CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Exploring gastric emptying rate in mini-pigs: Effect of food type and pre-dosing of metoclopramide
Authors	Henze, Laura J.;Griffin, Brendan T.;Christiansen, Martin;Bundgaard, Christoffer;Langguth, Peter;Holm, René
Publication date	2018-03-17
Original Citation	Henze, L. J., Griffin, B. T., Christiansen, M., Bundgaard, C., Langguth, P. and Holm, R. (2018) 'Exploring gastric emptying rate in mini-pigs: Effect of food type and pre-dosing of metoclopramide', European Journal of Pharmaceutical Sciences, In Press. doi: 10.1016/j.ejps.2018.03.017
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S0928098718301325 - 10.1016/j.ejps.2018.03.017
Rights	© 2018 Elsevier B.V. All rights reserved. This manuscript version is made available under the CC-BY-NC-ND 4.0 license. - https://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2024-03-29 16:00:49
Item downloaded from	https://hdl.handle.net/10468/5697



UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Accepted Manuscript



Exploring gastric emptying rate in mini-pigs: Effect of food type and pre-dosing of metoclopramide

Laura J. Henze, Brendan T. Griffin, Martin Christiansen, Christoffer Bundgaard, Peter Langguth, Rene Holm

PII: S0928-0987(18)30132-5
DOI: doi:10.1016/j.ejps.2018.03.017
Reference: PHASCI 4446

To appear in: *European Journal of Pharmaceutical Sciences*

Received date: 27 November 2017
Revised date: 15 March 2018
Accepted date: 16 March 2018

Please cite this article as: Laura J. Henze, Brendan T. Griffin, Martin Christiansen, Christoffer Bundgaard, Peter Langguth, Rene Holm , Exploring gastric emptying rate in mini-pigs: Effect of food type and pre-dosing of metoclopramide. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Phasci(2017), doi:10.1016/j.ejps.2018.03.017

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Exploring gastric emptying rate in mini-pigs: effect of food type and pre-dosing of metoclopramide

Laura J. Henze[1], Brendan T. Griffin[1], Martin Christiansen[2], Christoffer Bundgaard[3], Peter Langguth[4], Rene Holm[5,*]

[1]School of Pharmacy, University College Cork, Ireland

[2] Department of Pharmacy, University of Copenhagen, DK-2100 Copenhagen, Denmark

[3] Discovery DMPK, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen-Valby, Denmark

[4] Institute of Pharmacy and Biochemistry, Johannes Gutenberg University of Mainz, 55128, Mainz, Germany.

[5] Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium

*Correspondence to:

Dr. René Holm, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium. Email: rholm@its.jnj.com, Tel.: (+32) 01460 8107, Fax: (+ 32) 01464 1956

ACCEPTED MANUSCRIPT

Abstract

The present study investigated the gastric emptying rate in Göttingen minipigs pre- and post-prandial and evaluated the effect of metoclopramide on the same parameter, using paracetamol as an absorption marker. The pharmacokinetic evaluation of the obtained plasma concentration data for paracetamol demonstrated that the fastest gastric emptying rate was observed in the animals that were allowed access to normal pig food. There were no significant difference in the stomach emptying rate observed between fasted and fed minipigs, when fed either with a FDA standard breakfast or a nutritional energy drink. Pre-dosing minipigs with metoclopramide (0.2 or 0.4 mg/kg) did not demonstrate any effect on gastric emptying in either the fasted or fed state. The data in the present study demonstrated a relatively prolonged gastric emptying rate in mini-pigs both in the fasted and fed state, hence when conducting pharmacokinetic studies using mini-pigs, this should be taken into account in planning the plasma sampling time points. Further, as no difference could be observe in gastric emptying rate as a function of food the data also suggest that the Göttingen minipigs seems less suited to predict for food effect studies than other species.

Keywords; gastric emptying, mini-pigs, metoclopramide, fasted state, fed state

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1 Introduction

Reliable pre-clinical models for accurate evaluations of different formulations during drug development can be important to enable shorter development timelines. Here the pig model has shown good promise, given the physiological similarities to humans, where there is a general agreement that pigs could be a useful model for human oral absorption (Bode *et al.*, 2010; Kararli, 1995; Merchant *et al.*, 2011; Sjogren *et al.*, 2014; Walters *et al.*, 2011) and thereby a potential valuable tool in drug formulation development.

Gastric emptying is a fundamental factor influencing drug absorption, given that any change in stomach emptying will influence the rate at which drug is presented for absorption in the small intestine. The transit time between the stomach and the small intestine is, therefore an important characteristic in the oral absorption process. Gastric emptying rates in various races of pigs have been reported to show high inter-individual and inter-study variability. Hossain *et al.* (1990) and Oberle and Das (1994) observed a long gastric emptying rate in landrace pigs, in the range of 6-24 h. In contrast to this, Davis *et al.* (2001) reported gastric emptying times after a light meal to be closer to 6 h in landrace pigs. While historically, landrace pigs were the most widely used species, in recent times, minipigs and in particular the Göttingen minipig have become common in pre-clinical studies (Forster *et al.*, 2010). However, relatively few studies investigating gastric emptying in minipigs have been reported in the literature. Christiansen *et al.* (2015) investigated the potential of a fed state model developed for dogs in Göttingen minipigs, but the reported data demonstrated that the protocol was not able to differentiate between a drug with positive or negative food effect. The study also reported relatively long gastric emptying rates in minipigs. Suenderhauf and co-workers (2014) assessed the pharmacokinetics of paracetamol in fasted Göttingen minipigs to assess gastric emptying times. The study found that gastric transit times in overnight fasted minipigs were longer than those

ACCEPTED MANUSCRIPT

observed in humans. The study also highlighted the need of further work to develop effective fasting protocols for minipigs, as it was observed that a 12 hour fasting period was not sufficient to obtain an empty stomach in the animals based on the residual stomach contents measured post-mortem. Interestingly, minipigs that received 0.2 mg/kg metoclopramide i.m 2 h before necropsy had macroscopically empty stomachs, suggesting that this pre-treatment protocol with this pro-kinetic agent could be a feasible protocol to ensure consistent fasted state in minipigs.

The amount and type of food ingested can have a significant influence on gastric emptying and resultant oral drug bioavailability (Houghton *et al.*, 1990; McLaughlin *et al.*, 1998), and for this reason, it is a regulatory requirement to conduct fed and fasted state human clinical studies for all new oral medicines. Given the increasing need to streamline drug product development and avoid costly delays in clinical testing, pre-clinical *in vivo* models are an important tool to assess food dependent oral bioavailability– with a view to either predict likely food effects in humans or develop food independent formulation strategies. Currently, a pre-clinical food effect model is described in dogs (Lentz *et al.*, 2007), but given the similarities between humans and pigs gastrointestinal physiology, it is pertinent to consider the development of a protocol for food effect studies in the pigs. The Christiansen *et al.* (2015) study demonstrated that the protocol for dogs could not be transferred directly to minipigs due to the long gastric emptying rate observed relative to humans. However the observations by Suenderhauf *et al.* (2014) involving pre-treatment with 0.2 mg/kg metoclopramide to ensure gastric emptying represents a potentially interesting approach to ensure to developing a protocol in minipigs.

Paracetamol is known to be poorly absorbed in the stomach, but rapidly absorbed upon entry into the small intestine, which makes it a good marker when examining gastric emptying rate (Clements *et al.*, 1978; Heading *et al.*, 1973). The aim of the present study was to investigate the gastric emptying rate in Göttingen minipigs in the pre- and post-prandial state after

ACCEPTED MANUSCRIPT

administration of different food sources as well examining the impact of pre-administration of metoclopramide on paracetamol absorption under varying prandial conditions.

2 Materials and methods

2.1 Materials

500 mg paracetamol (Panodil®) film-coated tablets was obtained from Glaxo Smithkline (Brentford, UK). Fresubin energy drink ® was from Fresenius Kabi (Bad Homburg, Germany). Paracetamol and metoclopramide was purchased from Sigma-Aldrich Co (St.Louis, MO, USA) and sodium chloride from Fluka Chemie AG (Buchs, Switzerland). Purified water was obtained from a Millipore Milli-Q Ultrapure Water purification system (Billeria, MA, USA). All other chemicals were of analytical grade.

2.2 *In vivo* study

The protocol used for the in vivo study was approved by the institutional animal ethics committee in accordance with Danish law regulating experiments on animals and in compliance with EC directive 2010/63/EU and the NIH guidelines on animal welfare. Female Göttingen minipigs were obtained from Ellegaard Göttingen Minipigs A/S (Dalmose, Denmark) weighing 9.1-11.3 kg at the start and 15.5-18.3 kg at the end of the study. Animals were acclimatised for 14 days before initiation of the study. During the acclimatisation period, animals were trained to eat both FDA standard breakfast (800- 1000 kcal) and the Fresubin energy drink (375 kcal). The FDA standard breakfast consisted of two slices of bacon, two slices of buttered toast, two fried eggs, eight ounces of full milk, and four ounces of hash browns, which was homogenised and presented to the animals. Altromin 9023 (602 kcal; contains 25 fat kg^{-1}, 135 g protein kg^{-1}). was utilized as a normal pig food, the pigs were fed 120 g twice a day. The minipigs were examined weekly by a veterinarian and observed closely at the end of each experimental day.

ACCEPTED MANUSCRIPT

Before entering the experiment, minipigs were fasted for 18–20 h with free access to water. They were housed with straw as bedding and stabled individually from the fasting period until the last blood sample was taken. Minipigs were kept on a 12 h light and 12 h night cycle throughout the experiment.

The study design was a non-randomized one sequence design with a 7-day wash-out period between treatments. Minipigs (n = 6) were weighed on the day and orally dosed with 2 tablets of paracetamol (1000 mg). A total sample size of 6 animals was required to achieve significance for comparisons between groups, using a paired t-test to assess difference between at a significance level of p = 0.05. These calculations were performed on G Power (version 3.1.9.2 University Kiel, Germany) and were based on an effect size 1.5, α error probability 0.05, and with a power of 0.80.

The following treatment groups were conducted:

1. Fasted (Fasted)
2. FDA breakfast 30 min before dosing ($\mathrm{FED}_{\mathrm{FDA\ breakfast}}$)
3. 250 ml Fresubin energy drink 30 min before administration ($\mathrm{FED}_{\mathrm{Energy\ drink}}$)
4. Free access to normal pig food, fed in the mornings ($\mathrm{FED}_{\mathrm{pig\ food}}$)
5. Fasted and 0,2 mg/kg metoclopramide intramuscular 2 h before dosing ($\mathrm{FASTED}^{\mathrm{MCP\ 0.2\ mg/kg}}$)
6. Fasted and 0,4 mg/kg metoclopramide intramuscular 2 h before dosing ($\mathrm{FASTED}^{\mathrm{MCP\ 0.4\ mg/kg}}$)
7. Pre-treatment with 0.4 mg/kg metoclopramide intramuscular 2 h and FDA breakfast 30 min before dosing ($\mathrm{FED}^{\mathrm{MCP\ 0.4mg/kg}}_{\mathrm{FDA\ breakfast}}$)

ACCEPTED MANUSCRIPT

8. Pre-treatment with 0.4 mg/kg metoclopramide intramuscular 2 h and 250 mL Fresubin energy drink 30 min before dosing ($FED_{\text{Energy drink}}^{\text{MCP 0.4mg/kg}}$)

In addition, the animals were administered paracetamol intravenously. The intravenous paracetamol formulation contained 24 mg paracetamol/mL, which was dissolved in purified water containing 0.7% (w/v) NaCl, and the pH was adjusted to 8.0. Each animal received 10 mL, i.e. a dose of 240 mg.

4 mg/mL metoclopramide solution for intramuscular injection was prepared in 0.9% sterile NaCl by magnetic stirring at ambient temperature and subsequent aseptic filtering through a sterile 0.22 μm filter into a sterile vial. The intramuscular dose was therefore 0.5 or 1 mL/10 kg, for the 2 and 4 mg/kg dose, respectively. Metoclopramide was injected intramuscularly two hours before paracetamol dosing and the food or energy drink was presented to the animals 30 min prior to oral dosing of the paracetamol tablets. In all cases the animals finished their meals quickly.

Blood samples (3.0 mL) were obtained by individual vein puncture of the vena jugularis. Blood samples were collected at 5, 15, 30 min 1, 2, 4, and 7 h after drug administration. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) coated tubes and immediately centrifuged for 10 min at 4 ºC, 2765g (Centrifuge Multifuge 1 S-R; Heraeus, Hanau, Germany). Plasma was harvested and stored at −80ºC until analysis.

2.3 Bioanalysis

Plasma concentrations of paracetamol were measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) as previously described (Christiansen *et al.*, 2015). In

ACCEPTED MANUSCRIPT

short, calibration standards and quality control (QC) samples were prepared by spiking drug-free porcine plasma and an internal standard [deuterated paracetamol (Toronto Research Chemicals Inc., Toronto, Ontario, Canada), in 10% trichloroacetic acid] was added to 10 µL of the calibration standards. The inter-assay inaccuracy was within ±15 % for all concentrations.

The MS peak area correlated linearly with the plasma concentration of the paracetamol in the range of 10–2000 ng/mL. If plasma concentrations exceeded the linear range, appropriate dilution was performed.

2.4 Pharmacokinetic Analysis

The pharmacokinetics of paracetamol in plasma of individual pigs was determined. After intravenous administration, the pharmacokinetic parameters for paracetamol were determined using compartmental pharmacokinetic analysis. Following oral administration of paracetamol, the area under the plasma concentration time curve (AUC), and mean residence time (MRT) were determined from non-compartmental analysis. Absolute bioavailability (F_{abs}) is defined as follows:

$$F_{abs} = \frac{Cl_{tot} * AUC}{Dose} \qquad (1)$$

F was determined as the dose normalised peak areas ratio of the oral to intravenous plasma profiles as follows:

$$F_{abs}^{0-7h} = \left(\frac{AUC_{oral}^{0-7h}}{AUC_{i.v.}^{0-7h}}\right) * \left(\frac{Dose_{i.v.}}{Dose_{oral}}\right) (2)$$

The area under the curve during the 7 hours sampling period (0-7h) was not consistently >80% AUC total (calculated as $AUC_{0\text{-}7h}$ plus the AUC of the extrapolated tail segment from 7h- ∞) across all treatment groups. As a result, absolute bioavailability was expressed as F_{abs}^{0-7h} determined using the AUC ratios within the sampling period.

ACCEPTED MANUSCRIPT

Parameters representative of the gastric emptying, equal to the absorption profile included; a) the maximum plasma concentration (C_{max}), b) the time corresponding to maximum plasma concentration (t_{max}), c) the mean absorption time (MAT), and d) the cumulated absorbed amount of paracetamol calculated by deconvolution.

MRT for a drug administered by a non-instantaneous input involves a mean absorption time (MAT), and can be defined as follows:

$$MRT_{po} = MAT + MRT_{iv} \qquad (3)$$

where MRT_{po} and MRT_{iv} represent the mean residence time of oral paracetamol and intravenous paracetamol. The MRT_{iv} was based on the intravenous group and MRT_{po} was determined from the six individual animals in each group, hence each individual animal served as its own control.

2.5 Deconvolution

Correlation between time functions, such as absorption and plasma concentrations, can mathematically be treated by system analysis (Langenbucher, 1982). The plasma concentration profile obtained after oral administration can be treated as the response function R(t), i.e. the response observed when administering a compound. W(t) is the weighting function of the body system, as observed after intravenous or oral bolus administration. The correlation between the weighting and response function depends upon the input and can algebraically be described by the integral (Langenbucher, 1982):

$$R(t) = \int_0^t I(\vartheta) W(t-\theta) d\theta \qquad (4)$$

where R, I and W are the response, input and weighting functions, respectively. I(t) combines the release and absorption process and can be described as release time function. The integral of this form is known as the convolution integral, written as:

ACCEPTED MANUSCRIPT

$$R(t) = I(t) * W(t) \qquad (5)$$

where * is the convolution operator. In the current study, R(t) and W(t) were known, i.e. I(t) representing the absorption process was sought, denoted deconvolution. Langenbucher (1982) previously described, the response function R(t), deconvolved with the weight function W(t) as:

$$I(t) = \frac{R(t)}{W\,(t)} \qquad (6)$$

A number of approaches exists to solve the deconvolution problem (Madden *et al.*, 1996), but in the present study numerical deconvolution, as described by Langenbucher (1982), was used and approximated with the sum:

$$I(x_i) = \frac{[R(t)\,/T - \sum_{k=1}^{n} I(x_k) * W(x_{n-k+1})]}{W(x_i)} \qquad (7)$$

where T is the time interval, $I(X_k)$ and $W(X_k)$ the average input rate and weight between the times X_{k-1} and X_k. The numerical deconvolution was based on the obtained plasma concentration data. The deconvolution profiles provide a dynamic picture of the absorption process, and were used to determine the time taken to absorb various fractions of paracetamol. The data were normalised across the different treatments and presented as percentage of total absorbed paracetamol fraction. Mean time points for 25%, 50% and 75% absorbed paracetamol were calculated from the deconvolution profiles.

2.6 Statistical Analysis

The pharmacokinetic analysis was conducted using the PKPlus™ module in Gastroplus™ (ver. 9.0, Simulations Plus Inc., Lancaster, Ca.), and the deconvolution was conducted in Excel. Results are presented as means and their standard error mean (± S.E.M.). A nonparametric statistical test was used, the Kruskal Wallis rank test, with Dunn's multiple comparison rank sums to determine the significance of difference in t_{max}, AUC, bioavailability and C_{max}. All

ACCEPTED MANUSCRIPT

statistical analyses were performed using GraphPad Prism version 5, and 5% was considered significant.

3 Results and discussion

In the present study, the gastric emptying rate in Göttingen minipigs was investigated under a number of different prandial conditions, including investigation of the influence of pre-dosing of metoclopramide.

3.1 Intravenous administration

In order to assess the disposition kinetics of paracetamol in mini-pigs, a paracetamol solution containing 240mg of paracetamol was administered intravenously (i.v.). The paracetamol plasma concentration time profile obtained after i.v. administration is shown in Fig. 1. A two-compartmental model was found to adequately fit the data. The observed volume of distribution was 0.83 ± 0.12 L/kg, which was comparable to values reported for mini-pigs elsewhere (0.7 L/kg) (Suenderhauf *et al.*, 2014). In landrace pigs, a volume of distribution of 1.5 L/kg has been reported (Neirinckx *et al.*, 2010). The total clearance of paracetamol in this study was determined to be 0.5 ± 0.0 L/(h *kg), which was 50% lower compared to mean systemic clearance of paracetamol in landrace pigs of 0.9 L/(h *kg) (Neirinckx *et al.*, 2010).

3.2 Absorption of paracetamol pre- and post-prandial

Mean plasma concentration time profiles of paracetamol following the eight different dosing conditions are shown in Fig. 2 and 3 and the associated mean pharmacokinetic parameters are presented in Table 1. Mean absorption time (MAT) is summarized in Fig. 4, and deconvolution fractiles in Table 2. In general, a lag phase was observed between oral administration of paracetamol and the onset of absorption.

ACCEPTED MANUSCRIPT

3.2.1 Effect of food intake

Food intake leads to a slower gastric emptying rate, which generally leads to a longer t_{max} and lower C_{max} when drug is dosed post- rather than pre- prandially. This may also potentially affect the overall extent of drug absorbed (Christiansen *et al.*, 2015). Differences in t_{max} or other pharmacokinetic parameters for paracetamol when dosed either in fasted or fed state would therefore reflect how the ingested meal delays gastric emptying, given paracetamol is rapidly absorbed once it enters the small intestine. The current study explored the use of t_{max}, MAT, and deconvolution profiles to detect differences in the absorption profiles between the different treatment groups investigated, namely: (i) homogenised FDA breakfast, (ii) a nutritional energy drink, and (iii) normal pig food 120 g provided twice a day.

Fig. 2A displays the plasma concentration profiles for the four groups: Fasted, $FED_{\text{Pig food}}$, $FED_{\text{FDA breakfast}}$, $FED_{\text{Energy drink}}$. While there was a general trend towards a higher AUC in the fasted than in the fed state, there were no statistical differences in either AUC or C_{max} for each treatment group. The time to reach peak concentrations (t_{max}) of Fasted, $FED_{\text{FDA breakfast}}$ and $FED_{\text{Energy drink}}$ groups was approximately four hours (see Fig 2B), whereas for the group fed with pig food, t_{max} was reached at 1.6 hours.

The mean plasma concentration profile from the fasted minipigs, showed a second plasma peak, which was on closer evaluation of the individual plasma profiles, was present in three of the six pigs in the group. For the animals pre-administered with metoclopramide this was only observed in one of the six animals. Appearance of double peaks after oral administration is not an uncommon phenomenon and have been described previously in the literature for other compounds (Charman *et al.*, 1993; Lipka *et al.*, 1995; Metsugi *et al.*, 2008; Takamatsu *et al.*,

ACCEPTED MANUSCRIPT

2002; Languth *et al.*, 1994), and has also been reported for paracetamol (Clements *et al.*, 1978). The exact reason for the double peak is unclear, but variable gastric emptying has been previously suggested as a cause for the double peaks with paracetamol (Metsugi *et al.*, 2008). The occurrence of a second plasma peak in paracetamol plasma profiles in the present study may therefore be related to variable gastric emptying in pigs.

Additionally, one animal in the fasted group had a plasma concentration profile where the absorption was incomplete within the 7 hours sampling period, and therefore a distinct elimination phase could not be determined from the terminal phase of the plasma profile. This may reflect that a truly fasted state was not achieved with a fasting period of 20 hours, as suggested by Suenderhauf and co-workers (2014). Comparing the fasted group with the group dosed with the nutritional energy drink (Fig 2A), it was apparent that the plasma concentration profiles had similar shapes, but the fed state profile was shifted to the right with a lag time of one hour. After one hour, the absorption curve closely followed the fasted state curve.

MAT has previously been used to estimate the absorption time (Ikegami *et al.*, 2003; Mizuta *et al.*, 1990) with a short MAT indicating fast absorption. The lowest MAT was observed for the minipigs fed with pig food (see Fig 2C). The animals receiving the FDA breakfast had a MAT similar to fasted minipigs, whereas the group administered with the Fresubin energy drink had a slightly longer MAT than the fasted group. The differences in MAT were not statistically significant between the groups.

A similar rank order between groups was observed in the deconvolution profile of paracetamol, with the slowest absorption observed for the animals receiving energy drink, followed by fasted, $FED_{\text{FDA breakfast}}$ and $FED_{\text{pig food}}$ (see Fig 2D). For the groups receiving pig food or the FDA breakfast, there was no significant difference between the deconvolution profiles, and these

ACCEPTED MANUSCRIPT

groups had slightly faster absorption profiles than the animals receiving Energy drink. The deconvolution analysis demonstrated that the profile of the animals receiving the Fresubin energy drink showed an initial delay of approximately one hour, indicating a delayed absorption rate. The use of the energy drink as an energy source therefore led to a different absorption profile, relative to both the fasted and the fed stage induced with the FDA standard breakfast.

To compare the differences between the deconvolution profiles in more detail the exact time points for 25%, 50% and 75% absorbed paracetamol where calculated, see Table 2. For minipigs fed with pig food, 50% of the drug was absorbed after 1.9 hours, whereas for the fasted minipigs it took more than 3 hours to absorb 50% of the paracetamol dose. Similar to previous findings, this is again indicative of a faster gastric emptying rate in minipigs fed the normal pig feed. Possible reasons for this are unclear but may be related to animal habituation. Despite training of the animals with the different food sources, they still received normal pig food the majority of the time, hence, the animals may perceive the normal feed as less stressful or their digestive system may simply be better adjusted to normal pig food. This may be reflected in the faster gastric emptying rate in these animals.

In summary, the data presented here showed that the fasted group had a trend towards a higher extent of absorption when compared to the fed states, but all in all there was no clear and significant differences in paracetamol absorption between fasted and fed Göttingen minipigs. These findings are in accordance with previous studies (Christiansen *et al.*, 2015). A delayed gastric emptying in the fed state groups could be expected, as food has the characteristics to delay gastric emptying (Sjogren *et al.*, 2014). Lack of clear difference between the groups indicates that a 'true' fasted state could not be achieved within a fasting period of 18–20 hours, as also previously described in the literature (Suenderhauf *et al.*, 2014). The observed lag times for fed state treatment were comparable and similar to human lag times; 0.6-1.4 hours in various

ACCEPTED MANUSCRIPT

fed states (Hellmig *et al.*, 2006). Based on present knowledge the variable absorption rates and difficulties in obtaining a true fasted state in minipigs, suggest that the minipig may be less useful for food effect studies on pharmaceutical drug formulation development.

3.3 Effect of Metoclopramide on gastric emptying

Drug absorption in the pre- and post-prandial states are important parameters for new drug candidates and their associated formulations during the development process. Christiansen and co-workers (2015) reported relative slow gastric emptying in minipigs, which Suenderhauf *et al*. suggested may be overcome by pre-treatment with 0.2 mg/kg of metoclopramide. Metoclopramide is a prokinetic agent and accelerates gastric emptying in man (Behar and Ramsby, 1978). A similar dose of 0.2 mg/kg metoclopramide was previously used in pigs to control gastric emptying (Gregory *et al.*, 1995). The current study utilised doses of 0.2mg/kg and 0.4mg/mg administered 2 hours pre-dosing.

3.3.1 Influence of Metoclopramide in the fasted state

Administration of 0.2 mg/kg metoclopramide led to a marginally higher t_{max} and MAT (see Table 1), when compared to the fasted group, however the difference were not statistically significant and hence gastric emptying was not considered to be influenced by the pre-treatment. There was no clear differences in the plasma profiles with dosing at 0.4mg.kg metoclopramide. While a trend towards a lower AUC for the group dosed with the 0.4 mg/kg was evident, as shown in Table 1, the difference in AUC was not statistically significant. For both plasma profiles, the paracetamol absorption was not fully completed at the end of the plasma sampling and no clear elimination phase was identified (Table 1 and Fig. 3A).).

The deconvolution profiles and plasma concentration profiles of the fasted minipigs in comparison to the fasted pre-treated minipigs are presented in Fig 3. Overall there was no

ACCEPTED MANUSCRIPT

significant difference in the absorption of paracetamol between the groups (Fig. 3B, D, and F). Therefore, the effect of pre-treatment of metoclopramide on lowering food content in the stomach of the minipigs observed by Suenderhauf *et al.* (2014), did not translate into a faster gastric transit in our studies, as measured by paracetamol absorption. The reason for the lack of effect of metoclopramide on paracetamol absorption in fasted pigs are unclear but may be related to the longer fasting period of 18-20 hour applied in our study, compared to 12 hours in the Suendefhauf study, with the longer period of fasting allowing for sufficient time for complete gastric emptying.

3.3.2 Influence of metoclopramide in the fed state – FDA breakfast

To assess the influence of metoclopramide on fed state gastric motility, minipigs were pre-treated with 0.4 mg/kg of metoclopramide 1.5 hours before oral administration of a FDA standard breakfast and or a total of 2 hours before oral dosing of paracetamol. The pre-treatment of metoclopramide showed no significant effect on t_{max} or MAT relative to the animals just receiving the FDA standard breakfast (see Table 1). A direct comparison of the plasma concentration profiles for the two groups are presented in Fig. 3C and the deconvolution profiles in Fig. 3D. The group pre-treated with metoclopramide showed a slower absorption and a lag time of two hours, however, no significant differences was observed. These data therefore suggest that the pre-treatment with metoclopramide did not increase the gastric emptying rate in the animals when administered a FDA standard breakfast. The pre-treatment protocol investigated had no significant influence on the absorption profile of paracetamol in the fed state, and therefore does not provide an approach whereby food effects could be investigated in in fed-state minipigs.

ACCEPTED MANUSCRIPT

3.3.3 Influence of metoclopramide in the fed state – nutritional energy drink

For the animals fed the Fresubin energy drink no significant effect of pre-treatment of metoclopramide was observed on t_{max} and MAT, relative to the fasted group. (Table 1), i.e. the gastric emptying rates were equal. Similarly there were no mean plasma concentration time profiles comparing $\text{Fasted}^{\text{MCP 0.2 mg/kg}}$ and $\text{Fed}^{\text{MCP 0.4 mg/kg}}_{\text{Energy drink}}$ (Fig. 3A and E).

Fig. 3F summarizes the mean deconvolution profile of the animals receiving 250 mL Fresubin energy drink 30 min before the oral administration of paracetamol and minipigs with or without pre-treatment of metoclopramide two hours before paracetamol administration. The shape of the mean deconvolution profiles from the two groups were similar, but a trend towards an increased and faster absorption was observed for the metoclopramide pre-treated minipigs. The combination of metoclopramide pre-dosing and nutritional energy drink led to a deconvolution profile similar to the one obtained in the fasted animals, as shown in Fig. 3A and E. To reach a true fasted state in minipigs, the effect from the pre-treatment with metoclopramide was therefore insignificant both for the FDA breakfast and the energy drink. The use of metoclopramide, therefore, did not appear to influence the gastric emptying rate in either the fasted or any of the two investigated fed states. These data are not fully supportive of the observations presented by Suenderhauf and co-workers (2014), which could reflect the differences in the methodology between the two studies, namely a fasting period of 18-20 hours vs. 12 hours and estimating gastric emptying based on paracetamol absorption versus weighed post-mortem stomach contents.

Assuming that paracetamol is a relevant marker for gastric emptying in minipigs, the data in the present study confirmed that mini-pigs have a longer gastric emptying rate than man and that it was not affected by pre-treatment with 0.2 - 0.4mg/kg metoclopramide. Food did not

ACCEPTED MANUSCRIPT

significantly influence the gastric emptying rate, neither the standard FDA breakfast nor an energy drink, and the most reliable protocol was obtained by allowing the mini-pigs to follow their normal feeding regime i.e. standard pig food. One limitation of this study is that study is based on assessing food effect difference using paracetamol as a marker for gastric emptying, whereas it is possible that a drug with a more pronounced food effect in humans may be a more reliable marker. An additional limitation of the current study was that the most frequent blood sampling was conducted in the first hour after administration, which did not coincide the peak plasma concentrations observed for paracetamol (Cpeak) in minipigs. For more reliable estimates of t max, frequent blood sampling near the Cpeak are required. Therefore, future studies should take the delayed and variable absorption of paracetamol in minipigs into account, with more frequent sampling around an estimated window of tmax of 2-6 hours. Notwithstanding these limitations, it would appear that based on the current study, the impact of food on drug absorption in mini-pigs is highly variable and the investigated protocols are not supportive of fed versus fasted studies on oral dosage forms in this species.

4 Conclusion

The present study investigated gastric emptying rates in fasted and fed Göttingen minipigs and evaluated the effect of metoclopramide on the same parameter. The results indicated that a pre-treatment of metoclopramide did not regulate gastric emptying under any of the investigated conditions. In addition, the data demonstrated no significant difference in the stomach emptying rate between fasted and fed minipigs. None of the investigate protocols seemed able to induce a gastric emptying that would be extended when food was administered, as observed in humans. The mini-pig model appears to be less suited for exploring food effect interactions studies than other species.

ACCEPTED MANUSCRIPT

5 Acknowledgements

L.J. Henze, B. Griffin and R. Holm are part of the PEARRL European Training network, which has received funding from the Horizon 2020 Marie Sklodowska-Curie Innovative Training Networks programme under grant agreement No. 674909.

The experimental part of this study was conducted while R. Holm was employed at H. Lundbeck A/S, where the personnel in the animal facilities are highly acknowledged for their skilful handling of the minipig study.

References

Behar, J., Ramsby, G., 1978. Gastric emptying and antral motility in reflux esophagitis. Effect of oral metoclopramide. Gastroenterology 74, 253-256.

Bode, G., Clausing, P., Gervais, F., Loegsted, J., Luft, J., Nogues, V., Sims, J., Project, R., 2010. The utility of the minipig as an animal model in regulatory toxicology. J. Pharmacol. Toxicol. Meth. 62, 196-220.

Charman, W.N., Rogge, M.C., Boddy, A.W., Barr, W.H., Berger, B.M., 1993. Absorption of danazol after administration to different sites of the gastrointestinal-tract and the relationship to single-peak and double-peak phenomena in the plasma profiles. J. Clin. Pharmacol. 33, 1207-1213.

Christiansen, M.L., Müllertz, A., Garmer, M., Kristensen, J., Jacobsen, J., Abrahamsson, B., Holm, R., 2015. Evaluation of the use of Göttingen minipigs to predict food effects on the oral absorption of drugs in humans. J. Pharm. Sci. 104, 135-143.

Clements, J.A., Heading, R.C., Nimmo, W.S., Prescott, L.F., 1978. Kinetics of acetaminophen absorption and gastric emptying in man. Clin. Pharmacol. Ther. 24, 420-431.

ACCEPTED MANUSCRIPT

Davis, S.S., Illum, L., Hinchcliffe, M., 2001. Gastrointestinal transit of dosage forms in the pig. J. Pharm. Pharmacol. 53, 33-39.

Forster, R., Bode, G., Ellegaard, L., van der Laan, J.W., Project, R., 2010. The RETHINK project: minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment. J. Pharmacol. Toxicol. Meth. 62, 158-159.

Gregory, P.C., McFadyen, M., Rayner, D.V., 1995. Control of gastric emptying in the pig: influence of cholecystokinin, somatostatin and prokinetic agents. Exp. Physiol. 80, 159-165.

Heading, R.C., Nimmo, J., Prescott, L.F., Tothill, P., 1973. The dependence of paracetamol absorption on the rate of gastric emptying. Br. J. Pharmacol. 47, 415-421.

Hellmig, S., Von Schoning, F., Gadow, C., Katsoulis, S., Hedderich, J., Folsch, U.R., Stuber, E., 2006. Gastric emptying time of fluids and solids in healthy subjects determined by 13C breath tests: influence of age, sex and body mass index. J. Gastroenterol. Hepatol. 21, 1832-1838.

Hossain, M., Abramowitz, W., Watrous, B.J., Szpunar, G.J., Ayres, J.W., 1990. Gastrointestinal transit of nondisintegrating, nonerodible oral dosage forms in pigs. Pharm. Res. 7, 1163-1166.

Houghton, L.A., Mangnall, Y.F., Read, N.W., 1990. Effect of incorporating fat into a liquid test meal on the relation between intragastric distribution and gastric-emptying in human volunteers. Gut 31, 1226-1229.

Ikegami, K., Tagawa, K., Narisawa, S., Osawa, T., 2003. Suitability of the cynomolgus monkey as an animal model for drug absorption studies of oral dosage forms from the viewpoint of gastrointestinal physiology. Biol. Pharm. Bull. 26, 1442-1447.

Kararli, T.T., 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Disp. 16, 351-380.

ACCEPTED MANUSCRIPT

Langenbucher, F., 1982. Numerical convolution deconvolution as a tool for correlating in vitro with in vivo drug availability. Ind. 44, 1166-1172.

Langguth, P., Lee, K.M., Spahn-Langguth, H., Amidon, G.L., 1994. Variable gastric emptying and discontinuities in drug absorption profiles: dependence of rates and extent of cimetidine absorption on motility phase and pH. Biopharm. Drug Dispos. 15, 719-746.

Lentz, K.A., Quitko, M., Morgan, D.G., Grace, J.E., Jr., Gleason, C., Marathe, P.H., 2007. Development and validation of a preclinical food effect model. J. Pharm. Sci. 96, 459-472.

Lipka, E., Lee, I.D., Langguth, P., Spahn-Langguth, H., Mutschler, E., Amidon, G.L., 1995. Celiprolol double-peak occurrence and gastric motility: nonlinear mixed effects modeling of bioavailability data obtained in dogs. J. Pharmacokinet. Biopharm. 23, 267-286.

Madden, F.N., Godfrey, K.R., Chappell, M.J., Hovorka, R., Bates, R.A., 1996. A comparison of six deconvolution techniques. J. Pharmacokinet. Biopharm. 24, 283-299.

McLaughlin, J.T., Troncon, L.E.A., Barlow, J., Heggie, L.J., Thompson, D.G., 1998. Evidence for a lipid specific effect in nutrient induced human proximal gastric relaxation. Gut 43, 248-251.

Merchant, H.A., McConnell, E.L., Liu, F., Ramaswamy, C., Kulkarni, R.P., Basit, A.W., Murdan, S., 2011. Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea pig, rabbit and pig, and implications for their use in drug development. European J. Pharm. Sci. 42, 3-10.

Metsugi, Y., Miyaji, Y., Ogawara, K., Higaki, K., Kimura, T., 2008. Appearance of double peaks in plasma concentration-time profile after oral administration depends on gastric emptying profile and weight function. Pharm. Res. 25, 886-895.

Mizuta, H., Kawazoe, Y., Haga, K., Ogawa, K., 1990. Effects of meals on gastric emptying and small intestinal transit times of a suspension in the beagle dog assessed using

ACCEPTED MANUSCRIPT

acetaminophen and salicylazosulfapyridine as markers. Chem. Pharm. Bull. 38, 2224-2227.

Neirinckx, E., Vervaet, C., De Boever, S., Remon, J.P., Gommeren, K., Daminet, S., De Backer, P., Croubels, S., 2010. Species comparison of oral bioavailability, first-pass metabolism and pharmacokinetics of acetaminophen. Res. Vet. Sci. 89, 113-119.

Oberle, R.L., Chen, T.S., Lloyd, C., Barnett, J.L., Owyang, C., Meyer, J., Amidon, G.L., 1990. The influence of the interdigestive migrating myoelectric complex on the gastric emptying of liquids. Gastroenterology 99, 1275-1282.

Oberle, R.L., Das, H., 1994. Variability in gastric pH and delayed gastric emptying in Yucatan miniature pigs. Pharm. Res. 11, 592-594.

Sjögren, E., Abrahamsson, B., Augustijns, P., Becker, D., Bolger, M.B., Brewster, M., Brouwers, J., Flanagan, T., Harwood, M., Heinen, C., Holm, R., Juretschke, H.P., Kubbinga, M., Lindahl, A., Lukacova, V., Munster, U., Neuhoff, S., Nguyen, M.A., van Peer, A., Reppas, C., Hodjegan, A.R., Tannergren, C., Weitschies, W., Wilson, C., Zane, P., Lennernas, H., Langguth, P., 2014. In vivo methods for drug absorption - Comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. Eur. J. Pharm. Sci. 57, 99-151.

Suenderhauf, C., Tuffin, G., Lorentsen, H., Grimm, H.P., Flament, C., Parrott, N., 2014. Pharmacokinetics of paracetamol in Göttingen minipigs: in vivo studies and modeling to elucidate physiological determinants of absorption. Pharm. Res. 31, 2696-2707.

Takamatsu, N., Welage, L.S., Hayashi, Y., Yamamoto, R., Barnett, J.L., Shah, V.P., Lesko, L.J., Ramachandran, C., Amidon, G.L., 2002. Variability in cimetidine absorption and plasma double peaks following oral administration in the fasted state in humans: correlation with antral gastric motility. Eur. J. Pharm. Biopharm. 54, 255-255.

ACCEPTED MANUSCRIPT

Walters, E.M., Agca, Y., Ganjam, V., Evans, T., 2011. Animal models got you puzzled?: think pig. Ann. N. Y. Acad. Sci. 1245, 63-64.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1. Pharmacokinetic parameters of paracetamol, oral administration 1000 mg per animal (n = 4 - 6)

Pharmacokinetic parameters								
	Fasted	Fasted$^{MCP\ 0.2\ mg/kg}$	Fasted$^{MCP\ 0.4\ mg/kg}$	$FED_{Pig\ food}$	$FED_{FDA\ breakfast}$	$FED^{MCP\ 0.4\ mg/kg}_{FDA\ breakfast}$	$FED_{Energy\ drink}$	$FED^{MCP\ 0.4\ mg/kg}_{Energy\ drink}$
C_{max} (ng/mL)	27229± 17009	25911± 10808	17214± 17656	20821 ± 19355	14504± 9519	25488± 8824	15109± 7619	14641± 10252
t_{max} (h)	4.2 ± 1.6	5.8 ± 1.8	3.9 ± 3.4	1.6 ± 0.9	3.5 ± 2.7	5.6 ± 1.9	4.5 ± 1.2	2.8 ± 1.3
$AUC_{0\rightarrow7}$ (ng·h/mL)	101018 ± 51947	87240 ± 32829	44875 ± 41532	61265 ± 56062	42605 ± 33016	86275 ± 19041	54766 ± 27357	56306 ± 43684
F (%)	86.4 ± 61.1	72.5 ± 42.3	39.2 ± 38.8	51.1 ± 52.0	34.8 ± 28.2	71.0 ± 34.4	44.7 ± 28.8	46.6 ± 28.8
MAT (h)	2.15 ± 0.65	2.66 ± 1.26	2.00 ± 1.15	1.82 ± 0.86	1.59 ± 1.60	3.17 ± 0.50	2.62 ± 0.40	1.85 ± 0.57
MRT p.o. (h)	3.64 ± 0.68	4.10 ± 1.43	3.56 ± 1.32	3.36 ± 0.90	3.13 ± 1.67	4.60± 0.66	4.16 ± 0.54	3.40 ± 0.72

*MCP= Metoclopramide

ACCEPTED MANUSCRIPT

Table 2. Mean deconvolution time points (h) after oral administration of paracetamol 1000 mg (mean, n = 5 - 6)

Deconvolution summary								
Absorbed fraction (%)	Fasted (h)	Fasted$^{MCP\ 0.2\ mg/kg}$ (h)	Fasted$^{MCP\ 0.4\ mg/kg}$ (h)	$FED_{Pig\ food}$ (h)	$FED_{FDA\ breakfast}$ (h)	$FED^{MCP\ 0.4\ mg/kg}_{FDA\ breakfast}$ (h)	$FED_{Energy\ drink}$ (h)	$FED^{MCP\ 0.4\ mg/kg}_{Energy\ drink}$ (h)
25 %	1.6	3.1	1.0	0.8	1.2	3.3	2.7	1.8
50 %	3.2	4.6	2.8	1.9	2.4	4.6	3.7	2.8
75 %	4.0	5.8	5.1	3.6	5.0	5.8	5.3	4.4

*MCP= Metoclopramide

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

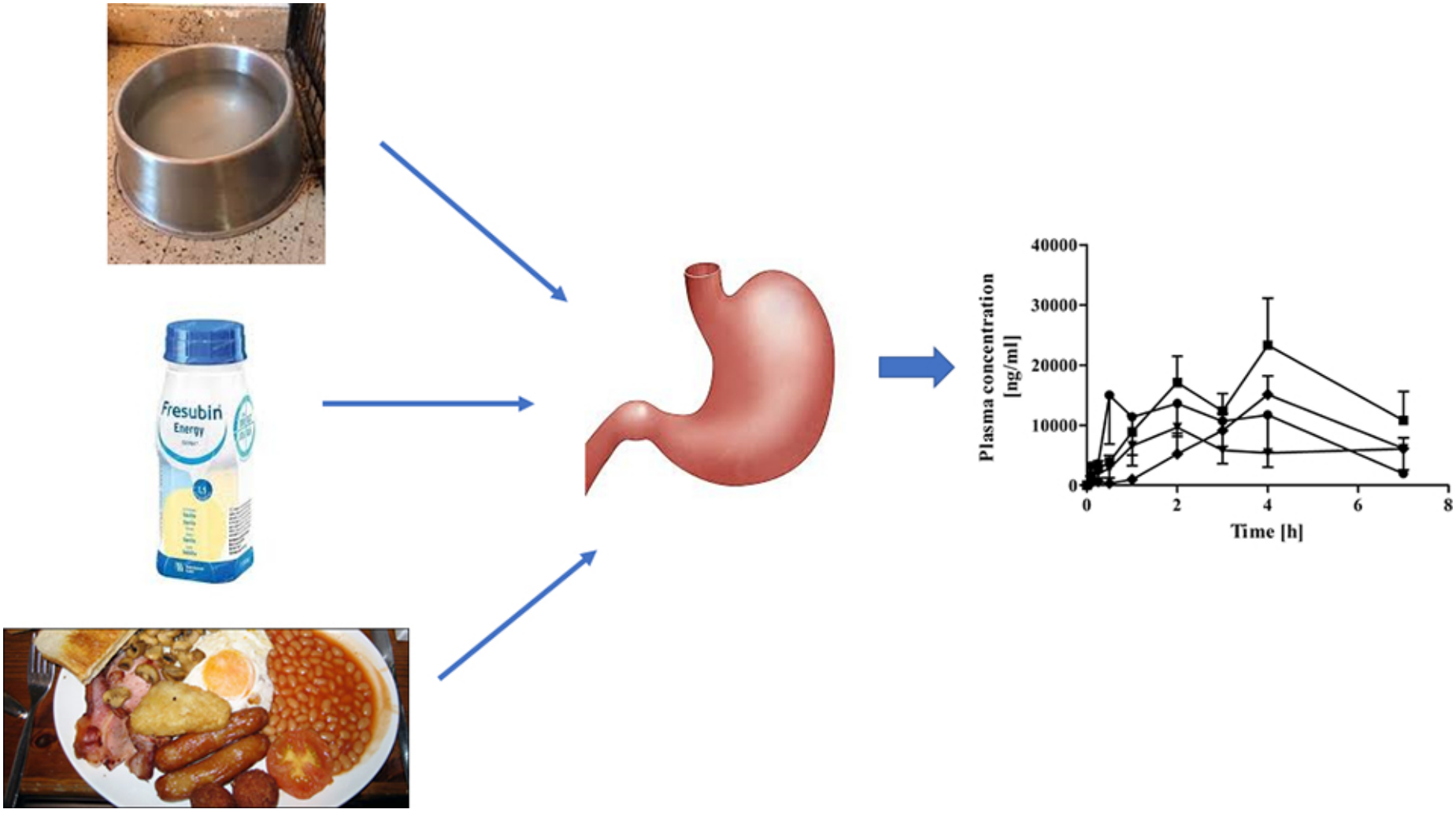
Figure legends

Fig. 1. Plasma concentration time profile following intravenous administration of 240 mg of paracetamol to pigs (mean ± SEM, n = 6).

Fig. 2. Fasted versus Fed. State, (A) plasma concentration profile after oral administration of paracetamol, ■= $FASTED$; ●= $FED_{Pig\ food}$; ◆= $FED_{Energy\ drink}$; ▼= $FED_{FDA\ breakfast}$ (B)tmax (median ± range). (C) mean absorption time (median ± range). (D) deconvolution profile, ■= $FASTED$; ●= $FED_{Pig\ food}$; ◆= $FED_{Energy\ drink}$; ▼= $FED_{FDA\ breakfast}$; (Mean ± SEM, n = 6).

Fig. 3. Effect of metoclopramide on fasted and fed states after oral administration of paracetamol of 1000 mg (Mean ± SEM, n = 5 - 6); A) plasma concentration profiles fasted state, $FASTED$ (■), $FASTED^{MCP\ 0.2\ mg/kg}$ (●), $FASTED^{MCP\ 0.4\ mg/kg}$(▲); B) mean deconvolution profiles fasted state, $FASTED$ (■), $FASTED^{MCP\ 0.2\ mg/kg}$ (●), $FASTED^{MCP\ 0.4\ mg/kg}$ (▲); C) plasma concentration profile, $FED_{FDA\ breakfast}$ (▼), $FED^{MCP\ 0.4\ mg/kg}_{FDA\ breakfast}$ (△); D) mean deconvolution profiles, $FED_{FDA\ breakfast}$(▼), $FED^{MCP\ 0.4\ mg/kg}_{FDA\ breakfast}$ (△);E) plasma concentration profiles, Fresubin energy drink, $FED_{Energy\ drink}$ (◆), $FED^{MCP\ 0.4\ mg/kg}_{Energy\ drink}$ (◇); F) mean deconvolution profiles, Fresubin energy drink, $FED_{Energy\ drink}$ (◆), $FED^{MCP\ 0.4\ mg/kg}_{Energy\ drink}$ (◇).

Fig. 4. Mean absorption times of paracetamol after oral administration of 1000 mg, plots show median ± range (n = 4 - 6).



Graphics Abstract

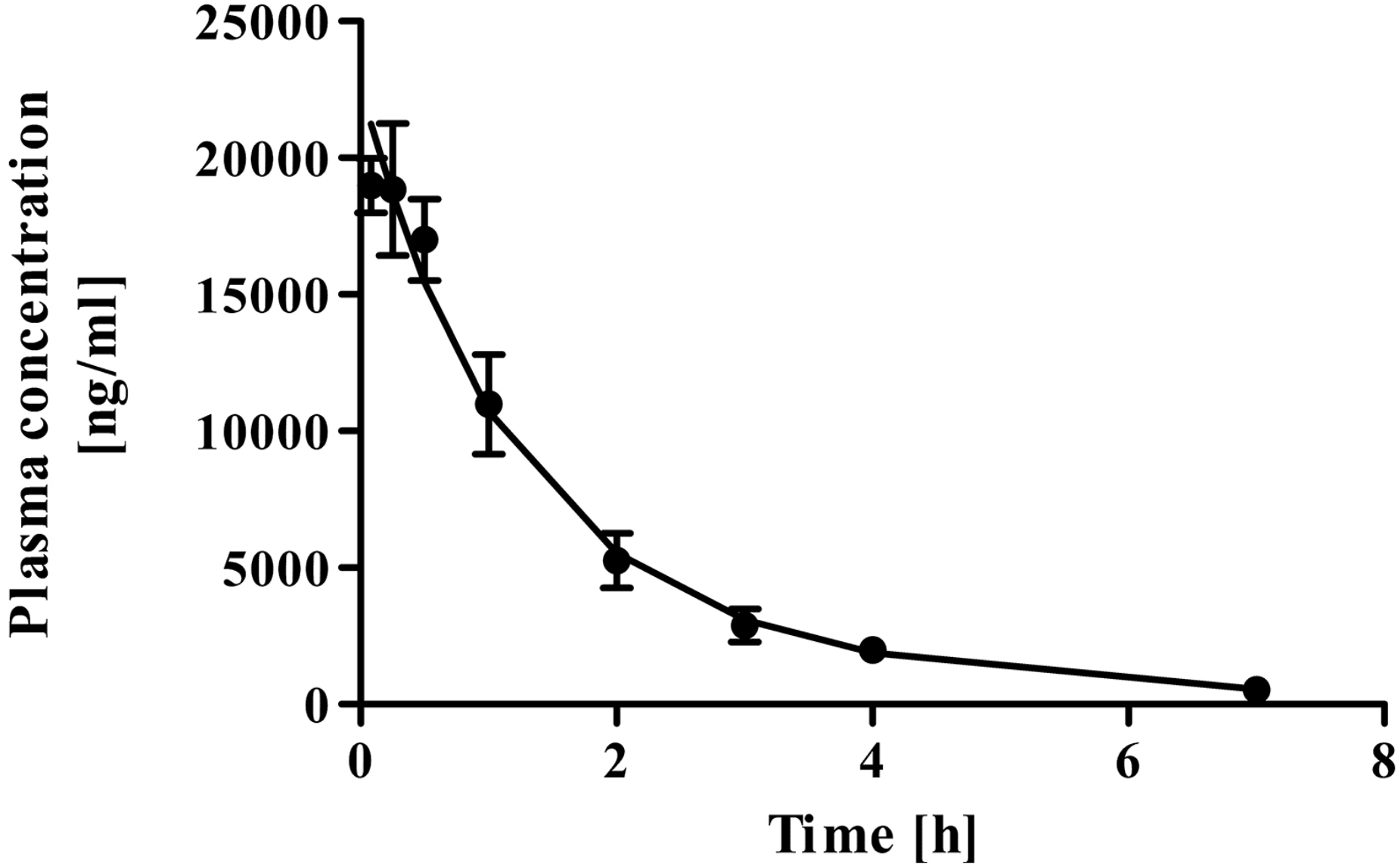

Plasma concentration
[ng/ml]
25000
20000
15000
10000
5000
0
0
2
4
6
8
Time [h]

Figure 1

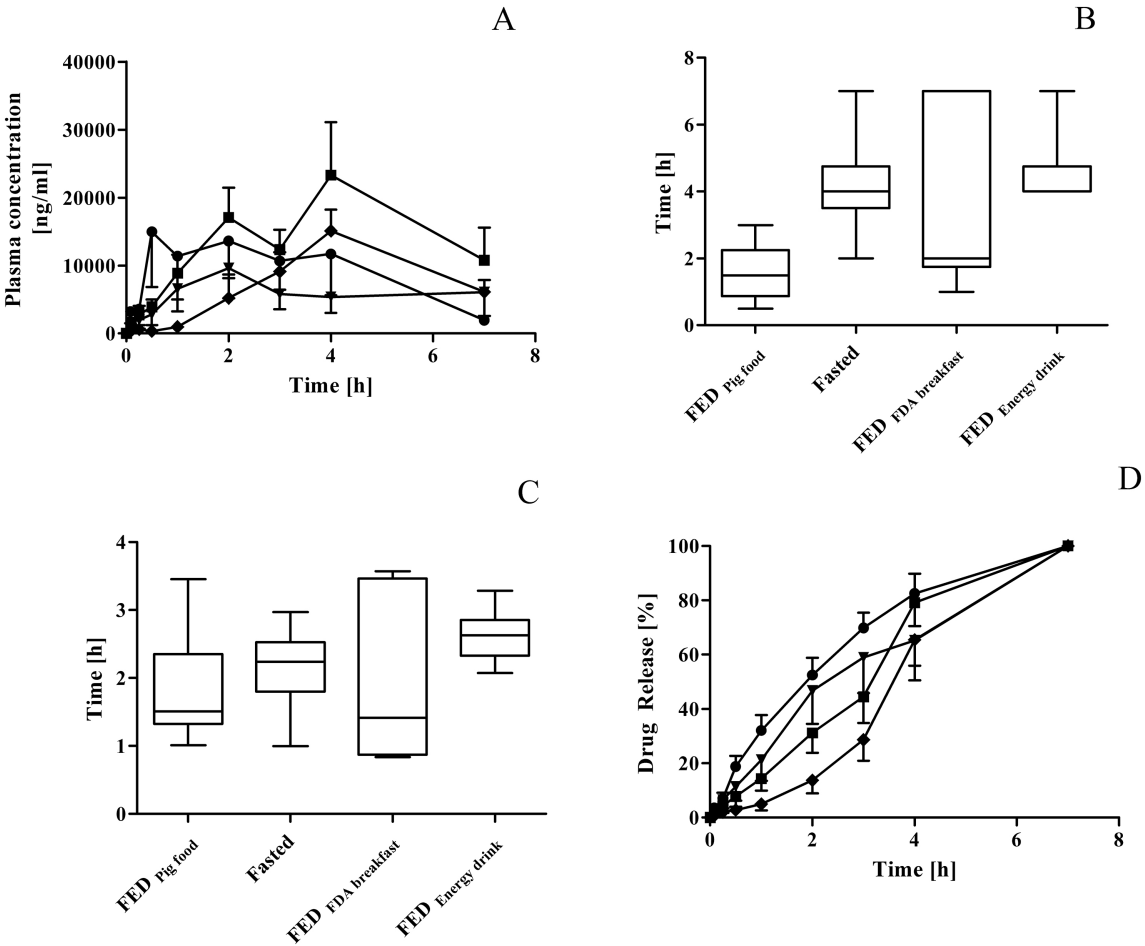

A
Plasma concentration [ng/ml]
40000
30000
20000
10000
0
0
2
4
6
8
Time [h]
B
Time [h]
8
6
4
2
0
FED Pig food
Fasted
FED FDA breakfast
FED Energy drink
C
Time [h]
4
3
2
1
0
FED Pig food
Fasted
FED FDA breakfast
FED Energy drink
D
Drug Release [%]
100
80
60
40
20
0
0
2
4
6
8
Time [h]

Figure 2

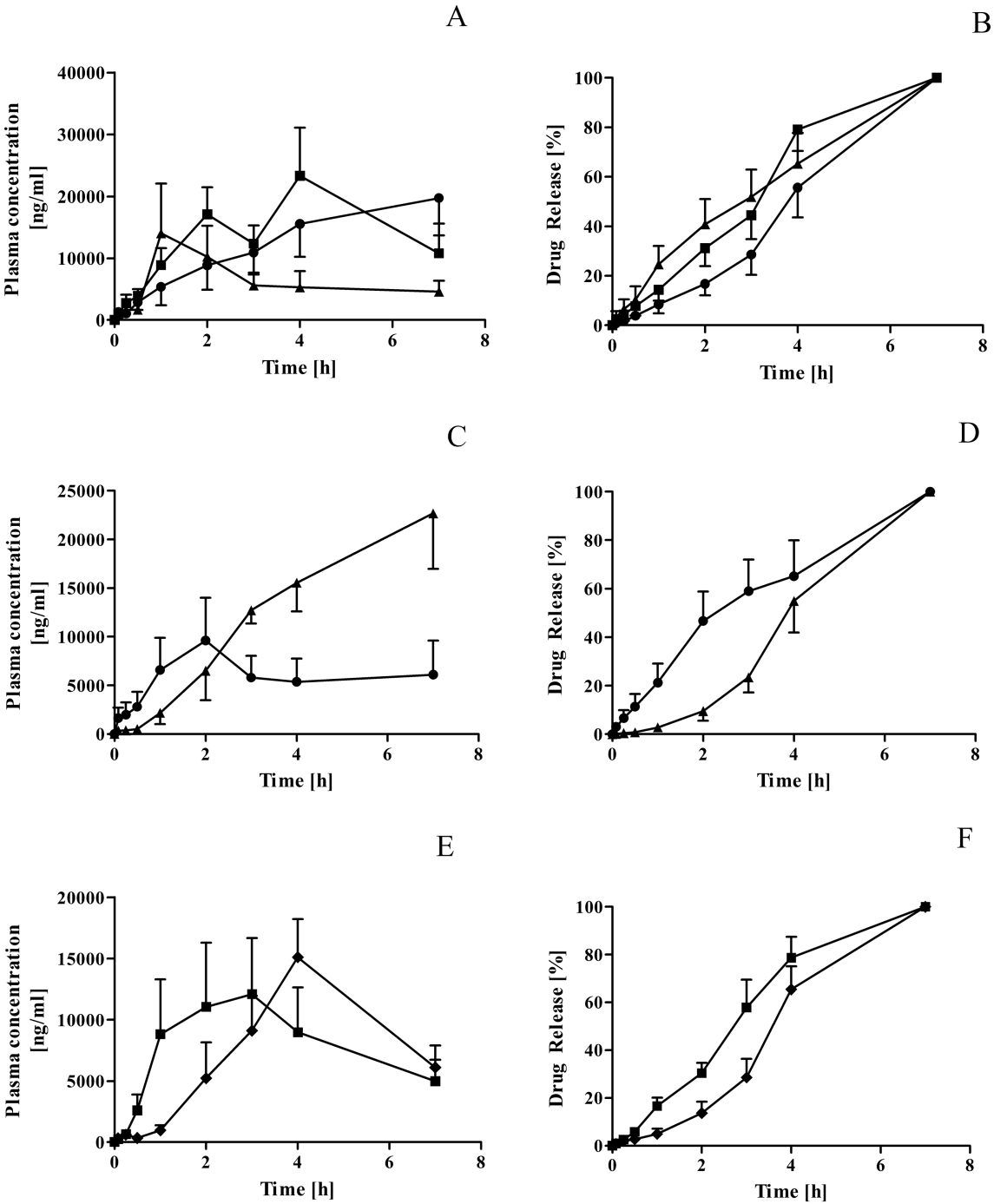

A
B
C
D
E
F
Plasma concentration [ng/ml]
Drug Release [%]
Time [h]
40000
30000
20000
10000
0
25000
15000
5000
100
80
60
40
20
0
2
4
6
8

Figure 3

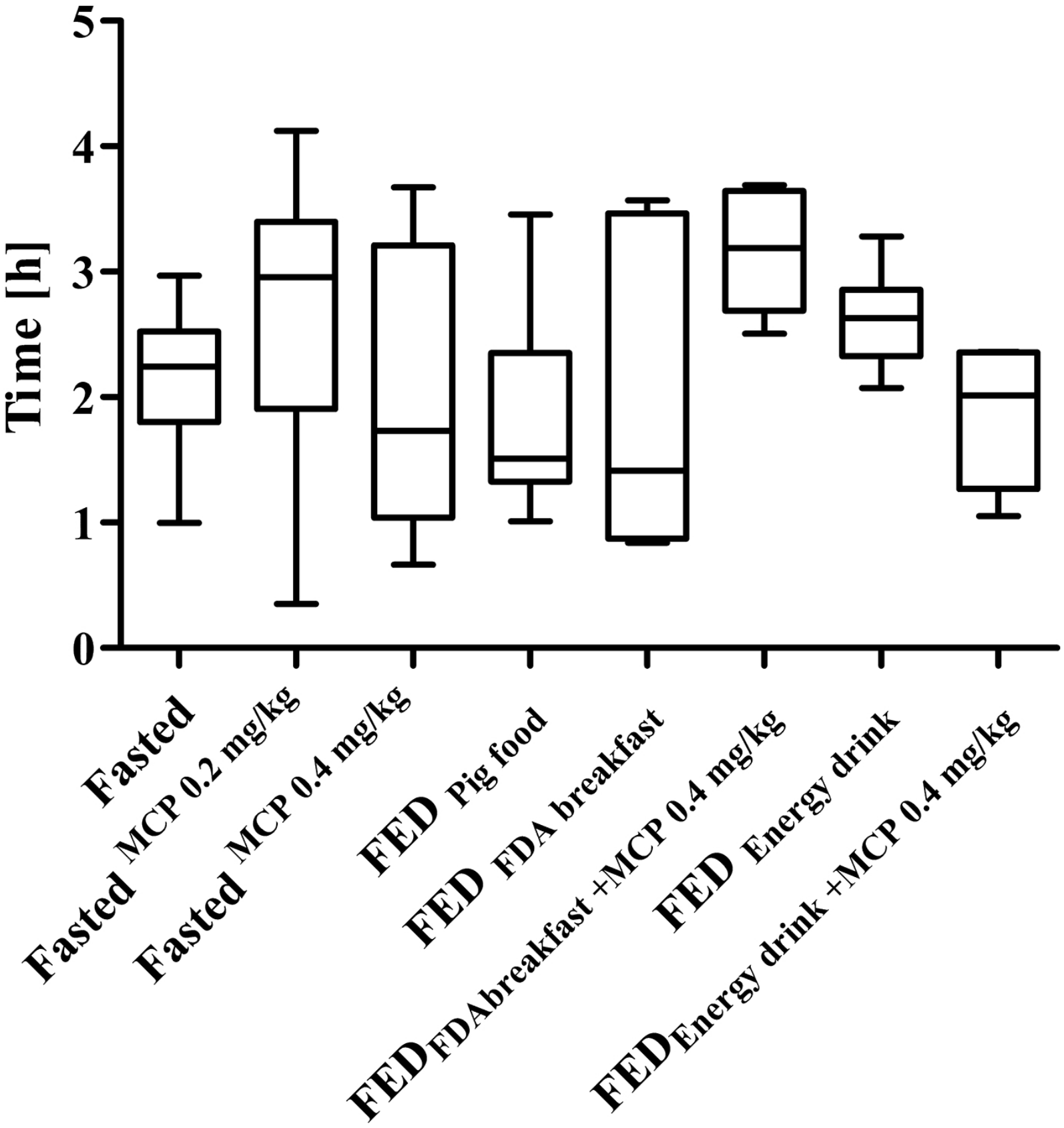

Time [h]
0
1
2
3
4
5
Fasted
Fasted MCP 0.2 mg/kg
Fasted MCP 0.4 mg/kg
FED Pig food
FED FDA breakfast
FED FDAbreakfast +MCP 0.4 mg/kg
FED Energy drink
FED Energy drink +MCP 0.4 mg/kg

Figure 4