

Theses of doctoral (PhD) dissertation

**EVALUATION OF FEED ADDITIVES AND THEIR
POTENTIAL IN ANTIMICROBIAL REPLACEMENT
IN FARM ANIMALS**

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1. Antecedents and objectives of the doctoral dissertation

Due to the growing demand, it is essential to improve animal food production, and the demand for healthy meat products free of harmful additives has increased tremendously in recent decades. In addition, a ban on the use of antimicrobial drugs for growth promotion will also facilitate the production of drug-free animal products. Further restrictions imposed by the authority are expected on the treatment of livestock with medicinal products, especially antimicrobials. One option to reduce drug use is to use beneficial agents that can be used in feed. A number of non-medicinal active substances have health-promoting and growth-promoting effects, and these feed additives have immunomodulatory activity or may have beneficial effects on the microbiota composition of the host. The beneficial effects of many non-drug substances have been observed and described, but their detailed mechanism of action and possible side effects are little known. More detailed knowledge would allow for more targeted application and more efficient use. Several feed supplements are available, of which the effects of fulvic acid, sanguinarine, drinking water acidifier, probiotic metabolites and beta-glucan have been investigated.

The objectives of the present doctoral dissertation are summarized below.

- Our aim is to investigate the protective effect of two probiotics and four commercially available feed supplements on endotoxin-induced inflammation in porcine intestinal epithelial cells. Furthermore, to determine the effect of the test feed supplements on the expression of porcine cytochrome P450 genes.
- Adaptation of CYP isoenzyme-specific luminescent substrates developed for the determination of the activity of human cytochrome P450 enzymes to measure the cytochrome P450 enzyme activity of rabbit (*Oryctolagus cuniculus*).

- The aim of this study is to investigate the effects of four feed supplements - β -glucan, drinking water acidifier, sanguinarine-containing product and fulvic acid - on the expression of hepatic cytochrome P450 genes in chickens (*Gallus gallus domesticus*).
- A further aim of our studies is to measure the cytochrome P450 enzyme activity of the liver after treatment with the above-mentioned additives and metabolites of a poultry probiotic; *Bacillus licheniformis* and to determine whether there is an interaction between the feed additives and tiamulin, a reference antibiotic known to inhibit CYP enzymes.
- A further aim of our research is to investigate the effects of two commercially available feed supplements, fulvic acid and a sanguinarine-containing product, on drug metabolism in rabbits. The effect on important cytochrome enzymes, CYP2C and CYP3A6 in drug biotransformation is investigated *ex vivo*. The antibiotic tylosin was used as a cytochrome P450 enzyme inhibitor model compound to monitor for feed-drug interactions on CYP enzymes.
- Finally, the aim of our study is to determine whether the administration of the four feed supplements - (1-3), (1-6) β -glucan, fulvic acid, a sanguinarine-containing preparation, and a drinking water acidifier - to chickens, affect the level of specific circulating antibody titer induced by *Pasteurella multocida* vaccination.

2. New scientific results of the thesis

I. Possible effects on infection and inflammatory processes

1. The inhibitory effect of all test feed additives (Wellmune WGP[®], Sangrovit[®] WS, Immunofort[®], and Fulvix pulvis[®]) was demonstrated in endotoxin-evoked inflammation in porcine intestinal cell culture; Hsp70 and TNF-alpha mRNA levels were reduced after the simultaneous LPS and feed additive treatment.
2. The immunomodulatory activity of beta-glucan and fulvic acid was exhibited in LPS-induced inflammation in IPEC-J2 cells; IL-8 gene expression was increased while TNF-alpha gene expression was decreased after the cell cultures were concurrently exposed to LPS and the feed additive.
3. Administration of beta-glucan via drinking water along with *Pasteurella multocida* vaccine to chickens did not influence the amount of serum *Pasteurella multocida* immunoglobulin formed.
4. The simultaneous use of fulvic acid or drinking water acidifier or the sanguinarine containing product with the inoculation of *Pasteurella multocida* vaccine leads to higher specific antibody level in the chicken flock.

II. Effects on drug metabolism

1. Porcine CYP3A29 mRNA levels were not affected by any of the feed additives tested; Wellmune WGP[®], Sangrovit[®] WS, Immunofort[®], and Fulvix pulvis[®].
2. The supplements with acidic properties increased the intestinal gene expression of porcine CYP1A1 and CYP1A2 genes, especially at higher doses.

3. The adapted cytochrome P450 luminescent method is a fast, safe, simple, and sensitive tool for testing the effect of active substances on the rabbit's cytochrome P450 system.
4. The *in vitro* and *in vivo* findings about rabbit CYP450 expression pattern were largely consistent, validating the short-term primary hepatocyte cell culture method in xenobiotic metabolism investigations.
5. The investigated drinking water supplements did not cause any alteration in the level of the measured rabbit hepatic microsomal CYP activity alone or in combination with tylosin tartrate *ex vivo*.
6. The *in vivo* applied water-soluble feed additives did not cause clinically important alterations in the gene expression level and the activity of the avian CYP2C.
7. Co-administration of investigated feed additives with *ex vivo* tiamulin exposure had no relevant effect on the action of both enzymes tested – neither CYP1A nor CYP2C in chickens.

3. List of the candidate's publications related to the topic of the dissertation certified by the Library, indicating all authors

List of full text papers in peer-reviewed journals:

Palócz Orsolya, Csikó György: Az antibiotikumok túlzott mértékű használatának csökkentését célzó szerek az állattenyésztési és klinikai gyakorlatban: Irodalmi áttekintés: Reduction of the excessive use of antibiotics in animal husbandry and in clinical practice, Literature review, MAGYAR ÁLLATORVOSOK LAPJA 136:(3) pp. 177-183. (2014), IF 0,185

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Palocz O, Paszti-Gere E, Galfi P, Farkas O: Chlorogenic Acid Combined with *Lactobacillus plantarum* 2142 Reduced LPS-Induced Intestinal Inflammation and Oxidative Stress in IPEC-J2 Cells, PLOS ONE 11:(11) Paper e0166642. (2016), IF 2,806

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Farkas O, Palócz O, Pászti-Gere E, Gálfi P: Role of chlorogenic acid combined with *Lactobacillus plantarum* in treatment LPS-induced intestinal inflammation, In: Edeas Marvin (szerk.), 9th World Congress on Polyphenols Applications. Konferencia helye, ideje: St. Julian's, Málta, 2015.06.03-2015.06.05. International Society of Antioxidants, 2015. p. 60.

Palócz Orsolya, Farkas Orsolya, Szentmiklósi Diána, Nagy Tamás és Csikó György: Xenobiotikumok hatása házinyúl eredetű citokróm P450 enzimrendszerre *in vivo* vs. *in vitro*, 42. Állatorvostudományi Akadémiai Beszámolók, 2016. január

Somogyi Zoltán, Palócz Orsolya, Csikó György: Baromfikolera vakcina védőhatásának támogatása béta-glükánnal, 42. Állatorvostudományi Akadémiai Beszámolók, 2016. január

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Csikó György, Palócz Orsolya: Enhancing the efficiency of immunization via drinking water supplementation in broiler chickens, In: M Francesch, D Torrallardona, J Brufau (szerk.), Proceedings of the 21st European Symposium on Poultry Nutrition. 308 p., Konferencia helye, ideje: Vila-seca, Spanyolország, 2017.05.08-2017.05.11., 2017. p. 202.

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Farkas O., Palócz O., Pásztiné Gere E., Gálfi P.: Anti-inflammatory effect of probiotics – a complex *in vitro* intestinal model, International Scientific Conference of Probiotics and Prebiotics (IPC 2017) Budapest, 2017. június 20-22.

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Palócz Orsolya, Csikó György: Cytochrome P450 gene expression of porcine jejunal cells exposed to feed additives, JOURNAL OF VETERINARY PHARMACOLOGY AND THERAPEUTICS 41: Suppl 1, 14th Congress of the European Association for Veterinary Pharmacology and Toxicology in Wrocław, Poland, 2018.06.23-2018.06.27.

Csikó György, Palócz Orsolya: Examination of the interspecies differences in microsomal drug metabolism in chickens and rabbits, JOURNAL OF VETERINARY PHARMACOLOGY AND THERAPEUTICS 41: Suppl 1, 14th Congress of the European Association for Veterinary Pharmacology and Toxicology in Wrocław, Poland, 2018.06.23-2018.06.27.

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