Figure 3**. The mean body temperature of the control group demonstrated hypothermia during the first 4h following the second LPS injection. In the control group, the lowest body temperature was observed at $T_{25.5}$ (40.2 ± 0.89°C). After

hypothermia, the mean body temperature raised ($T_{26\text{-}27.5}$) and fluctuated around 41.4 ± 0.88°C until the end of the experiment ($T_{28\text{-}34}$). The evolution of the mean body temperature of the cockatiels treated with meloxicam was comparable to the control group. However, hypothermia was less pronounced (lowest body temperature ($T_{26}$): 40.6 ± 0.47°C). A mild hypothermia was observed in the birds treated with mavacoxib and celecoxib with the lowest temperature observed at $T_{25.5}$ (41.5 ± 0.51°C) and $T_{26}$ (41.3 ± 0.74°C), respectively.

No significant difference in AUC was observed between the different groups for the period during hypothermia ($T_{24\text{-}28}$, $p = 0.76$) and the period after hypothermia ($T_{28\text{-}34}$, $p = 0.28$). When comparing the individual time points during hypothermia, a significant difference between the different groups could only be observed at $T_{25}$, $T_{25.5}$, $T_{26}$ and $T_{26.5}$. No significant difference in mean body temperature could be observed during hypothermia ($T_{24\text{-}28}$) between the control group and the cockatiels treated with meloxicam ($p = 0.22\text{-}0.99$). The mean body temperature of the birds treated with mavacoxib was significantly different from the control group at $T_{25}$, $T_{25.5}$ and $T_{26}$ ($p < 0.01$). A significantly higher mean body temperature was observed in the group treated with celecoxib in comparison with the control group at $T_{25}$ ($p = 0.03$) and $T_{25.5}$ ($p = 0.01$).

### *3.2.2 State of consciousness and signs of illness*

A significant decrease in alertness, and increase in dyspnea and ruffled feathers was observed in all groups during hypothermia ($T_{24\text{-}28}$) in comparison with the period after hyperthermia ($T_{28\text{-}34}$)($p < 0.01$) (**Table 1**). No significant differences were observed between the cockatiels treated with one of the three NSAIDs (mavacoxib ($p = 0.22$), celecoxib ($p = 0.47$), meloxicam ($p = 0.88$)) and the control group in the percentage of time showing alertness, dyspnea and ruffled feathers during the entire experiment ($T_{24\text{-}34}$). Only the cockatiels treated with mavacoxib were significantly more alert during hypothermia ($p = 0.04$) and showed less

294 ruffled feathers during the period after hypothermia ($p = 0.02$) in comparison with the control
295 group.
296
297 Little activity (less than 50% of the time) was observed during the entire experiment in all
298 groups (**Table 2**). No significant differences in activity ($p = 0.18$) and grooming ($p = 0.29$)
299 was observed between the different groups. In general, the time spent with feed- and water
300 uptake was low during the trial.
301
302 The preferential location in the cage of the cockatiels after the second LPS injection was on
303 the floor and on the perch (**Table 3**). No significant differences in percentage of time spend
304 on the floor, wire mesh or perch was observed between the different treatment groups
305 compared to the control group ($p = 0.93$, $p = 0.67$ and $p = 0.95$, respectively).
306
307 *3.2.3 Plasma prostaglandin $E_2$ concentration*

308 The mean plasma $PGE_2$ concentration (+SD) of the control and NSAID treated groups are
309 depicted in **Figure 4**. The mean plasma $PGE_2$ concentration of the control group described a
310 maximum at $T_{24.5}$, followed by a plateau phase and a steady decrease after $T_{28}$. The average
311 AUC of the control group (1038.0 h.pg/mL) was higher in comparison with the birds treated
312 with mavacoxib (628.3 h.pg/mL), celecoxib (526.3 h.pg/mL) and meloxicam (222.0
313 h.pg/mL), respectively.

## 4. DISCUSSION

For the first time an *in vivo* cockatiel LPS-induced inflammation model was developed to study the PD parameters (body temperature, clinical appearance and plasma $PGE_2$ concentration) of the selective COX-2 inhibitors celecoxib, mavacoxib and meloxicam. In the present study, hypothermia was observed after IV LPS administration to cockatiels (*Psittaciformes*). This was in accordance with the LPS-induced body temperature changes detected by Burness et al.[26] in *Passeriformes* (Zebra finches, *Taeniopygia guttata*). These observations were in contrast with the results obtained in chickens and pigeons, where a short period of hypothermia was followed by hyperthermia. In ducks and Japanese quails, only hyperthermia occurred after IV LPS administration[27]. In accordance with the *Passeriformes*, the observed hypothermia might be linked with the high body surface area-volume ratio in association with the high body temperature of cockatiels. Consequently, relative heat loss is higher in cockatiels in comparison to larger birds species, which complicates thermoregulation. Moreover, small birds, such as cockatiels, are characterized by a higher basal metabolic rate compared to larger bird species. The mean body temperature of cockatiels is already high (41.7°C), whereby an increase in body temperature is complicated, since an increase in body temperature of 1°C requires an increase of 10% in metabolism[28,29].

During the period after hypothermia, body temperature fluctuated around 41.4 °C, probably due to an increase in alertness and activity (flying and climbing) of the birds, leading to a stress-induced increase in body temperature during measurements. The decrease in time spent on grooming behavior, and feed- and water consumption was probably due to stress caused by the frequent handlings performed during the experiments.

The changes in body temperature and clinical appearance are more pronounced when administering mavacoxib in comparison to the other NSAIDs tested in the current study. This

might be associated with the higher oral bioavailability of mavacoxib compared to the other NSAIDs (mavacoxib: 111-113%, celecoxib: 56-110%, meloxicam: 11%)[14,17]. Besides, the clearance of mavacoxib is slower (mavacoxib: 0.033 L/h.kg, celecoxib: 4.32 L/h.kg, meloxicam: 3.38 L/h.kg), leading to a longer elimination half-life (mavacoxib: 135.41 h, celecoxib: 0.88 h, meloxicam: 0.90 h) and a prolonged therapeutic effect[17]. Finally, tissue distribution of mavacoxib is higher due to its larger volume of distribution (mavacoxib: 6.35 L/kg, celecoxib: 5.49 L/kg, meloxicam: 4.40 L/kg), possibly influencing the local prostaglandin production[17,30].

The extent of inhibition of the COX-2 enzyme activity was determined by measuring the $PGE_2$ plasma concentration. The lowest and highest $PGE_2$ plasma concentrations were achieved after administration of meloxicam and mavacoxib, respectively. These results are in contrast with the other observed PD effects, where the effects on body temperature and clinical appearance of mavacoxib were more pronounced than meloxicam. $PGE_2$ production is both expressed constitutively as induced by inflammation[31,32]. Meloxicam has an influence on both mechanisms, possibly leading to lower $PGE_2$ plasma concentrations. Whereas mavacoxib and celecoxib are COX-2 selective inhibitors and have only an influence on $PGE_2$ production induced by inflammation. The lack of correlation between changes in body temperature and plasma $PGE_2$ concentration, was in contrast with the results obtained in mammals, but was similar with the results observed in broiler chickens[20,21,22]. A first explanation might be that in birds other prostaglandin systems might be involved in LPS-induced temperature and behavioral changes. Whether the changes in body temperature are caused by peripheral or central production of prostaglandins remains unknown. A second explanation might be the high lipophilicity of mavacoxib and celecoxib, enabling better penetration of the central nervous system, influencing thermoregulation[33]. This theory was

364 also opted by Johnson et al.[34], who administrated indomethacin centrally and was able to
365 inhibit the LPS-induced hyperthermia. Moreover, Guo et al.[35] administered celecoxib to rats
366 and discovered an inhibition of the central COX-2 expression. Consequently, the changes in
367 body temperature and behavior might be explained by the inhibition of the production of
368 cerebral prostaglandins.
369

370 **5. CONCLUSIONS**

371 In conclusion, an *in vivo* cockatiel LPS-induced inflammation model to study the PD of the
372 COX-2 selective inhibitors celecoxib, mavacoxib and meloxicam was developed. The present
373 study demonstrated that the birds treated with mavacoxib and celecoxib are less prone to LPS-
374 induced hypothermia in comparison to meloxicam. Despite the lack of a clear correlation
375 between illness and changes in body temperature, an increased alertness was observed after
376 administration of mavacoxib. Consequently, suggesting that mavacoxib was more effective
377 for the treatment of LPS-induced hypothermia than meloxicam and celecoxib. The absence of
378 a correlation between the change in body temperature and plasma $PGE_2$ concentration
379 demonstrated that different mechanisms might be involved in thermoregulations. Further
380 research is required to determine the specific role of the prostaglandins in hypothermia
381 (central or peripheral $PGE_2$ production) in birds.

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**Declaration of interests**

None.

**Authors' contributions**

EG: general coordination, animal study, preparation, review and final approval of the manuscript

REH: study design, animal study, data interpretation, preparation, review and final approval of the manuscript

ROH: animal study, data interpretation, review and final approval of the manuscript

SDB: analytical analysis, review and final approval of the manuscript

SS: data interpretation, statistical analysis, review and final approval of the manuscript

GA: general coordination, study design, animal study, review and final approval of the manuscript

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**Table 1.** Mean percentage (± SD) of time the cockatiels (n = 7, for each group) showed a certain state of consciousness and signs of illness after a second LPS injection combined with no (control), mavacoxib, celecoxib and meloxicam treatment.

| **Period during hypothermia ($T_{24-28}$)** | | | | |
|---|---|---|---|---|
| | **Control** | **Mavacoxib** | **Celecoxib** | **Meloxicam** |
| **alertness (%)** | 22.2 ± 5.9 | 54.0 ± 11.2* | 36.5 ± 10.2 | 23.8 ± 5.6 |
| **apathetic (%)** | 17.5 ± 5.9 | 11.1 ± 5.4 | 27.0 ± 4.8 | 20.6 ± 6.1 |
| **soporose (%)** | 60.3 ± 10.0 | 34.9 ± 10.1 | 36.5 ± 11.8 | 55.6 ± 10.6 |
| **ruffled feathers (%)** | 38.1 ± 10.0 | 66.7 ± 10.8 | 57.1 ± 15.0 | 41.3 ± 10.2 |
| **dyspnea (%)** | 14.3 ± 9.9 | 20.6 ± 10.7 | 1.6 ± 1.6 | 9.5 ± 9.5 |
| **Period after hypothermia ($T_{28-34}$)** | | | | |
| | **Control** | **Mavacoxib** | **Celecoxib** | **Meloxicam** |
| **alertness (%)** | 60.7 ± 15.8 | 73.8 ± 17.0 | 63.1 ± 13.1 | 51.2 ± 14.5 |
| **apathetic (%)** | 13.1 ± 6.5 | 1.2 ± 1.2 | 20.2 ± 7.0 | 20.2 ± 8.7 |
| **soporose (%)** | 26.2 ± 16.9 | 25.0 ± 16.2 | 16.7 ± 13.1 | 28.6 ± 12.2 |
| **ruffled feathers (%)** | 11.9 ± 3.1 | 60.7 ± 16.7* | 47.6 ± 15.9 | 11.9 ± 3.6 |
| **dyspnea (%)** | 0.0 ± 0.0 | 4.8 ± 4.8 | 0.0 ± 0.0 | 2.4 ± 2.4 |
| **Entire experiment ($T_{24-34}$)** | | | | |
| | **Control** | **Mavacoxib** | **Celecoxib** | **Meloxicam** |
| **alertness (%)** | 41.4 ± 10.7 | 63.6 ± 13.0 | 45.7 ± 8.0 | 36.4 ± 8.3 |
| **apathetic (%)** | 15.7 ± 4.3 | 5.7 ± 2.3 | 24.3 ± 4.7 | 21.4 ± 6.8 |
| **soporose (%)** | 42.9 ± 13.3 | 30.7 ± 13.2 | 30.0 ± 9.5 | 41.4 ± 8.0 |
| **ruffled feathers (%)** | 73.6 ± 12.9 | 50.7 ± 14.5 | 50.7 ± 15.8 | 79.3 ± 5.9 |
| **dyspnea (%)** | 7.9 ± 4.3 | 12.1 ± 7.1 | 0.7 ± 0.7 | 5.7 ± 5.7 |

***results are significantly different from the control group ($p < 0.05$)**

**Table 2.** Mean (±SD) percentage of time the cockatiels (n = 7, for each group) were active (climbing or flying), grooming and consume feed-and water after a second LPS injection combined with no (control), mavacoxib, celecoxib and meloxicam treatment.

| | **Control** | **Mavacoxib** | **Celecoxib** | **Meloxicam** |
|---|---|---|---|---|
| **activity (%)** | 33.8 ± 5.0 | 33.3 ± 4.4 | 44.2 ± 5.1 | 44.0 ± 3.5 |
| **grooming (%)** | 0.3 ± 0.2 | 3.9 ± 1.9 | 0.9 ± 0.7 | 2.1 ± 1.9 |
| **feed- and water consumption (%)** | 0.8 ± 0.4 | 0.0 ± 0.0 | 1.7 ± 0.4 | 0.7 ± 0.5 |

**Table 3.** Mean (±SD) percentage of time the cockatiels (n = 7, for each group) spent on a certain location in the cage after a second LPS injection combined with no (control), mavacoxib, celecoxib and meloxicam treatment.

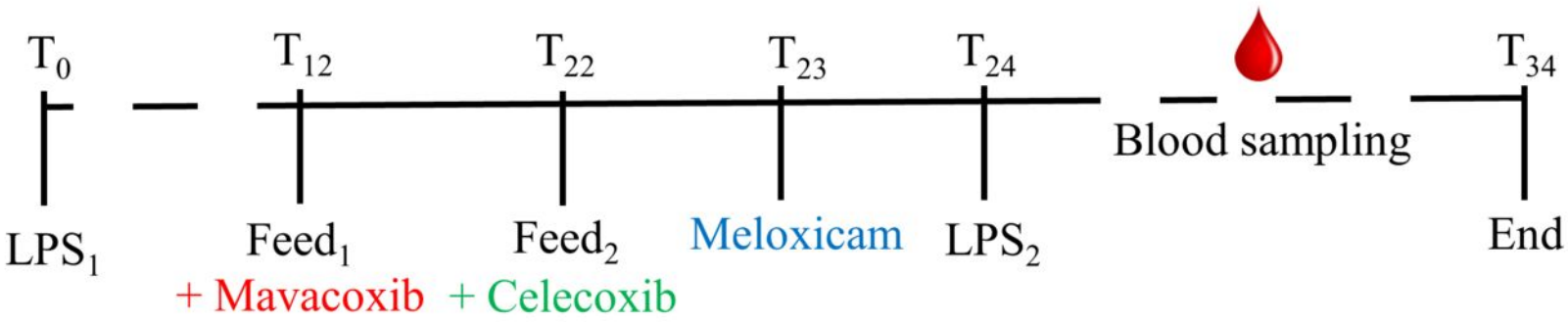
| | Control | Mavacoxib | Celecoxib | Meloxicam | Mean |
|---|---|---|---|---|---|
| **floor (%)** | 45.8 ± 13.5 | 33.8 ± 9.6 | 36.0 ± 13.4 | 37.7 ± 11.3 | 38.3 ± 11.5 |
| **wire mesh (%)** | 16.1 ± 5.7 | 24.7 ± 6.8 | 21.3 ± 12.4 | 13.8 ± 5.8 | 19.0 ± 7.9 |
| **perch (%)** | 38.0 ± 13.2 | 41.4 ± 12.6 | 42.7 ± 12.2 | 48.5 ± 8.9 | 42.7 ± 11.3 |

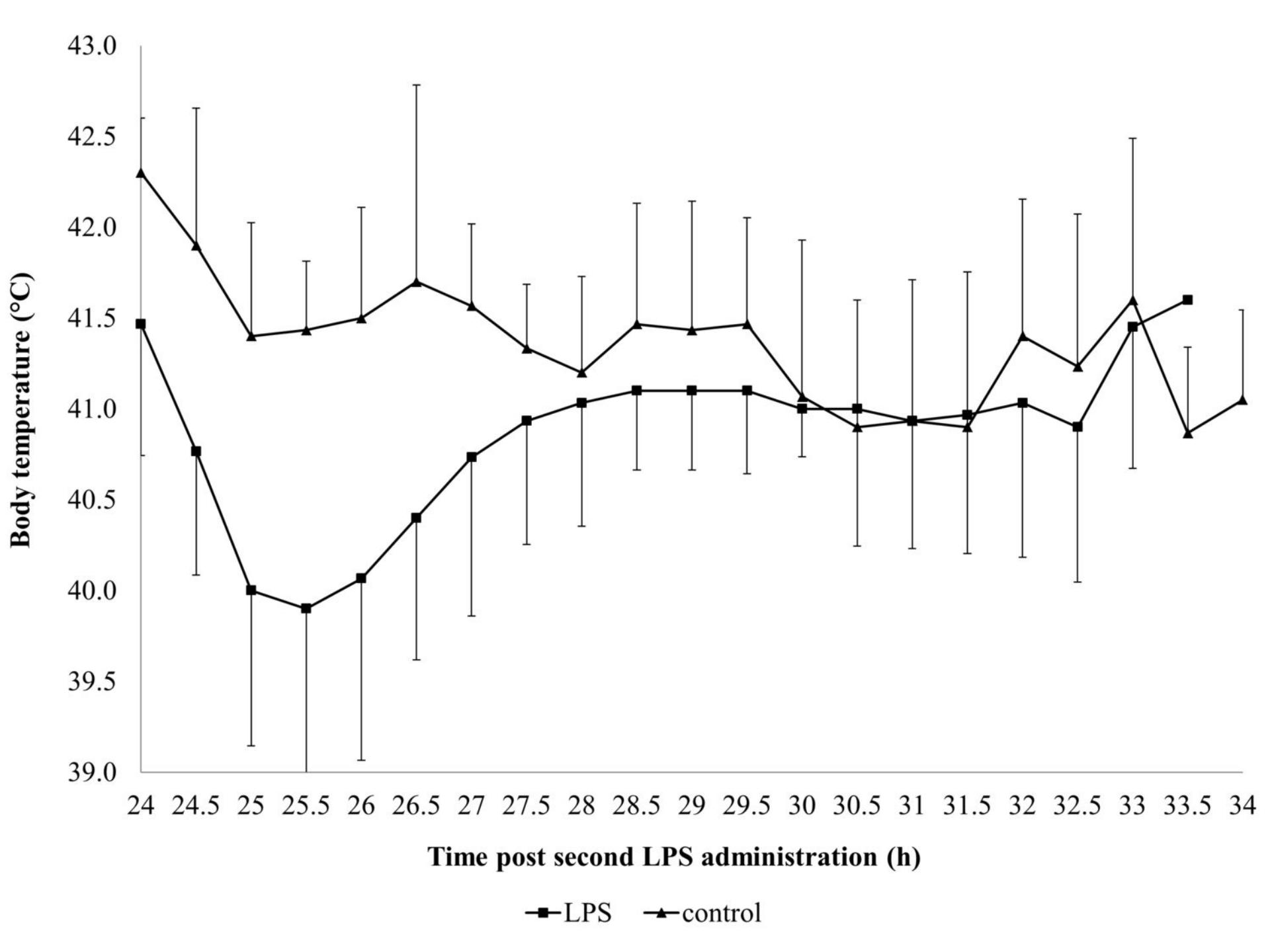
**Figure 1.** Experimental design pharmacodynamic study. $LPS_{1/2}$: lipopolysaccharide dose 1 and 2, respectively; T: time point post first LPS administration (h).

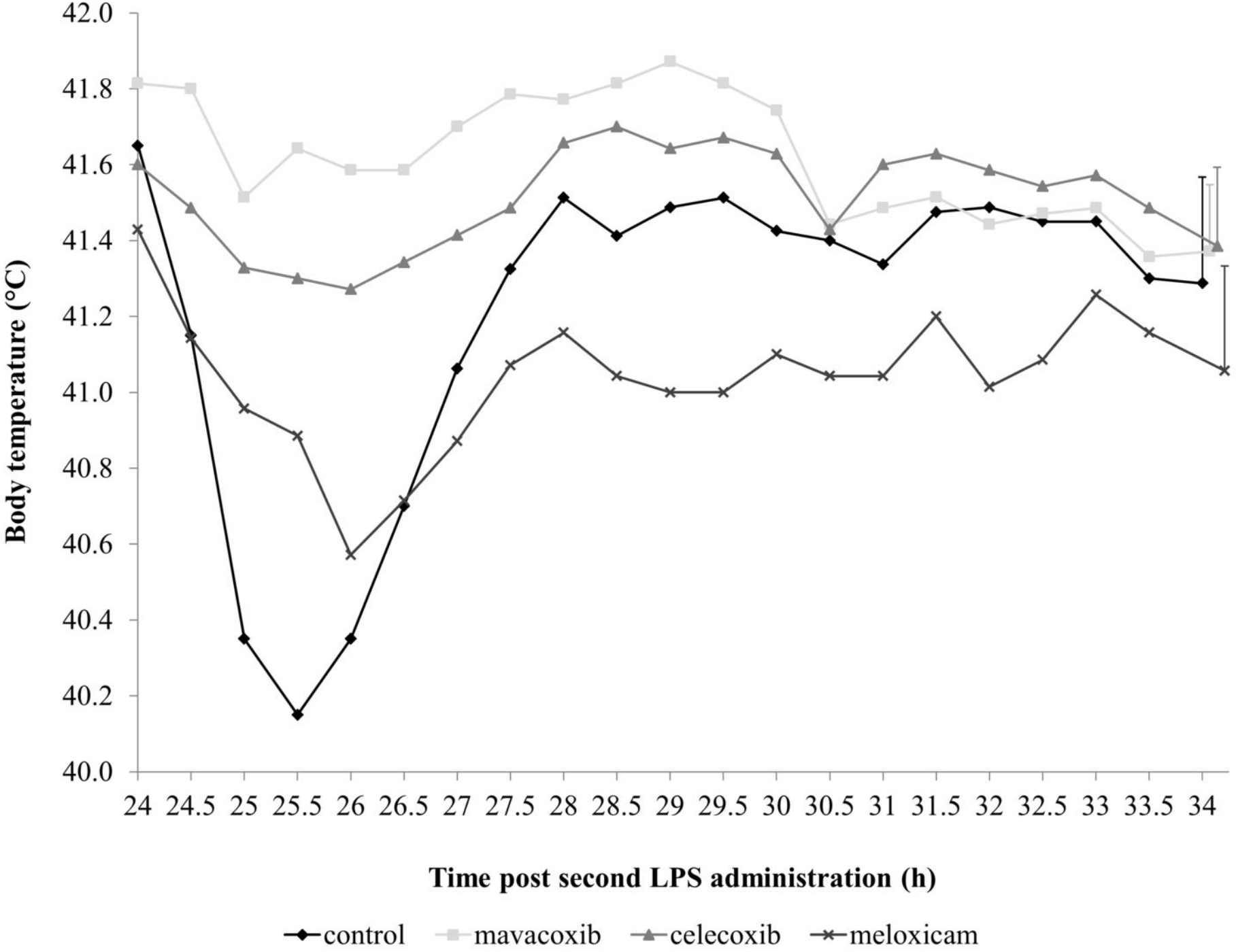
**Figure 2.** Evolution of mean (±SD) body temperature after second LPS (7.5 mg/kg BW) (LPS, □) and an equivalent 0.9% NaCl bolus (control, Δ) administration in cockatiels (n = 3 for each group).

**Figure 3**. Evolution of mean (+SD last time point) body temperature of cockatiels (n = 7, for each group) after receiving a second LPS injection combined with no (control), mavacoxib, celecoxib and meloxicam treatment.

**Figure 4.** Mean (+SD) plasma prostaglandin E2 concentration versus time curves of cockatiels (n = 7, for each group) after receiving a second LPS injection combined with no (control), mavacoxib, celecoxib and meloxicam treatment.


T0
T12
T22
T23
T24
T34
Blood sampling
LPS1
Feed1
+ Mavacoxib
Feed2
+ Celecoxib
Meloxicam
LPS2
End


43.0
42.5
42.0
41.5
41.0
40.5
40.0
39.5
39.0
Body temperature (°C)
24
24.5
25
25.5
26
26.5
27
27.5
28
28.5
29
29.5
30
30.5
31
31.5
32
32.5
33
33.5
34
Time post second LPS administration (h)
LPS
control


42.0
41.8
41.6
41.4
41.2
41.0
40.8
40.6
40.4
40.2
40.0
Body temperature (°C)
24 24.5 25 25.5 26 26.5 27 27.5 28 28.5 29 29.5 30 30.5 31 31.5 32 32.5 33 33.5 34
Time post second LPS administration (h)
control
mavacoxib
celecoxib
meloxicam

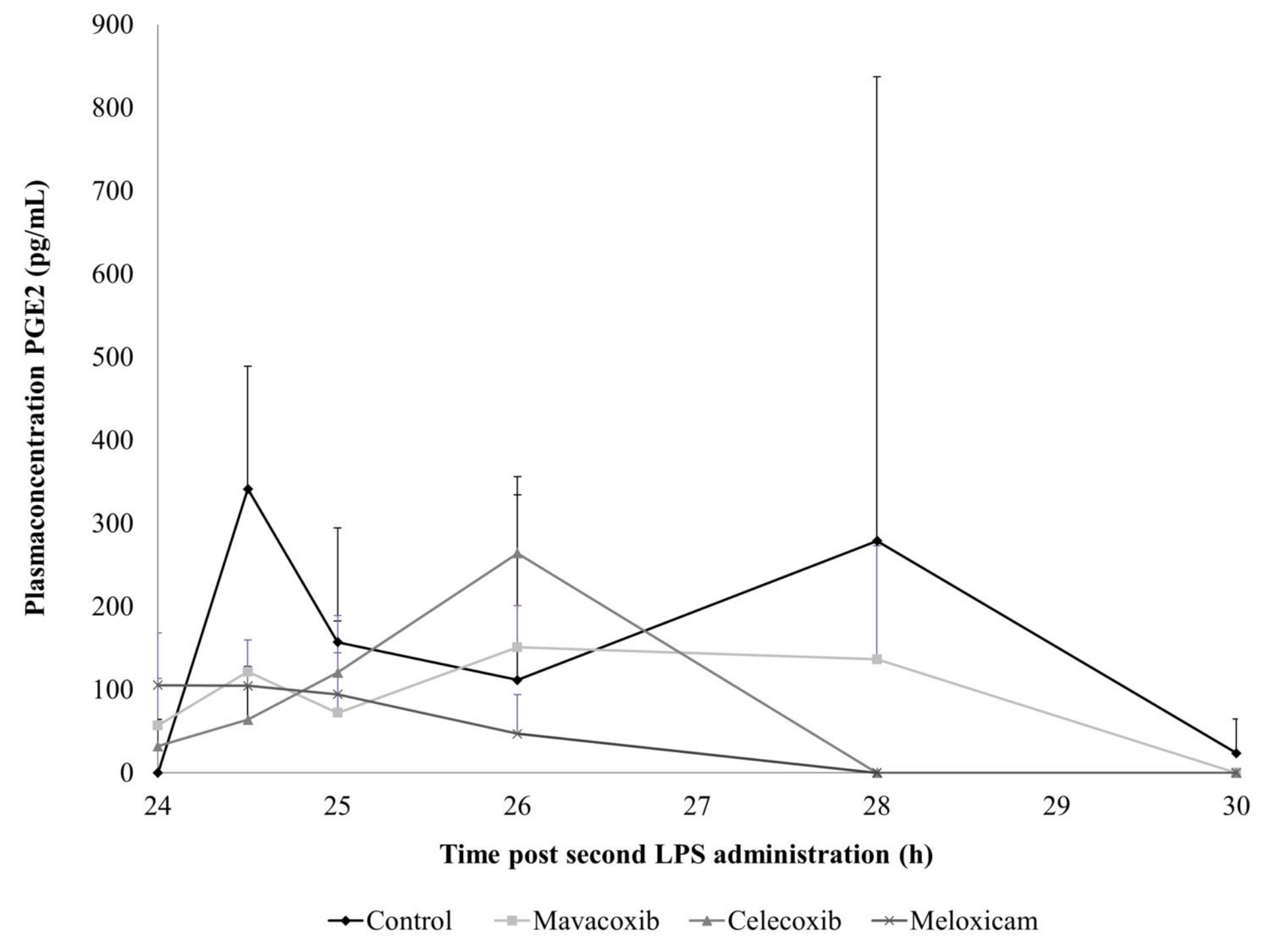

900
800
700
600
500
400
300
200
100
0
Plasmaconcentration PGE2 (pg/mL)
24
25
26
27
28
29
30
Time post second LPS administration (h)
Control
Mavacoxib
Celecoxib
Meloxicam

**Table 1.** Mean percentage (± SD) of time the cockatiels (n = 7, for each group) showed a certain state of consciousness and signs of illness after a second LPS injection combined with no (control), mavacoxib, celecoxib and meloxicam treatment.

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| | **Control** | **Mavacoxib** | **Celecoxib** | **Meloxicam** |
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| | **Control** | **Mavacoxib** | **Celecoxib** | **Meloxicam** |
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