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Effect of *Silybum marianum* fruit constituents on the health status of rabbits in repeated 42-day fattening experiment

Pavel Kosina[a,*], Adéla Dokoupilová[b], Karel Janda[b], Karolína Sládková[b], Petra Silberová[b], Veronika Pivodová[a], Jitka Ulrichová[a]

[a] *Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University in Olomouc, Hněvotínská 3, 775 15 Czechia*

[b] *Department of Husbandry and Ethology of Animal, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21Praha 6 – Suchdol,Czechia*

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ABSTRACT

Rabbits are an important source of high-quality dietary proteins. Intensive farming is connected with the protection of rabbits against diseases, mainly digestive disorders. Previously used antibiotics have been replaced with other types of compounds with prophylactic or curative effects, substances or mixtures of natural origin are preferred. Mechanically processed *Silybum marianum* fruits – 0.2% or 1% (w/w), were added to the standard diet of HYLA broiler rabbits in a 42-day fattening experiment repeated in quadruplet. At the end of the experiment, the main compounds of interest – selected flavonolignans and taxifolin, were subject of HPLC/MS analyses in feed, plasma, urine and faeces. Data showed a mild effect on the growth performance of rabbits (carcass weight and carcass yield in the group fed 1% mechanically processed *S. marianum* fruits), but no effect on the majority of selected blood biochemical parameters and markers of oxidative stress after the application of the feed containing *S. marianum* constituents. However, when in one repetition a higher incidence of health problems connected with digestive disorders occurred, the diet containing the highest content of *S. marianum* constituents was able to attenuate the morbidity and mortality of rabbits. The addition of phytopreparations based on *S. marianum* constituents to the animal diet is promising for conventional methods of rabbits farming.

Abbreviations: *S. marianum*, *Silybum marianum*; SM, silymarin; SB, silybin; GAP, good agricultural practices; FRAP, ferric reducing antioxidant power; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSR, glutathione reductase; GST, glutathione S-transferase; CAT, catalase; GSH, reduced glutathione; MF A, mobile phase A; MF B, mobile phase B; HESI, heated electron spray ionization; TBA, thiobarbituric acid; TCA, trichloroacetic acid; DTNB, 5,5-dithiobis-2-nitrobenzoic acid; PMS, phenazine methosulfate; NBT, nitro blue tetrazolium; CDNB, 1-chloro-2,4-dinitrobenzene; GSSG, oxidized glutathione; MIC, minimal inhibition concentration; HAV, hepatitis A virus; HCV, hepatitis C virus; TA, taxifolin; SC, silychristin; SD, silydianin; SB A, silybin diastereomer A; SB B, silybin diastereomer B; ISB A, isosilybin diastereomer A; ISB B, isosilybin diastereomer B; GIT, gastrointestinal tract.

* Corresponding author.
E-mail addresses: pavel.kosina@upol.cz (P. Kosina), dokoupilova@af.czu.cz (A. Dokoupilová), janda@af.czu.cz (K. Janda), sladkovak@af.czu.cz (K. Sládková), Petra.Silberova@seznam.cz (P. Silberová), VeronikaPivodova@seznam.cz (V. Pivodová), JitkaUlrichova@seznam.cz (J. Ulrichová).

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1. Introduction

Milk thistle, *Silybum marianum* (L.) Gaertn, is well known as a medicinal plant and has been used since ancient times as a natural remedy with positive effects on the human organism. The active components presented in the fruits (seeds) of this plant as well as in a standardized extract of fruits (seeds) – Silymarin, are flavonolignans (Silybin A, Silybin B, Isosilybin A, Isosilybin B, Silychristin, Silydianin), together with the flavonoids Taxifolin, Quercetin and polymeric and oxidized polyphenolic compounds derived from flavonolignans (Gazak et al., 2007). A large number of individual mechanisms of action have been observed to date, frequently acting in an additive manner to produce the general effect of the flavonolignans of *S. marianum* on the target organism. These actions include antioxidant properties, free radical scavenging abilities, stabilization of cell membranes, proteosynthesis stimulation as well as specific interactions with cell receptors and signalling pathways on a molecular level. (Gazak et al., 2007; Zholobenko and Modriansky, 2014; Surai, 2015). Phytopreparations containing flavonolignans/Silymarin are widely used as hepatoprotectants in human medicine (Abenavoli et al., 2010).

Rabbits are important farm animals; they are valued for their dietetic type of meat with high-quality proteins, low fat and cholesterol content, suitable for human nutrition. On the other hand rabbits are sensitive to various diseases, the quality of their care and conditions of their farming. Digestive disorders account for 70% of rabbit diseases (Carabano et al., 2008). These disorders cause high morbidity, inhibition in animal growth, and worse feed conversion, resulting in economic losses. To solve the above-mentioned problems, antibiotics were previously frequently applied. Due to the ban on antibiotics usage in animal husbandry, an alternative approach is the application of compounds obtained from natural sources, phytoadditives or extracts of plants, containing e.g. mannanoligosacharides, organic acids, and enzymes as well as probiotics (Falcao-e-Cunha et al., 2007).

Several studies were carried out with *S. marianum* extracts, silymarin (SM) or silybin (SB) on rabbits as standard laboratory experimental animals. There are studies of silymarin pharmacokinetics (Carceles et al., 1987a,b; Voruganti et al., 2014). Other studies focus on the protective effects of SM or SB after the application of drugs (anticancer, hypertension treating) with side effects causing hepatotoxicity (Abdel-Rahman and Abdel-Hady, 2013; Jahan et al., 2015). In many studies SM or SB serve as standard reference drugs with a known hepatoprotective effect (Saleem et al., 2015).

The effect of SM on the treatment of diseases caused by the main types of rabbit pathogens (protozoa, viruses, and bacteria) was previously confirmed *in vitro* as well as in clinical studies. The anti-protozoan effect of SM was observed for Leishmaniosis during combined human treatment with SM and glucantime (Jabini et al., 2015). In human medicine, several studies were previously focused on the application of SM/SB in Hepatitis A virus (HAV) and Hepatitis C virus (HCV) treatment (Wagoner et al., 2010; Dahari et al., 2015). SM/SB was effective at inhibiting HAV and HCV infection (Polyak et al., 2007; Esser-Nobis et al., 2015) as well as against the Chikunqunya virus (Lani et al., 2015). The antibacterial and anti-adherent activities of SM on the biofilm viability of standard bacterial strains were studied (Evren and Yurtcu, 2015), where gram-positive bacteria were inhibited with MIC 60–241 μg/mL, gram-negative bacteria were not affected by up to 1000 μg/mL.

Only limited knowledge is available about the usage of flavonolignans/SM in the field of agriculture and veterinary science, its bioavailability, pharmacokinetics and safety for livestock (Gazak et al., 2007; Hackett et al., 2013; Andrzejewska et al., 2015). To the best of the authors' knowledge, no data from fattening experiments have been published until now.

The aim of the fattening study was to evaluate the effect of constituents of *S. marianum* fruits on the growth of broiler rabbits, their health status with the impact on antioxidant parameters, and possible prophylactic action in standard fattening experiments. Rabbit broilers were fed a diet supplemented with 0.2% or 1% mechanically processed *S. marianum* fruits in comparison to the standard diet until they achieved 2600 g of live weight or 42 days of fattening.

2. Materials and methods

2.1. Chemicals

Standards of flavonolignans were kindly provided by Dr. L. Cvak (IVAX Pharmaceuticals, Ltd. Opava, Czech Republic) and prof. V. Křen (Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic). Pure mechanically processed fruits of *Silybum marianum* (Silyfeed® Basic) were obtained from IREL, Ltd. Miroslavské Knínice, Czech Republic. Buffer components, L-ascorbic acid, and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetonitrile and β-glucuronidase/arylsulfatase from *Helix pomatia* were obtained from Merck (Darmstadt, Germany). Other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared using reverse-osmosis deionized water (Ultrapur, Watrex, Prague, Czech Republic). Nitrogen and helium (99.999 % for all) were obtained from Linde Gas (Prague, Czech Republic).

2.2. Animals

HYLA broiler rabbits aged 35 days were taken from the farm of Mr. Kočár (Genetic Centre HYLA in Ratibořice, Czech Republic), for the experiment. This study was conducted in accordance with Good Agricultural Practices (GAP), published by the Food and Agriculture Organization of the United Nations (Nations, F.a.A.O.o.t.U., 2003) and is not classified as an experiment in accordance with Act No 246/1992 Coll., on the protection of animals against cruelty.