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Effects of butyrate on hepatic epigenetics and microsomal drug-metabolizing enzymes in chicken

Brief version of the PhD thesis

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1. Introduction

The short chain fatty acid butyrate is one of the major end products of the anaerobic microbial fermentation of carbohydrates in the forestomachs of ruminants and in the large intestine of monogastric mammals, birds and humans. Butyrate is also a widely used feed additive as an alternative growth promoter due to its beneficial effects on growth performance, first of all in poultry and pig nutrition. This is of special importance due to the banning of the traditional antibiotic growth promoters in the European Union.

Butyrate is the most important energy source of the gastrointestinal mucosa, playing also a predominant role in proliferation and differentiation of the epithelial cells and providing a protective effect against colorectal cancer. It has a significant improving action on the composition of the intestinal microflora because of its selective antimicrobial effect on most enteral pathogens. Butyrate is efficiently metabolized by the intestinal epithelial cells, but a certain amount can be absorbed and transported into the liver by the portal vein. Butyrate is an important energy source of the hepatocytes as a substrate of the β-oxidation, but it can be considered as a potent effector of the hepatic metabolism as well. In addition to its other biochemical effects, butyrate can influence plasma insulin concentration and increase insulin sensitivity of various tissues.

Butyrate may provoke its effects on metabolism via many different, yet not completely defined pathways. One of those pathways is that butyrate is known as a histone deacetylase (HDAC) inhibitor, inducing histone hyperacetylation *in vitro* and playing a predominant role in the epigenetic regulation of gene expression and cell function. During posttranslational modifications of histones, the balance of histone acetyltransferase and HDAC activity determines the acetylation state of core histones. If HDAC is blocked by butyrate, it causes histone hyperacetylation in the N-terminal histone tails, which results in modified chromatin structure, influencing the transcriptional pattern of certain genes. Butyrate-triggered histone modifications may be highly involved in butyrate's antitumor, antibacterial and metabolic effects as well.

It is already known for a long time that butyrate causes hyperacetylation of histones H3 and H4 in all examined vertebrate cell lines, while H2A and H2B are also affected in certain rat-derived cell types. In contrast to the wide range of *in vitro* results, only very little data are available in the literature regarding *in vivo* effects of butyrate on histone acetylation, and absolutely no data about such action in chicken. It was hypothesized in this study that orally applied butyrate might cause *in vivo* modifications in the chromatin structure of the hepatocytes of chickens in the early post-hatch period.

Hepatic microsomal cytochrome P450 (CYP) enzymes, forming a superfamily of hemoproteins, are primarily involved in the oxidative metabolism of numerous endogenous

and exogenous compounds. They play a predominant role in the phase I reactions of xenobiotic biotransformation and in steroid metabolism. It is known that the expression of certain drug-metabolizing microsomal CYP enzymes can be affected by histone modifications, altering the chromatin structure and the affinity and binding possibility of some transcriptional factors to the promoter region of CYP genes. Based on these findings, the enteral microbiome-produced or the orally added butyrate may also alter the activity of CYP enzymes, having an impact on hepatic detoxification capacity and drug metabolism, defined as possible pharmacoepigenetic influences. However, such possible effects of butyrate on hepatic microsomal CYP enzymes were not examined yet.

Macrolide antibiotics, such as erythromycin are also potent effectors of the CYP enzymes. Erythromycin may have multiple effects on various microsomal CYPs, modifying the metabolism of other xenobiotics, which can give rise to serious drug-drug interactions. Nevertheless the effects of erythromycin on CYP gene expression in chicken have not been investigated yet. Erythromycin is a commonly applied antibiotic in poultry medicine, therefore studying its effects on hepatic CYP enzymes and its possible interaction with simultaneously applied butyrate in chicken would be of special importance.

In spite of the wide range of the known biological actions of butyrate, numerous aspects of its epigenetic, trophic and metabolic effects still remained unclear, especially *in vivo*, after oral butyrate application. Since butyrate is widely used in poultry nutrition, chickens are not only target species of butyrate administration as feed additive, but they can also serve as model for the investigation of butyrate's actions. Young chickens have a large capacity of growing, intensive hepatic metabolism and quite low rates of butyrate production in the large intestine, so they can be proper candidates to study the effects of the orally applied butyrate.

Two different forms of oral butyrate application were compared as well in this study. Butyrate was added orally to broiler chickens either as feed additive or in daily bolus after overnight fasting. The latter treatment provided a fast, but short-term release of greater amount of butyrate to the portal vein and an intense butyrate exposure for the liver and therefore served as a model for investigating butyrate's possible *in vivo* epigenetic and metabolic action.

Summarized in points, the most important goals of this PhD study were:

Ad 1

(1a) to investigate histomorphological changes in the small intestinal epithelium caused by oral butyrate application and to compare these data in chicken and rat, the latter as a monogastric mammalian model. This would provide more information beyond the beneficial effects of butyrate on growth.

(1b) to study the butyrate uptake of primary cultures of hepatocytes as well as the first-pass hepatic clearance of butyrate in chicken and rat.

Ad 2

to evaluate the *in vivo* epigenetic effects of butyrate added orally to broiler chickens either as feed additive or in daily bolus. It was aimed to monitor the modifications in the acetylation state of hepatic core histones at the most frequent acetylation sites triggered by butyrate stimuli following both forms of application.

Ad 3

to detect the effects of butyrate on gene expression of hepatic microsomal drug-metabolizing CYP enzymes firstly *in vitro*, in primary cultures of chicken hepatocytes, then *in vivo*, after oral butyrate supplementation to broilers. Finally, the activity of CYP enzymes was aimed to be measured to screen, whether butyrate could influence the detoxification capacity and xenobiotic biotransformation of the liver.

Ad 4

to study the possible pharmacoepigenetic interaction between butyrate and erythromycin *in vitro* on CYP gene expression of cultured chicken hepatocytes. Additionally, pharmacokinetic properties of erythromycin were measured following oral butyrate application in broiler chickens *in vivo* to study how butyrate could influence drug metabolism, having a huge impact from pharmacotherapeutical and food safety point of view.

2. Materials and methods

2.1. In vitro studies on primary cultures of hepatocytes

2.1.1. Butyrate uptake of primary cultures of chicken and rat hepatocytes

Primary cultures of chicken and rat hepatocytes were prepared and confluent monolayers were gained after 24 h cultivation. Cell cultures were characterized by the immunohistochemical detection of glutaminase. Cells were treated for 24 h with various concentrations of sodium butyrate (0, 1, 5 and 10 mM), dissolved in the appropriate cell culture medium. Butyrate concentration of media was measured at the end of the 24 h treatment period by gas chromatography using a 30 m (0.25 mm i.d.) fused Nukol silica column.

2.1.2. CYP gene expression of primary cultures of chicken hepatocytes

Primary cultures of chicken hepatocytes were treated for 24 h according to the following protocol: cell culture media contained six different concentrations of sodium butyrate (0, 1, 2.5, 5, 7.5, 10 mM) and each sodium butyrate concentration was combined with the following concentrations of erythromycin: 0, 10, 50 and 100 μM, respectively. Cells were harvested and lysed in TRIzol reagent after the incubation time. Following RNA isolation and reverse transcription, relative gene expression of CYP1A, CYP2H1 and CYP3A37 enzymes was determined by qRT-PCR examinations.

2.1.3. Erythromycin elimination of primary cultures of chicken hepatocytes

Cultured chicken hepatocytes were treated with various concentrations of sodium butyrate and erythromycin as described in section 2.1.2. After the 24 h treatment period, concentration of erythromycin from culture media of cell cultures was determined with validated HPLC using a Merck-Hitachi LaCrom Elite HPLC system combined with Nucleosil C18 5 μm 25x0.46 column.

2.2. In vivo studies

2.2.1. Effects of butyrate applied as feed additive in chicken

Broiler chickens in the early post-hatch period were fed by a commercial stock diet or received sodium butyrate supplementation of the feedstuff (1.5 g/kg) for 21 days. On the last day of the experiment, blood samples were drawn from the brachial vein in order to determine plasma butyrate concentration by gas chromatography. Experimental animals were slaughtered on day 21 and small intestinal samples were taken from the proximal jejunum and were investigated by histometrical examinations. The liver was exsanguinated by the perfusion of the portal vein and liver samples were taken for the determination of CYP gene expression (qRT-PCR) and for the isolation of subcellular organelles, applying multi-

step differential centrifugation. Hepatic histone acetylation was screened by western blotting from cell nuclei, while drug-metabolizing CYP enzyme activity was detected by specific enzyme assays (aminopyrine N-demethylation, aniline hydroxylation and testosterone 6β-hydroxylation). Intracoelomal phenobarbital (PB) treatment (80 mg/kg BW) was conducted to serve as positive control for CYP activity measurements.

Comparative examinations were also performed with rats after weaning, receiving butyrate supplementation of the diet (1.5 g/kg). After slaughtering on day 21 of the experiment, measurement of plasma butyrate concentration, histometrical examination of small intestinal micromorphology and enzyme assays on hepatic microsomal CYP activity were carried out.

2.2.2. Effects of butyrate applied as daily bolus in chicken

Broiler chicks in the early post-hatch period were treated once daily with orally administered bolus of sodium butyrate following overnight fasting with two different doses (0.25 or 1.25 g/kg body weight per day) for five days. The lower dose of butyrate boli provided approximately the same amount of sodium butyrate on each day of treatment, which was taken up daily as feed additive in the previous experiment. After slaughtering and tissue sampling, blood plasma butyrate concentration, extent of hepatic histone acetylation and microsomal CYP activities were determined as described in section 2.2.1.

2.2.3. Additional effects of butyrate and phenobarbital in chicken

The effect of concomitant butyrate and PB application on hepatic microsomal CYP activity was tested in broiler chickens, receiving oral butyrate treatment in daily bolus (0.25 g/kg BW) and intracoelomal PB injection (80 mg/kg BW) parallelly. CYP activities were measured from microsomal fractions of livers as mentioned in section 2.2.1.

2.2.4. Effects of butyrate on pharmacokinetics of erythromycin in chicken

Broiler chickens were fed by a normal stock diet, with or without sodium butyrate supplementation (1.5 g/kg diet). At the end of the six-week long feeding period chickens were treated with a single intramuscular dose (30 mg erythromycin base/kg BW) of erythromycin injection. Blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 8 and 12 h after the injection and plasma erythromycin concentration was determined by validated HPLC method. The pharmacokinetic parameters of erythromycin were calculated applying noncompartmental analysis with the software Kinetica 4.4.1.

2.3. Statistical analysis

Statistical analysis of data was performed with R 2.14.0 software. One-way ANOVA or ANCOVA and Student's t-test were used for the comparison of results of the treated groups with those of controls at normal distributions, while non-parametric Mann-Whitney's test was approved at not normal distributions. Level of significance was set at $P<0.05$.

3. Results and Discussion

3.1. Effects of oral butyrate application on the small intestinal histomorphology

Growth promoting effects of butyrate might be based partially on the morphological changes of the small intestinal mucosa: butyrate tended to increase depth of crypts in both species, while butyrate had a positive trophic effect on the enterocytes in chicken, but not in rat. Increased height of enterocytes after dietary butyrate supplementation, observed in our study, makes butyrate not only a potent regulator of cell division, but a molecule that can cause hypertrophic changes in the gastrointestinal epithelial cells due to its direct trophic effect. All these morphological alterations can lead to a larger surface of small intestine and consequently more efficient nutrient absorption, which may enhance the growth rate of these animals. However, it is clear that such improvements in growth performance are based on many other different effects as well, such as balancing the gut flora or increasing the immune response.

3.2. Butyrate uptake of cultured hepatocytes and fate of orally applied butyrate beyond the gut

Regarding our *in vitro* examinations, butyrate was greatly taken up from cell culture media by cultured hepatocytes, but this uptake was limited at higher butyrate concentrations in chicken by reaching a maximal amount of butyrate to be able to be taken up. In contrast, no such limitation could be found in primary cultures of rat hepatocytes, where higher amount of butyrate could be taken up even at 10 mM initial butyrate concentration. According to this difference, we can suggest that different transport mechanisms may exist in the two examined species.

However, butyrate is partly metabolized in the liver, we found that a certain amount is passed through to the systemic circulation, causing elevation of its plasma concentration in chickens and rats receiving oral butyrate treatment either as feed additive or in case of chickens as daily bolus. In this later form of administration, significant dose-dependency of plasma butyrate concentration was also detected. Based on our results it can be stated that alimentary added butyrate could reach the extrahepatic tissues as well and might act as a biologically active molecule.

3.3. Epigenetic effect of orally applied butyrate on hepatic histone acetylation

Orally added butyrate, applied either as feed additive or in bolus, had a remarkable impact on nucleosome structure of hepatocytes of broiler chickens *in vivo*. Independently from the form of application or the dose, butyrate caused hyperacetylation of histone H2A, but no changes were monitored in the acetylation state of H2B at the examined lysine residues. Intensive, approximately 18-fold hyperacetylation of H3 was caused by the higher administered dose in bolus, while the lower concentration altered the acetylation of H3 neither in bolus nor as feed additive. Acetylation ratio of H4 tended to be increased only by the lower dose of butyrate boli, but interestingly, no alterations could be observed after the application of the higher dose or as feed additive.

Summarizing our results, orally applied butyrate either as feed additive or in bolus influenced hepatic histone acetylation *in vivo*; but the butyrate-provoked acetylation pattern was partly application- and dose-dependent. Since butyrate relevantly modified the chromatin structure, it can be considered as an important epigenetic effector of gene expression of hepatocytes.

3.4. Effects of butyrate on hepatic CYP gene expression and enzyme activity in vitro and in vivo

Butyrate had a pronounced effect on gene expression of certain CYP enzymes in primary cultures of chicken hepatocytes *in vitro* as well as *in vivo*, applied as feed additive. Expression of CYP2H1 gene increased in both cultured hepatocytes and the liver of butyrate-fed chicks, while CYP3A37 gene expression declined after *in vitro* butyrate treatment, but this down-regulation was ameliorated *in vivo*. Interestingly, CYP1A gene was observed to be suppressed by butyrate in primary hepatocyte cultures, but in contrast, it was overexpressed *in vivo* in butyrate-fed animals compared to the controls.

In spite of the observed *in vivo* modifications in histone acetylation and CYP gene expression, no significant changes were observed in the activity of hepatic microsomal CYP2H and CYP3A37 enzymes in any cases of oral butyrate application in both chickens and rats, tested by specific enzyme assays. It means that the butyrate-triggered alterations in the transcription of the examined CYP genes were finally not realized on the level of enzyme activity. However, butyrate in bolus attenuated the stimulatory effect of the simultaneously administered PB on CYP2H and CYP3A37 enzymes, possibly due to the partly common regulatory pathways of CYP gene expression.

3.5. Interaction of butyrate and erythromycin in vitro and in vivo

Regarding the *in vitro* interaction of butyrate and erythromycin, this macrolide antibiotic showed an additive action with concomitant butyrate treatment on gene expression of CYP2H1, but antagonized butyrate's effect on CYP3A37 gene expression of primary cultures of chicken hepatocytes. Notwithstanding that butyrate concentration was found to be a potent effector of erythromycin metabolism, the drug-metabolizing activity of cultured hepatocytes was not affected significantly by butyrate.

In the *in vivo* trial longer absorption half-life, T_{max} and shorter elimination half-life of erythromycin were observed in the butyrate-treated group of chickens. Based on C_{max} values, the two groups were non-bioequivalent. It can be stated that although there were differences in certain pharmacokinetic parameters of erythromycin, the dietary administered butyrate did not alter relevantly the therapeutic activity nor the terminal elimination of erythromycin in chickens.

Based on our results, it can be concluded that orally added butyrate acted as an epigenetic factor by increasing the acetylation of core histones in the liver and had an impact on hepatic CYP gene expression of chicken *in vitro* and *in vivo* as well. Nevertheless these alterations did not affect the microsomal CYP activity of the liver, and the concomitant application of butyrate with veterinary pharmaceuticals possibly would not cause a major feed-drug metabolic interaction *in vivo* in chickens. So butyrate is suggested to be applied in safe as feed additive in poultry industry, from pharmacotherapeutical and food safety point of view as well, possibly not having any relevant pharmacoepigenetic interactions with simultaneously applied xenobiotics.

4. New scientific results

Ad 1

Butyrate applied as feed additive (1.5 g/kg diet) increased the height of enterocytes in the jejunum of broiler chickens in the early post-hatch period, having a positive trophic effect on the gastrointestinal epithelium.

Ad 2

Orally applied butyrate had a remarkable *in vivo* epigenetic effect on nucleosome structure of hepatocytes of chickens. Butyrate supplementation of the diet with the dose of 1.5 g/kg caused hyperacetylation of hepatic histone H2A, while it did not alter the acetylation state of the other core histones. Butyrate, administered in daily bolus for five days with the dose of 1.25 g/kg body weight increased the acetylation state of H2A and H3, but no significant changes could be monitored in the acetylation state of H2B and H4.

Ad 3

Butyrate had a pronounced effect on gene expression of certain hepatic cytochrome P450 (CYP) enzymes of chicken *in vitro* on primary hepatocyte cultures (in the concentration of 1 to 10 mM) as well as *in vivo*, applied as feed additive (1.5 g/kg diet). Expression of CYP2H1 gene was increased by butyrate in both cultured hepatocytes and the liver of butyrate-fed chicks, while CYP3A37 gene expression declined after *in vitro* butyrate treatment, but this down-regulation was ameliorated *in vivo*. CYP1A gene was observed to be suppressed by butyrate in primary hepatocyte cultures, but in contrast, it was overexpressed *in vivo* in butyrate-fed animals compared to the controls. Orally applied butyrate, either as feed additive (1.5 g/kg diet) or in bolus (0.25 or 1.25 g/kg body weight), did not affect the activity of hepatic microsomal CYP2H and CYP3A37 enzymes in the liver of chickens, tested by aminopyrine N-demethylation (CYP2H/CYP3A37), aniline hydroxylation (CYP2H) and testosterone 6β-hydroxylation (CYP3A37) assays. However, butyrate in bolus attenuated the stimulatory effect of simultaneously administered phenobarbital on CYP2H and CYP3A37.

Ad 4

The macrolide antibiotic erythromycin (10 to 100 μM) increased the expression of CYP2H1 gene of cultured chicken hepatocytes, which was additive with the effect of simultaneous butyrate (1 to 10 mM) treatment. Erythromycin ameliorated butyrate's suppressing action on CYP3A37 gene in the same primary cell culture model. Alimentary supplemented butyrate (1.5 g/kg diet) modified the pharmacokinetic parameters of erythromycin followed by its single intramuscular injection (30 mg/kg body weight) in chicken: increased absorption half-life, T_{max} and shorter elimination half-life could be observed in chickens receiving such butyrate application. However, these alterations modified relevantly neither the therapeutic activity nor the terminal elimination of erythromycin.

5. Own scientific publications

5.1. Publications related to the topic of the present dissertation

Full text papers in peer-reviewed journals:

Mátis, G., Neogrády, Zs., Csikó, Gy., Kulcsár, A., Kenéz, Á. and Huber, K.: **Effects of orally applied butyrate bolus on histone acetylation and cytochrome P450 enzyme activity in the liver of chicken – a randomized controlled trial**, Nutr. Metab., 10. 12, 2013. (**IF:** 2.89)

Mátis, G., Neogrády, Zs., Csikó, Gy., Gálfi, P., Fébel, H., Jemnitz, K., Veres, Zs., Kulcsár, A., Kenéz, Á. and Huber, K.: **Epigenetic effects of dietary butyrate on hepatic histone acetylation and enzymes of biotransformation in chicken**, Acta Vet. Hung., 61. (4), 2013. (DOI: 10.1556/AVet.2013.033) (last known **IF:** 0.642)

Csikó, Gy., Nagy, G., **Mátis, G.**, Neogrády, Zs., Kulcsár, A., Jerzsele, Á., Szekér, K. and Gálfi, P.: **Possible effects of dietary n-butyrate on hepatic biotransformation and pharmacokinetic behaviour of drugs in chicken**, J. Vet. Pharmacol. Ther., [submitted] (last known **IF:** 1.181)

Mátis, G., Csikó, Gy., Jemnitz, K., Veres, Zs., Fébel, H., Kulcsár, A., Petrilla, J. and Neogrády, Zs.: **A takarmányba kevert butirát citokróm P450 enzimekre gyakorolt hatásának vizsgálata patkány májban (Investigation of the effect of butyrate supplementation of the diet on hepatic cytochrome P450 enzymes in rats)**, Magy. Állatorv. Lapja, 135. 109-116, 2013. (last known **IF:** 0.3)

Oral presentations on international conferences:

Csikó, Gy., Nagy, G., **Mátis, G.**, Neogrády, Zs. and Gálfi, P.: **The effect of dietary sodium butyrate on the pharmacokinetics of erythromycin in broiler chickens**, 12th International Conference of the European Association for Veterinary Pharmacology and Toxicology, Utrecht, The Netherlands, 2012.

Mátis, G., Kulcsár, A., Petrilla, J., Kenéz, Á., Csikó, Gy., Neogrády, Zs. and Huber, K.: **Epigenetic consequences of oral butyrate application in chicken**, GfE Conference, Göttingen, Germany, 2013.

Poster presentations on international conferences:

Mátis, G., Kenéz, Á., Kulcsár, A., Csikó, Gy., Neogrády, Zs. and Huber, K.: **Trophic and epigenetic effects of dietary butyrate supplementation in chicken**, GfE Conference, Göttingen, Germany, 2012.

Mátis, G., Csikó, Gy., Jemnitz, K., Veres, Zs., Szabó, M., Kulcsár, A., Kenéz, Á., Gálfi, P. and Neogrády, Zs.: **Effects of dietary butyrate supplementation on hepatic microsomal cytochrome P450 activity in chicken and rat**, FEBS3+ Conference, Opatija, Croatia, 2012.

Oral presentations on Hungarian national conferences:

Mátis, G., Csikó, Gy., Annus, K., Neogrády, Zs. and Gálfi, P.: **A nátrium-n-butirát metabolizmusa patkány és csirke primer májsejttenyészetben**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2010.

Mátis, G., Neogrády, Zs., Csikó, Gy. and Annus, K.: **A butirát hatásának vizsgálata májsejttenyészetben: a primer tenyészet jellemzése immunhisztokémia és western blot segítségével**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2011.

Mátis, G., Csikó, Gy., Fébel, H. and Neogrády, Zs.: **A takarmánykiegészítőként adott butirát növekedési teljesítményre és egyes vérparaméterekre gyakorolt hatásának vizsgálata brojlercsirkében és patkányban**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2012.

Mátis, G., Kenéz, Á., Kulcsár, A., Csikó, Gy., Neogrády, Zs. and Huber, K.: **A takarmánykiegészítőként adott butirát epigenetikus hatásának vizsgálata brojlercsirkében**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2012.

Mátis, G., Csikó, Gy., Kulcsár, A., Kenéz, Á., Jemnitz, K., Veres, Zs., Szabó, M. and Neogrády, Zs.: **A takarmánykiegészítőként adott butirát máj citokróm P450 izonezimekre gyakorolt hatásának vizsgálata brojlercsirkében és patkányban**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2012.

Mátis, G., Kenéz, Á., Kulcsár, A., Csikó, Gy., Neogrády, Zs. and Huber, K.: **A bolusban adott butirát májsejtek hiszton-acetilációjára gyakorolt hatásának vizsgálata brojlercsirkében**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2013.

Mátis, G., Csikó, Gy., Kulcsár, A., Petrilla, J., Pleva, D., Neogrády, Zs. and Gálfi, P.: **Máj citokróm P450 enzimek aktivitásának vizsgálata bolusban adott butirátkezelést követően brojlercsirkében**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2013.

5.2. Publications not related to the topic of the present dissertation

Full text papers in peer-reviewed journals:

Domokos, M., Ígyártó, B., Glávits, R., Pécsi, A., **Mátis, G.**, Földi, J., Kulcsár, M., Huszenicza, Gy., Neogrády, Zs. and Gálfi, P.: **Az apoptotikus sejt arányának csökkenése hyperketonaemiás tehenek endometriumában az involúció korai időszakában**

(Decreasing the proportion of apoptotic cells in the endometrium in early period of involution in hyperketonaemic cows), Magy. Állatorv. Lapja, 132. 641-646, 2010. (**IF:** 0.3)

Pászti-Gere, E., **Mátis, G.**, Farkas, O., Kulcsár, A., Palócz, O., Csikó, Gy., Neogrády, Zs. and Gálfi, P.: **The effects of intestinal LPS exposure on inflammatory responses in porcine enterohepatic co-culture system**, Inflammation, [submitted] (last known **IF:** 1.747)

Oral presentations on Hungarian national conferences:

Mátis, G., Mitze, S., Neogrády, Zs. and Gálfi, P**.: Bakteriális lipopoliszacharidok által kiváltott interleukin-6 termelés bendőhámsejteken**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2009.

Mátis, G., Mitze, S., Vonza, É., Neogrády, Zs. and Gálfi, P.: **Bendőnyálkahártya hám- és kötőszöveti eredetű sejtjeinek bakteriális lipopoliszacharidok által kiváltott interleukin-6 termelése**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2010.

Mátis, G., Csikó, Gy., Kulcsár, A., Petrilla, J., Farkas, O., Palócz, O., Neogrády, Zs. and Gálfi, P.: **Bakteriális lipopoliszacharidok által kiváltott oxidatív stressz hatása sertés primer májsejttenyészetre**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2013.

Farkas, O., Palócz, O., Csikó, Gy., Pászti-Gere, E., **Mátis, G.**, Kulcsár, A., Petrilla, J., Neogrády, Zs. and Gálfi, P.: **Oxidatív stressz és gyulladás hatásának vizsgálata in vitro bélhám és májsejt ko-kultúra modellen**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2013.

Kulcsár, A., Molnár, A., **Mátis, G.**, Petrilla, J., Pál, L., Jerzsele, Á., Neogrády, Zs. and Dublecz, K.: **A különböző koncentrációban adott butirát gátló hatásának *in vitro* vizsgálata *Campylobacter jejuni* törzsekre**, MTA Akadémiai Beszámolók, Budapest, Hungary, 2013.

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