

Резюмета

на научните трудове на

гл. ас. д-р Тончо Господинов Динев

представени за участие в конкурс за „Доцент” по Научна специалност „Микробиология“, Професионално направление 4.3. „Биологически науки”, Област 4. „Природни науки, математика и информатика”

6. *Journal of Veterinary Pharmacology and Therapeutics*, 2008, 31, 167–170. (IF=1.581)

Pharmacokinetics of pefloxacin and its metabolite norfloxacin in male and female ducks

D. Dimitrova¹, R. Moutafchieva¹, I. Kanelov¹, T. Dinev¹, S. Yanev², B. Pandova², L. Lashev¹

¹Department of Pharmacology, Physiology of Animals and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria;

²Department of Drug Toxicology, Institute of Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Abstract: Pefloxacin (PFL) is a fluoroquinolone with a broad spectrum of antimicrobial activity against both Gram-negative and Gram-positive microorganisms including intracellular bacteria. The purpose of the present study was to determine the serum concentrations and pharmacokinetic parameters of PFL and its main metabolite norfloxacin (NFL) in male and female ducks after intravenous (i.v.) and oral (p.o.) administration. The study was performed on 10-month-old 12 healthy ducks (6 male and 6 female stock), weighing 3.5–4.5 kg. Pefloxacin was administered i.v. and orally in a dose of 10 mg/kg of body weight. High performance liquid chromatography (HPLC) with fluorescence detection was used as the method of analysis for PFL. Pefloxacin was absorbed rapidly, with insignificant differences between male and female ducks. Norfloxacin appeared in the blood immediately after i.v. or p.o. PFL administration. In both cases the lag time was very short. The rate of elimination of NFL was comparable to that of PFL. The blood levels of NFL remained very low, with slightly higher rates of metabolism in males. Generally PFL demonstrates a kinetic behaviour typical for fluoroquinolones after i.v. administration, but both its distribution and elimination in our experiments are faster than such behaviour for the other members of the group as per the data published.

7. *Journal of Veterinary Pharmacology and Therapeutics*, 2009, 32 (Suppl. 1), 148–149. (IF = 1.408)

26. Abstracts of the 11th European Association for Veterinary Pharmacology and Toxicology (EAVPT 2009), July 12-16, 2009, Leipzig, Germany.

Gender related differences in the pharmacokinetics of antibacterials in poultry

L. Lashev, D. Dimitrova, A. Haritova, I. Kanelov, R. Moutafchieva, **T. Dinev**
Department of Pharmacology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Abstract: *Sulfonamides.* Previous investigations have shown various differences of the behavior in both sexes after i.v. introduction of sulfadimidine (SDD) and sulfachlorpyrazine (SCP), sulfamethoxazole (SMZ), sulfathiazole (STZ), sulfacetamide (SAM) to adult chickens, pheasants, and quails. In roosters the total body clearance is higher for SCP, but for STZ, SDD and SAM no statistical difference exists. In all cases the elimination is faster. The same is registered for SDD, SMZ and trimethoprim in male quails. No statistically significant difference was found for SDD in pheasants. Faster absorption and elimination were found in roosters after oral (p.o.) administration of SDD, sulfadiazine, SMZ, STZ and SCP. *Fluoroquinolones.* Danofloxacin, marbofloxacin, enrofloxacin and pefloxacin applied i.v. and p.o. in chickens, turkeys, ducks and quails did not show any statistically significant sex related pharmacokinetic distinctions.

Penicillins. The rate and the level of ampicillin distribution as well as the total body clearance have lower values in cocks. In quails ampicillin do not show any differences. In the cases of p.o. treatment of hens and cocks with ampicillin, amoxicillin and penicillin V, faster absorption, higher maximal blood levels and faster elimination were found in the males. Similar results were found for male and female ducks, treated with amoxicillin. *Aminoglycosides, aminocyclitols.* Roosters, treated i.v. with kanamycin, gentamicin and apramycin (APR) slower distributed and eliminated all of the antibiotics than the hens. The values of the total body clearance are higher in the hens. The same characterise tobramycin disposition in mallard ducks, but quails treated i.v. with APR did not show any differences between both sexes.

8. *The Veterinary Journal*, 2009, 179, 462–464. (IF = 2.323)

Pharmacokinetics of tobramycin in ducks and sex-related differences

D. Dimitrova, R. Moutafchieva, I. Kanelov*, **T. Dinev**, L. Lashev
Department of Pharmacology, Physiology of Animals and Physiological Chemistry, Trakia University, 6000 Stara Zagora, Bulgaria

Abstract: The aims of the present study were to determine the disposition of tobramycin after single intravenous (IV) and intramuscular (IM) injections in ducks, and to establish any sex-related differences. Tobramycin sulfate was administered as a 2.5% water solution in a crossover design at a dose of 5 mg/kg to 12 healthy ducks (six males and six females). Concentrations of the drug in serum were determined by a microbiological assay. The serum pharmacokinetic values for tobramycin were best represented using a one- or two-compartment open model, depending on the method of administration. Non-compartment analysis was also performed after IV administration. Tobramycin had a low degree of

distribution and a relatively fast elimination. The mean volume of distribution in ducks (males and females) was higher than that reported in pigeons but lower than in chickens, with a slower rate of elimination. The IM injection resulted in a fast and complete absorption. The rate of elimination after IM administration was about twice as slow as in other avian species. Sex-related variations in tobramycin pharmacokinetics were similar to those reported for kanamycin and apramycin in hens and roosters.

9. *Journal of Veterinary Pharmacology and Therapeutics*, 2012, 35 (Suppl. 3), 110-111. (IF = 1.349)

27. The 12th International Congress of the European Association for Veterinary Pharmacology and Toxicology (EAVPT 2012), July 8-12, 2012, Noordwijkerhout, The Netherlands.

Pharmacokinetics of enrofloxacin and marbofloxacin in Japanese quails and pheasants

L. Lashev, D. Dimitrova, A. Haritova, R. Moutafchieva, **T. Dinev**
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

Abstract: The aim of the present investigation was to study and compare the pharmacokinetics of enrofloxacin (EFC) and marbofloxacin (MFC) in Japanese quails and pheasants. EFC (10 mg kg⁻¹) and MFC (5 mg kg⁻¹) were administered intravenously (i.v.) and orally (p.o.) after 12 h of food deprivation to Japanese quails (202.1 ± 20.5 g b.w.) and common pheasants (0.92 ± 0.1 kg b.w.). Serum concentrations of MFC, EFC and its main metabolite ciprofloxacin (CFC) were determined by an in-house validated HPLC method. Pharmacokinetic analyses were performed with a compartmental model using WinNonlin 5.0.1. The values of AUC_{CFC}/AUC_{EFC} are as follows: quails: i.v. – 0.13; p.o. – 0.48; pheasants: i.v. – 0.22; p.o. – 0.20. MFC metabolites were not detected neither in quails nor in pheasants. After i.v. administration quails eliminate EFC and MFC faster than pheasants. EFC is distributed and eliminated faster than MFC from both species. EFC is metabolized to a higher degree in quails, compared to chickens and turkeys. After p.o. administration quails absorb and eliminate both substances faster than pheasants, which showed pharmacokinetic data comparable to that reported from chickens and turkeys. Metabolite formation in quails is extremely high, in comparison to pheasants and other poultry species. The bioavailability of both substances in pheasants is higher. In conclusion, these results contribute to the elucidation of species difference in avian species.

10. *Journal of Avian Medicine and Surgery*, 2013, 27(1), 23–31. (IF = 0.672)

Comparative pharmacokinetics of enrofloxacin, danofloxacin, and marbofloxacin after intravenous and oral administration in Japanese quail (*Coturnix coturnix japonica*)

A. Haritova, D. Dimitrova, **T. Dinev**, R. Moutafchieva, L. Lashev
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria;

Abstract: A population approach was used to evaluate the pharmacokinetic parameters of 3 fluoroquinolones administered to Japanese quail (*Coturnix coturnix japonica*). Healthy adult quail (n = 50) were divided into 3 groups, each administered a separate intravenous and oral dose of the compounded drug: enrofloxacin at 10 mg/kg (n = 18; 9 male, 9 female), danofloxacin at 10 mg/kg (n = 12; 6 male, 6 female), and marbofloxacin at 5 mg/kg (n = 20; 10 male, 10 female). A fourth group was used as a control (n = 5). Enrofloxacin was metabolized extensively to ciprofloxacin, while no metabolites of either danofloxacin or marbofloxacin were detected. The volume of distribution was high, greater than 1 in all cases, and highest for danofloxacin, followed by enrofloxacin, then marbofloxacin. The total body clearance was higher in quail than that reported for other avian species with the exception of ostriches. As in mammals, the lowest clearance rate of the 3 fluoroquinolones was observed for marbofloxacin. Enrofloxacin was absorbed most rapidly, followed by marbofloxacin, then danofloxacin. The highest bioavailability was observed for danofloxacin followed by marbofloxacin, while very low bioavailability with significant conversion to ciprofloxacin was observed for enrofloxacin. Population analysis showed low intersubject variability for danofloxacin and marbofloxacin in contrast to that for enrofloxacin and its main metabolite, ciprofloxacin. Because of their more favorable pharmacokinetic properties after oral administration, either danofloxacin or marbofloxacin appears to be preferable to enrofloxacin for the treatment of susceptible bacterial infection in Japanese quail.

11. *British Poultry Science*, 2014, 55(1), 120–125. (IF = 0.936)

Comparative pharmacokinetics of danofloxacin in common pheasants, guinea fowls and Japanese quails after intravenous and oral administration

D. Dimitrova, A. Haritova, **T. Dinev**, R. Moutafchieva, L. Lashev
Department of Pharmacology, Physiology of Animals and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

Abstract: 1. The pharmacokinetics of danofloxacin was investigated in common pheasants, guinea fowls and Japanese quails after intravenous (i.v.) and oral (p.o.) administration at a dose of 10 mg kg⁻¹ body weight. Concentrations of the drug in serum were determined by high-performance liquid chromatography. The values of the pharmacokinetic parameters after both applications were calculated on the basis of a one-compartment model. The elimination half-lives after i.v. injection were 6.82 ± 1.87, 3.31 ± 0.13 and 3.84 ± 0.89 h in pheasants, guinea fowls and quails, respectively. Total body clearance values were 0.45 ± 0.16, 1.23 ± 0.07 and 1.61 ± 0.34 l h⁻¹ kg⁻¹ in pheasants, guinea fowls and quails, respectively. After p.o. administration, maximum serum concentrations were 0.54 ± 0.26, 0.51 ± 0.12 and 0.78 ± 0.11 µg ml⁻¹ respectively, reached at 2.04 ± 0.23, 10.4 ± 5.64 and 5.35 ± 0.47 h. Oral

bioavailability values were 82.32% for pheasants, 79.46% for guinea fowls and 83.5% for Japanese quails. Pharmacokinetic/pharmacodynamic (PK/PD) predictive indices were also calculated and compared.

12. *Bulgarian Journal of Veterinary Medicine*, 2015, 18(4), 325–337. (SJR = 0.181)

Interspecies and gender-related variations of some haematological parameters in Galliformes bird species

L. Lashev¹, S. Atanasova², T. Dinev¹

¹Department of Pharmacology, Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

²Department of Biochemistry, Microbiology and Physics, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

Abstract: An analysis of literature data about routine haematological parameters of avian species from the Galliformes order is made. Data for the following gallinaceous birds were included: wild and domesticated chicken breeds, common pheasant, quail, turkey, peafowl and guinea fowl. Data for red and white blood picture indices such as haemoglobin, red blood cells (RBC), packed cell volume (PCV), total and differential white blood cell (WBC) counts from the literature as well as own data were used. Gender-related differences were reported by most of authors for RBC, haemoglobin and PCV with higher values in male birds from the *Gallus gallus* species. Similar results were not found in other species of the order. In most of the cases no statistically significant interspecies or gender-related differences in WBC counts were registered. Tendencies for higher heterophil/lymphocyte ratios (H/L) in male birds were also registered. Differences related to the breed, result of various degree of domestication, were not identified.

13. *Istanbul Üniversitesi Veteriner Fakültesi Dergisi*, 2016, 42(2), 186-189. (SJR = 0.209)

Comparative study of erythrocyte sedimentation rate after aminoglycoside and aminocyclitol treatment in goats (*Capra hircus*)

T. Dinev¹, D. Kanakov², G. Beev¹, N. Rusenova³, S. Denev¹

¹Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, Trakia University, 6000 Stara Zagora, Bulgaria.

²Faculty of Veterinary Medicine, Department of Internal Diseases, Trakia University, 6000 Stara Zagora, Bulgaria.

³Faculty of Veterinary Medicine, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Trakia University, Stara Zagora 6000, Bulgaria.

Abstract: The aim of the present study was to follow up the erythrocyte sedimentation rate (ESR) in healthy female goats during and after 5-day parenteral treatment with amikacin (10 mg/kg), tobramycin (5 mg/kg), apramycin (20 mg/kg), gentamicin (4 mg/kg), kanamycin (10 mg/kg) and spectinomycin (20 mg/kg). Gentamicin and tobramycin caused an initial increase followed by a significant decrease of ESR on the 5th day for gentamicin and the 10th day for tobramycin, respectively, followed by recovery after the treatment. Reversely, amikacin and especially spectinomycin produced an increase of ESR without recovery several days post treatment. Kanamycin caused decrease of ESR on the 5th day without recovery in the subsequent days. Only apramycin did not give rise to increasing of ESR. In conclusion, the aminoglycosides, especially tobramycin and gentamicin, caused more severe alterations of ESR than the aminocyclitols.

14. *Trakia Journal of Sciences*, 15(1), 50-55.

Pharmacokinetics and allometric analysis of spectinomycin

T. Dinev

Department of Biochemistry, Microbiology and Physics, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

Abstract: In this study were compared microbiological and HPLC methods for determination of spectinomycin pharmacokinetics in goats. The goats were subject to intravenous administration of spectinomycin at 20 mg/kg body weight. For the microbiological assay was used test microorganism *Sarcina lutea* ATCC 9341. Limit of quantification in the microbiological assay was 6.25 µg/mL and in the HPLC method was 0.1 µg/mL. Spectinomycin concentrations following microbiological assay were initially higher than HPLC-determined and gradually the differences decreased. During HPLC analysis spectinomycin was found until 12 h and during microbiological assay until 4 h. As a result, in HPLC analysis the values of $V_d(\text{area})$ (0.527 L/kg), $t_{1/2\beta}$ (1.74 h) and Cl_B (3.512 mL/kg/min) were considerably higher in comparison with microbiological method ($V_d(\text{area})$ - 0.147 L/kg, $t_{1/2\beta}$ - 0.8 h and Cl_B - 2.174 mL/kg/min). The data from method validation also showed the advantage of HPLC method. Because of that can be concluded that HPLC is more sensitive and accurate method for spectinomycin determination.

Regarding the allometric equation for elimination half-life ($t_{1/2\beta} = 1.19.W^{0.02}$) the values are showing lack of correlation to body weight. Volume of distribution allometric equation ($V_d(\text{area}) = 0.37.W^{0.96}$) and total body clearance allometric equation ($Cl_B = 1.92.W^{1.09}$) have high level of correlation to body weight (<0.001) and therefore can be used.

15. *Journal of Central European Agriculture*, 2017, 18(2), 369-387. (SJR = 0.161)

28. 9th Scientific conference of the Bulgarian Focal Point of EFSA, October 24-25, 2016, Hissar, Bulgaria, pp. 66-67.

Reproduction impact of mancozeb on rainbow trout (*Oncorhynchus mykiss* W.) and accumulation of its carcinogen metabolite, ethylene thiourea in fish products

M. Tzanova¹, V. Atanasov¹, B. Zaharinov², G. Beev¹, **T. Dinev¹**, E. Valkova¹

¹Department of Biochemistry, Microbiology and Physics, Faculty of Agriculture, Trakia University, Studentski grad, 6000 Stara Zagora, Bulgaria.

²Department of Natural sciences, New Bulgarian University, Mondevideo Str 21, 1618 Sofia, Bulgaria

Abstract: Pesticides can be taken up from the water and accumulated in tissues of hydrobionts, often multiplying thousands of times higher in the organism than in the surrounding water. The dithiocarbamate mancozeb is applied in plant protection as fungicide. In recent years the amount of mancozeb used in Europe significantly increased. It is carcinogen due to its metabolite - ethylene thiourea (ETU), which causes thyroid and pituitary tumors. The purpose of this study is to determinate the quantity of ethylene thiourea in products of rainbow trout (*Oncorhynchus mykiss* W.), reared in environment containing permissible, according to the European law, amount of mancozeb. Seeking an answer to the question: is this concentration limit really safe for the reproduction of rainbow trout and can the more toxic metabolite - ETU, be accumulated in the fish eggs and fillet and afterwards make them harmful to the consumers? The study included 3 stages: feeding, analysis of ethylene thiourea in fish eggs and fillet by a new developed and validated HPLC (high performance liquid chromatography) method and study of the reproductive indicators. The assays of ETU in all analyzed samples (fish and water) were below the limit of quantification of the method, 0.05 mg·l⁻¹, so fish do not accumulate the carcinogen degradation product of mancozeb and the maximum residue level of mancozeb is really safe for the humans as consumers. But these environmental conditions caused reproductive disorders. They can be partly compensated by using sperm activation medium for artificial insemination of trout eggs, but successful fertilization does not guarantee successful hatching, especially of eggs in trout farms with presence of mancozeb in water, even in allowable concentration. The presented results confirm previous investigation, that Salmonidae are very sensitive fish species, react to the lowest deviations in concentration levels of xenobiotics and are used for indicator of non-polluted water.

16. Bulgarian Journal of Veterinary Medicine, 2018, 21(3), 253–268. (SJR = 0.167)

Antimicrobial activity of *Lactobacillus plantarum* against pathogenic and food spoilage microorganisms: a review

T. Dinev¹, G. Beev¹, M. Tzanova¹, S. Denev¹, D. Dermendzhieva², A. Stoyanova³

¹Department of Biochemistry, Microbiology and Physics; Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

²Department of Applied Ecology and Animal Hygiene; Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

³Department of Plant Production; Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

Abstract: One of the most important properties of probiotic bacteria is their antimicrobial activity against many species of microorganisms which could be useful to prevent food spoilage caused by certain sensitive bacteria and fungi as well as to control the speed of

propagation of potentially pathogenic bacteria by probiotic application. *Lactobacillus plantarum* is considered one of the probiotic bacteria with broadest spectrum of antibacterial activity which makes it useful in veterinary, human medicine and food industry. According to a number of studies *Lactobacillus plantarum* exerts inhibitory activity against many Gram-positive and Gram-negative bacteria – *Escherichia coli* (including *E. coli* 0157:H7), *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Klebsiella*, *Salmonella*, *Shigella*, *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus spp.*, etc. as well as a number of moulds and yeasts – *Aspergillus*, *Fusarium*, *Mucor*, *Candida spp.*, etc. The main antibacterial compounds of *Lactobacillus plantarum* are the bacteriocins and organic acids whereas the antifungal compounds are the organic acids, hydroxy fatty acids and cyclic dipeptides. Because of the high antifungal activity of some *L. plantarum* strains against food spoilage microorganisms they can be used as effective biopreservatives in food industry. Also, some *L. plantarum* strains could be applied as supporting therapeutic agents in treatment of infections caused by the corresponding susceptible microorganisms.

17. *Romanian Biotechnological Letters*, 2019, 24(1), 176-183. (IF2019 = 0.59)

***In vitro* test of inhibition effect of extracts from three seaweed species distributed at Black Sea on different pathogens potentially dangerous for aquaponics**

I. Sirakov¹, K. Velichkova¹, N. Rusenova², T. Dinev³

¹Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, Students campus, Stara Zagora, Bulgaria.

²Trakia University, Faculty of Agriculture, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Students campus, Stara Zagora, Bulgaria.

³Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, Students campus, Stara Zagora, Bulgaria.

Abstract: Aquaponics is innovative recirculation system where hydrobionts are cultivated together with the plants. The possibilities for control of pathogens in these systems are highly restricted. One possible strategy for inhibition of pathogenic microorganisms in aquaponics is the usage of seaweeds extracts. The study connected with the investigation of inhibition effect of seaweeds from Bulgarian Black Sea coast on different pathogens is rare. The aim of current study was to test *in vitro* the inhibition effect of three seaweed species (*Ulva rigida*, *Cladophora vagabunda*, and *Ceramium rubrum*) distributed at Black Sea in front of Bulgarian coast on different pathogens (bacteria and fungi) which are potentially harmful to hydrobionts, plants and consumer of aquaponics products. The ethanol and methanol extracts from investigated seaweed species were prepared. They were tested with agar well diffusion method against the following fish, food borne and plant pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Salmonella typhimurium*, *Candida albicans*, *Penicillium verrucosum var. verrucosum*, *Fusarium graminearum*, *Fusarium moniliforme* and *Aspergillus ochraceus*. The current study showed that the following extracts from seaweeds distributed in front of Bulgarian Black sea coast possess high inhibition effect (size of inhibition zone higher than 10 mm) against potentially pathogenic microorganisms in aquaponics: ethanol extract of *C. vagabunda* against *B. cereus* and *A. ochraceus*, methanol extract of *C. vagabunda* against *C. albicans*, ethanol extract of *C.*

rubrum against *E. coli*, *B. cereus* and *C. albicans*.

18. *Ecologia Balkanica*, 2019, 11(2), 167-180. (SJR = 0.134)

Agro-ecological assessment of Ovcharitsa Dam (Bulgaria) water used for thermal power plant cooling

D. Dermendzhieva¹, G. Zhelyazkov², G. Beev³, G. Kostadinova¹, **T. Dinev**³, G. Petkov¹

¹Trakia University, Faculty of Agriculture, Department of Applied Ecology and Animal Hygiene, Stara Zagora 6000, Bulgaria.

²Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, Stara Zagora 6000, Bulgaria.

³Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, Stara Zagora 6000, Bulgaria.

Abstract. Ecological (as a natural source) and agricultural (as a resource for fish farming and irrigation of crops) assessment of Ovcharitsa Dam water, used for thermal power plant (TPP) cooling was carried out in one monitoring point by measurement of 12 physicochemical parameters (temperature, transparency, pH, EC, DO, COD, BOD₅, unionized NH₃, NO₂⁻, NO₃⁻, total N and PPO₄), one biological parameter (chlorophyll-a), 9 pesticides and volatile organic compounds /VOC/ (atrazine, simazine, diuron, 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene, tetrachloroethane, trichloromethane, hexachlorobutadiene) and 6 microbiological parameters (aerobic mesophilic microorganisms, coliforms, total coli titer, *Escherichia coli* counts, *E. coli* titer, *Salmonella* spp. counts), stipulated in Bulgarian legislation in 2016-2017 (REGULATIONS: No. 4, 2000; No. 18, 2009; On EQS for priority substances and certain other pollutants, 2010 and No. H-4, 2012). The water samples were taken periodically during a two- year period: for physicochemical and biological analysis – in February, April, June, August and November; for microbiological observation – in June, August and November; for pesticides and VOC – in April. Water sampling, sample preparation and analysis were performed according international ISO and BSS standards. It was found that: a) the dam water ecological status was determined as “poor” by chlorophyll-a content and “very poor” by orthophosphates content based on the lowest estimates for the monitored parameters; b) with regard to the content of pesticides and VOC, the dam water was defined as water “in good chemical status”; c) the values of all monitored parameters were within the ranges (recommended and mandatory) for carp fish water with exception of temperature and unionized NH₃, which exceeded the norms during some months of the year; d) according to measured water transparency, the trophic state of dam water was determined as hypereutrophic; e) the microbial status of the analyzed water demonstrated that it was not suitable for irrigation because it exceeded norms for total coli-titer and *E. coli*-titer, and due to the presence of intestinal pathogens (*Salmonella* spp.), which are not allowed in the water for irrigation.

19. *Bulgarian Journal of Agricultural Science*, 2019, 25 (Suppl. 3), 3-12. (SJR = 0.191)

34. 3rd *International Conference of Bio-antioxidants "Natural Bio-antioxidants as an Inspiration for Food Chemistry and Pharmacy"*, September 17-21, 2019, Nessebar, Bulgaria, p. 42.

Antioxidant constituents and antioxidant activity of some red wine and red table grape varieties, cultivated in different regions of Bulgaria

M. Tzanova¹, V. Atanasov¹, M. Ivanov², A. Iliev², S. Atanassova¹, P. Peeva¹, N. Grozeva¹, M. Gerdzhikova¹, **T. Dinev¹**

¹Trakia University, Faculty of Agriculture, 6000 Stara Zagora, Bulgaria,

²Agricultural Academy, Institute of Viticulture and Enology, 5800 Pleven, Bulgaria

Abstract: Foods that have a positive effect on human health are becoming more and more popular nowadays. Such foods are the grapes - rich in phenolic compounds, which are known as very potent bio-antioxidants. This paper presented a research made for the first time-twelve red wine varieties (Rubin, Kaylashki Rubin, Storgozia, Mavrud, Nikopolski Mavrud, Melnik 55, Bouquet, Cabernet Sauvignon, Merlot, Syrah, Mourvedre and Malbec) and eight red table varieties (Velika, Dunav, Siyana, Hybrid V5-1, Muskat Hamburg, Moldova, Palieri and Black Pearl), cultivated in different regions of Bulgaria were tested for their antioxidant activity (AA) and contents of total phenols (TPC), *trans*-resveratrol (t-RVT) and quercetin (QU). In the grape skins the parameters ranged: t-RVT- from 2.05 ± 0.21 to 14.34 ± 1.35 and from 1.44 ± 0.17 to 23.71 ± 2.53 mg/kg FW; QU - from 0.27 ± 0.03 to 1.98 ± 0.18 and from 0.63 ± 0.07 to 2.12 ± 0.20 mg/kg FW; TPC – from 17 ± 2 to 371 ± 33 and from 21 ± 2 to 444 ± 43 mmol GAE/kg dm and AA – from 23.2 ± 1.9 to 59.9 ± 5.5 and from 32.4 ± 2.8 to 66.4 ± 6.3 mmol TE/kg dm for wine and table grapes, respectively. The table grape varieties had higher mean values of the parameters examined than the wine grape varieties. South Bulgaria have grape varieties with higher values of the antioxidant parameters with comparison to North Bulgaria. But, Danubian region, North Bulgaria, have wine and table grape varieties with very good antioxidant parameters and can't be ignored. The reason for this is the agrometeorological conditions. The correlations between the determined values were positive with very high correlation coefficients.

20. *Bulgarian Journal of Agricultural Science*, 2019, 25 (Suppl. 3), 109-115. (SJR = 0.191)

31. *International Conference on Agricultural Science and Business*, May 30-31, 2019, Stara Zagora, Bulgaria, p. 46.

Physicochemical, sanitary and safety indicators changes during the ripening of Bulgarian white brined cheese from local farms

G. Beev¹, T. Kolev², N. Naydenova³, **T. Dinev¹**, M. Tzanova¹, G. Mihaylova³

¹Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, 6000 Stara Zagora, Bulgaria.

²Agricultural Institute, Department of Breeding and Technologies in Cattle Breeding, 6000 Stara Zagora, Bulgaria

³Trakia University, Faculty of Agriculture, Department of Dairy Science, 6000 Stara Zagora, Bulgaria.

Abstract: The present study aims to determine the physicochemical and microbiological changes of white-brined cheese from local farms during manufacturing and ripening. Milk pasteurization for white-brined cheese production leads to a severe reduction of microorganisms in milk. Thus, after pasteurization the total number of microorganisms decreases from 480 000 to 810 cfu/cm³, *Salmonella* spp. from 800 to 2 cfu/cm³ and *E. coli* from 4000 to 0 cfu/cm³. Ripening processes lead to a drastic reduction of cheese microflora with prevalence of specific lactic microflora (lactobacilli and lactococci) on the 45th day and complete annihilation of *E. coli* and *Salmonella* spp. These changes in the cheese microflora made the final product safe for consumption. On the other hand, the experimental data shows a strong multiplication of *Salmonella* spp. on the 7th day (10 cfu/cm³ at the 24th hour reached 0 cfu/cm³ on the 7th day) and insufficient decrease of the number of other microorganisms, making fresh white-brined cheese at its early ripening stages unsafe for consumption. Ripening of the cheese brings about an increase of the dry matter percentage (from 33.5% at 24th hour to 38.5% at 45th day), the fat content (from 13.3% to 16.4%), salt content (from 4.1% to 5.8%) and total protein content (from 13.7% to 16.7%) and reduction of moisture in non-fat substance (from 76.7% to 73.8%) of the final product. These changes are in accordance with the accepted standards for white-brined cheese production.

21. *Bulgarian Journal of Agricultural Science*, 2019, 25 (Suppl. 3), 120-123. (SJR = 0.191)
33. *International Conference on Agricultural Science and Business*, May 30-31, 2019, Stara Zagora, Bulgaria, p. 75.

Antimicrobial activity of *Amaranthus* spp. extracts against some mycotoxigenic fungi

S. Terzieva¹, K. Velichkova¹, N. Grozeva¹, N. Valcheva², T. Dinev²

¹Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, 6000 Stara Zagora, Bulgaria.

²Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, 6000 Stara Zagora, Bulgaria.

Abstract: Plants, their parts and products have been used to treat diseases and pathogens. The aim of the present study was to test different extracts from three species of genus *Amaranthus* L. – *A. deflexus* L., *A. retroflexus* L. and *A. hybridus* L. for antifungal activities. The plant extracts (methanol and ethanol) from ground and underground plant parts were tested for antimicrobial activity by agar well diffusion method. Five fungal strains (*Penicillium verrucosum* var. *verrucosum* NBIMCC 2003 NRRL F-143, *Penicillium expansum*, *Fusarium graminearum* NBIMCC 2294 IMI 155426, *Aspergillus ochraceus* NBIMCC 2002 IM-BAS and *Aspergillus niger*) were used. Antimicrobial activity was evaluated by measuring zones of inhibition of microbial growth surrounding plant extracts in the wells. The most effective extracts, which showed activity against all tested strains of microorganisms, were *A. deflexus* and *A. hybridus* ethanol flower extract, *A. retroflexus* ethanol root extract and *A. retroflexus* methanol leaves and stem extract.

Removal of indicator and pathogenic bacteria by *Lemna minuta* Kunth in an aquaponic recirculation system.

K. Velichkova¹, I. Sirakov¹, T. Dinev²

¹Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria.

²Department of Biochemistry Microbiology and Physics, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria.

Abstract: The use of eco-technologies, such as duckweed insetting, for wastewater treatment is becoming popular because of its affordability and efficiency of pathogen removal. The purpose of the current research was to study the influence of *Lemna minuta* as a single plant in aquaponic recirculation system on the indicator microorganisms and pathogens in water. The aquaponic recirculation system consisted of 10 fish cultivation tanks and 4 plant tanks. The cleaning block of the system consisted of one mechanical filter (settling tank) and moving bed biofilter. Weekly samples of control water, fish tanks, and tanks with *L. minuta* were taken in four replicates. For the quantitative detection of some sanitary indicator microorganisms (coliforms, Enterobacteriaceae), pathogens (*Salmonella* spp.) and total count in the treated water, medium plates (Compact Dry EC; Compact Dry ETB; Compact Dry SL and Compact Dry TC, R-Biopharm AG, Germany) coated with dry culture medium were used. Cultivation of *L. minuta* as a single plant of the aquaponic recirculation system showed that the species had a very good elimination effect on pathogenic and indicatory microorganisms. *L. minuta* can be applied to purify water in aquaponic recirculation system and, in addition, the cultivated biomass can be used as feed in various agricultural industries.

23. Monograph, Dema Press, Rousse, Bg, 2020, pp. 157.

Antimicrobial potential of probiotic lactic acid bacteria

T. Dinev

Abstract: One of the most important properties of probiotic bacteria is their antimicrobial activity against many species of microorganisms, which could be useful to prevent food spoilage caused by certain sensitive bacteria and fungi as well as to control the speed of propagation of potentially pathogenic bacteria. The objective of this research was to evaluate the antimicrobial potential of important probiotic lactic acid bacteria (LAB) (*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*).

Although *in vitro* activity of probiotic bacteria (based on the production of bacteriocins, organic acids and some other inhibitory substances such as hydroxy fatty acids, carbon dioxide and hydrogen peroxide) usually cannot be compared to the degree of activity of specific chemotherapeutic agents (such as antibiotics, fluoroquinolones, etc.), LAB exert also *in vivo* activity against pathogens, which is based on competition for nutrients and binding sites of the intestinal mucosa. The literature analysis indicates that the production of antimicrobial substances by probiotic LAB is species and strain specific. For example, some

strains produce mainly organic acids; others – bacteriocins; third, some other antimicrobial substances. Most commonly, *in vitro* antimicrobial activity is based on the simultaneous production and action of several of the aforementioned antimicrobial compounds.

It was found that *Lactobacillus plantarum* was the most extensively studied probiotic bacteria with excellent antibacterial and antifungal activity. As a whole, *Lactobacillus acidophilus* exhibited the widest spectrum and highest degree of antimicrobial activity, while the others probiotic bacteria (*Lactobacillus gasseri*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) also have promising strains with good antibacterial activity. However, these four species were lacking enough data about their antifungal activity, which is a good perspective for future studies.

Based on published experimental data, it can be concluded that certain strains of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, which exhibit high specific antimicrobial activity (*in vitro* and *in vivo*), have great potential to participate in the composition of novel organic functional additives with dietary, prophylactic and therapeutic activity. Strains of the above-mentioned probiotic bacteria, which have good antagonistic activity against important sanitary microorganisms, food spoilage microorganisms and animal and human pathogens, could be used for the production of dairy, meat and plant foods and feeds in order to improve their quality, shelf life and safety. In other words, lactic acid bacteria and their compounds with high antimicrobial activity are a safe and promising biological alternative to chemical preservatives that are currently widely used in the food industry. These bacteria are also suitable for the production of modern functional foods on a probiotic and synbiotic basis, with valuable nutritional, dietary and probiotic characteristics.

25. 7th Xenobiotic Metabolism and Toxicity Workshop of Balkan Countries, June 3-6, 2008, Novi Sad, Serbia. Published in *European Journal of Drug Metabolism and Pharmacokinetics*, 2008, 33 (Special Issue), p. 30. (IF = 0.738)

Pharmacokinetics of enrofloxacin in Japanese quails

D. Dimitrova¹, T. Dinev¹, A. Gentchev², S. Yanev³, B. Pandova³, L. Lashev¹

¹Department of Pharmacology, Physiology of Animals and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University. Stara Zagora, Bulgaria.

²Department of Animal Husbandry — Poultry-farming, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

³Department of Drug Toxicology, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Abstract: The pharmacokinetics of enrofloxacin was investigated in 20 healthy mature, male and female, Japanese quails. The tested quinolone was administered orally (in the crop) at a dose 10 mg/kg of body weight. Blood samples were collected from v. *brachialis* prior to medication and then at 0.25, 0.50, 1, 2, 4, 6, and 8 h after the treatment. The concentrations of enrofloxacin and its active metabolite ciprofloxacin were determined by high performance liquid chromatography (HPLC). Pharmacokinetic parameters were calculated using compartmental analysis with WinNonlin v. 4.0.1 computer programme. The pharmacokinetic parameters of enrofloxacin were as follows: $t_{1/2B}$ was 5.17 ± 1.40 h, $MRT - 7.87 \pm 2.04$ h, T_{max} was 1.01 ± 0.36 h, $AUC_{0 \rightarrow \infty}$ 1.04 ± 0.20 $\mu\text{g}\cdot\text{h}/\text{mL}$ and C_{max} was 0.18 ± 0.04 $\mu\text{g}/\text{mL}$, respectively. The metabolite (ciprofloxacin) was eliminated ($t_{1/2B}$ was 1.77 ± 0.41 h) from the

body faster than the parent compound (enrofloxacin). The MRT was 3.11 ± 0.51 h, $T_{\max} = 0.46 \pm 0.04$ h, C_{\max} was 0.23 ± 0.05 $\mu\text{g/mL}$, and $\text{AUC}_{0 \rightarrow \infty}$ was 0.63 ± 0.18 $\mu\text{g.h/mL}$, respectively.

29. *Workshop on Food-borne Pathogens and Food Safety*, May 26-27, 2016, Sofia, Bulgaria, p. 32.

Antimicrobial activity of *Lactobacillus acidophilus* against foodborne pathogens

T. Dinev¹, G. Beev¹, S. Denev¹, S. Danova², M. Tzanova¹

¹Department of Biochemistry, Microbiology and Physics, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria.

²Department of General Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.

Abstract: The foodborne diseases are major cause for morbidity and mortality in the population of the world. Most of the deaths occur in developing countries, although they are not limited only to them. Lactic acid bacteria (LAB) are considered as potentially promising in the strategy to combat food-borne infections. There are three mechanisms that could explain the antimicrobial efficacy of LAB: the production of ribosomally synthesized antimicrobial peptides (bacteriocins), the yield of organic acids and hydrogen peroxide, the competition for nutrients etc. Organic acid lowers the local pH and therefore inhibits the growth of foodborne bacteria, sensitive to acidic conditions. The bacteriocins usually have narrow spectrum of activity against closely related bacteria, including foodborne pathogens. Most of the bacteriocins kill the susceptible bacteria by membrane permeabilization or by interactions with essential enzymes.

Lactobacillus acidophilus is considered the main probiotic species in the intestinal tract of healthy humans and is widely used in functional dairy foods. It produces a variety of metabolic products with antimicrobial properties, including: organic acids and bacteriocins as lactacin B, F, acidophilin, acidocin, acidophilucin, acidophilicin, which are active against many pathogenic and spoilage organisms - *Escherichia coli* (including *E. coli* 0157:H7), *Klebsiella*, *Salmonella*, *Shigella*, *Bacillus* and *Clostridium* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, etc. Because of the reasons above *L. acidophilus* is frequently used as a biocontrol agent in the gastrointestinal ecosystem in humans and animals. On the other hand, the above mentioned antimicrobial products have been applied to food products to prevent the growth of certain undesirable bacteria and foodborne pathogens. To better understand the mode of action and the possible applications of *L. acidophilus* against foodborne pathogens more clinical and laboratory studies of different strains are required.

30. *VII International Symposium of Livestock Production*, September 14-16, 2017, Skopje, Macedonia, pp. 46-47.

Hygienic and ecological assessment of the microclimate in a farm for intensive breeding of rabbits

D. Dermendzhieva¹, G. Kostadinova¹, G. Petkov¹, **T. Dinev**², V. Vasilev³

¹Trakia University, Faculty of Agriculture, Department of Applied Ecology and Animal Hygiene, 6000 Stara Zagora, Bulgaria.

²Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, 6000 Stara Zagora, Bulgaria.

³Agricultural Institute, 6000 Stara Zagora, Bulgaria.

Abstract: The aim of the present study was to investigate and to make a hygienic and ecological assessment of the microclimate (temperature/T – °C, relative humidity/RH – %, air velocity/AV – m/s, NH₃ – mg/m³, H₂S – mg/m³, total dust/TD – mg/m³ and aerobic plate count/APC (x 10³ CFU/m³) in a farm for intensive breeding of rabbits. The rabbits (in number of 600 - mating, pregnant, does, fattening) were reared in cages, in a building (494 m²) with 4 premises: Ist – 218 m², IInd – 134 m², IIIrd – 77 m² and IVth – 65 m², for 265, 160, 100 and 70 rabbits, respectively. The microclimate parameters were determined for a year, twice per season in 5 monitoring points (MPs): MP-1, MP-2, MP-3 and MP-4, in the middle of the individual premises, and in MP-5 at 5 m, leeward of the barn. All monitored parameters were determined by Bulgarian State Standard's and other routine methods. It was found that: a) the barn provides optimal microclimate in terms of T (16.0–25.0°C) in winter, spring and autumn and RH (65–70 %) in winter at MP-1, MP-2 and MP-3, and in autumn at MP-3 and MP-4, and non-optimal microclimate in terms of T in summer (>25.0°C), RH in spring (< 65%) and summer (>70%) and AV (>2.0 m/s) in all seasons; b) the air of all premises contains significant quantities of NH₃ (14.3–41.6 mg/m³), H₂S (1.59–7.15 mg/m³), TD (0.36–5.00 mg/m³) and APC (22.0–83.0 x 10³ CFU/m³), which requires their standardization and animal hygiene evaluation; c) rabbits in Ist premise in the summer are exposed to moderate heat stress (THI=27.8–28.9°C); d) the working environment air quality (in the barn) meets the requirements for H₂S (<7.0 mg/m³) and TD content (<5.0 mg/m³) and deviates from them on NH₃ content (>14.0 mg/m³); d) ambient air quality (at MP-5) doesn't meet the NH₃ (>0.1 mg/m³), H₂S (>0.003 mg/m³) and TD (>0.25 mg/m³) content requirements; e) the working environment air and ambient air contain significant quantities of TD and APC, which requires their standardization and hygienic and ecological assessment, respectively.

32. *International Conference on Agricultural Science and Business*, May 30-31, 2019, Stara Zagora, Bulgaria, p. 54.

Vermicomposting of sewage sludge from different types wastewater treatment plants

D. Dermendzhieva, G. Beev, G. Kostadinova, G. Petkov, **T. Dinev**

Faculty of Agriculture, Trakia University, Bulgaria.

Abstract: Vermicomposting is environmentally friendly and cost-effective method of treatment of organic waste materials through the joint action of earthworms and microorganisms. The aim of the present study is to evaluate the vermicomposting ability (120 days) of the epigeic red earthworm *Lumbricus rubellus* through physicochemical and

microbiological analysis of sewage sludge from Municipal and Poultry meat processing enterprise/unit wastewater treatment plants. Physicochemical analysis was done by the classical methods described by ISO standards, and microbiological analysis by plating of 1ml of the sample dilutions on selective, chromogenic culture medium sheets. The values of pH, TOC, N-NH₄⁺ and C:N ratio decreased during vermicomposting. In regard to EC, TKN, TP, TK, N-NO₃⁻, P₂O₅ and K₂O values, they gradually increased during vermicomposting with the highest increment on the 120th day. The heavy metal content of Fe, Mn, Cu, Zn, Cr, Ni, Pb, Cd in sewage sludge always decreased after vermicomposting for 120 days, but the change is negligible. There were an overall decrease of the number of bacteria examined (total plate count, coliforms, *E. coli*, Enterobacteriaceae, *Salmonella* spp.) during vermicomposting. However, bacterial spore forms were not eliminated by vermicomposting.

02.09.2020
гр. Стара Загора

Подпис:.....
/гл. ас. д-р Тончо Динев/