

# Animal welfare, etológia és tartástechnológia



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## EFFECT OF CHRONIC APPLICATION OF QUERCETIN AND ACUTE DOSE OF T-2 TOXIN ON CONTENT OF SERUM BILIRUBIN AND ALBUMINS OF RABBITS

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### Abstract

The aim of the present study was to investigate the effect of long-term exposure of quercetin and acute dose of T-2 toxin on content of bilirubin and albumins of rabbits. Adult rabbits were divided into three experimental groups (E1, E2, E3) and the control group without quercetin addition. Quercetin was applied intramuscularly in various concentrations; 10  $\mu\text{g}\cdot\text{kg}^{-1}$  in E1 group, 100  $\mu\text{g}\cdot\text{kg}^{-1}$  in E2 group, and 1000  $\mu\text{g}\cdot\text{kg}^{-1}$  in E3 group for 90 days, 3 times per week. T-2 toxin was applied at dose 0.08 mg per kg of body weight 72 hours before slaughter. Application of quercetin insignificantly decreased content of bilirubin and decreased content of albumins in experimental groups compared to the control group. In conclusion, as the bilirubin serves in organism as antioxidant with the ability to scavenge free radicals, our results could contribute to the positive effect of quercetin on antioxidant balance, however further studies are needed.

**Key words:** quercetin, T-2 toxin, rabbits, bilirubin, albumins.

### Introduction

Flavonoids are widely distributed in the plant kingdom and are categorized as flavonol, flavanol, flavanone, flavone, anthocyanidin, and isoflavone and they are absorbed from food (**Murota and Terao, 2003**). Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a member of naturally occurring widely distributed compounds, the flavonoids, which are ubiquitous phenolic secondary metabolites found in plants, fruits, flowers and plant derived foods (**Harborne and Williams, 2000**). Numerous *in vitro* studies have revealed diverse biological effects of quercetin, including apoptosis induction, antimutagenesis, protein kinase C (PKC) inhibition, lipoxygenase inhibition, histamine-release inhibition, superoxide dismutase (SOD)-like activity, modulation of cell cycle, angiogenesis inhibition, and inhibition of angiotensin converting enzyme II (**Formica and Regelson, 1995**). Quercetin intake is therefore suggested to be beneficial for human health and its antioxidant activity should, at least partly, yield such a variety of biological effects (**Rice-Evans and Packer, 2010**).

Cells seem to use several systems for protection against oxidative stress (**Sedlak and Snyder, 2004**). Examples include repair enzymes (to repair damaged biomolecules), preventative antioxidants as albumin (to prevent the formation of free radicals) and scavenging antioxidants as bilirubin (to remove reactive species once formed). Albumin may represent the major and predominant circulating antioxidant in plasma (**Cha and Kim, 1996**). Albumin represents the quantitatively most important source of thiol in plasma, and this circulating store may be altered in situations where antioxidants become limiting, resulting in changes in the redox status (**Durand et al., 1997**).

Bilirubin is a bile pigment that may have an important role as an antioxidant. Bilirubin, through a hydrogen donation mechanism, participates as a scavenger of secondary oxidants formed in the oxidative process and thereby might alleviate oxidant stress in the blood. When a molecule of bilirubin acts as antioxidant, it is itself oxidized to biliverdin. Endogenous biliverdin

reductase should suffice to reduce newly formed biliverdin back to bilirubin and might primarily protect cells against lipid peroxidation (**Sedlak and Snyder, 2004**). In the serum, bilirubin may have a direct therapeutic action in coping with oxidative stimuli within the blood stream, such as quenching oxidized low-density lipoproteins (**Neuzil and Stocker, 1994**).

T-2 toxin is some of the most important and toxic trichothecene mycotoxin occurring in various agriculture products (**Iwahashi et al. 2008**). Lipophilic nature of T-2 toxin suggests that they are easily absorbed through skin, gut, and pulmonary mucosa (**Bunner and Morris, 1998**). Trichothecene causes multiorgan effect including emesis, and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, and bone marrow damage (**Wannemacher and Neufeld, 1991**).

The aim of the present study was to determine effect of application of quercetin in various doses and single dose of T-2 toxin on concentration of albumins and bilirubin in rabbit's blood.

## Materials and Methods

### *Animals and diet*

Adult female rabbits (n = 20) and male rabbits (n = 20) of meat line M91, maternal albinotic line (crossbreed Newzealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in experiment. Rabbits were healthy and their condition was judged as good at the commencement of the experiment. Water was available ad libitum. Groups of adult animals were balanced for age (150 days) and body weight ( $4 \pm 0.5$  kg) at the beginning of the experiment. Adult rabbits were fed diet of a  $12.35 \text{ MJ.kg}^{-1}$  of metabolizable diet (Table 2) composed of a pelleted concentrate.

Animals were divided into control group C and experimental groups (E1 – E3). Experimental groups received quercetin in injectable form at  $10 \text{ }\mu\text{g.kg}^{-1}$  in E1,  $100 \text{ }\mu\text{g.kg}^{-1}$  in E2 and  $1000 \text{ }\mu\text{g.kg}^{-1}$  in E3 group T-2 toxin for 90 days. T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) at dose 0.08 mg per kg of body weight 72 hours before slaughter was applied.

In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3.

Table 1

<b>Application of quercetin and T-2 toxin in rabbit blood <i>in vitro</i>.</b>		
Group	Quercetin ( $\mu\text{g.kg}^{-1}$ )	T-2 toxin ( $\text{mg.kg}^{-1}$ of body weight, 72 hours before slaughter)
C	0	0,08
E1	10	0,08
E2	100	0,08
E3	1000	0,08

C – control group, E1 – E3 – experimental groups with various doses of quercetin and combination with T-2 toxin.

### *Blood sampling and analyses*

After 3 mounts of intramuscular application of quercetin, rabbits were slaughtered and blood samples were obtained.

The blood serum was separated from whole blood by centrifugation at 3000g for 30 min. The concentrations of bilirubin (BR) was determined using automatic analyzer Microlab 300

(Merck®, Germany) and concentration of albumins (ALB) was determined by spectrophotometric analysis (Genesys 10, Thermo Fisher Scientific Inc., USA).

#### Statistical analyses

The data used for statistical analyses represent means of values obtained in blood collection (end of the experiment). One-way ANOVA test was applied to calculate basic statistic characteristics and for determination of significant differences between the experimental and control groups. Statistical software SIGMA PLOT 11.0 (Jandel, Corte Madera, CA, USA) was used. Differences were compared for statistical significance at the level  $P < 0.05$ .

Table 2

#### Chemical composition (g.kg<sup>-1</sup>) of the experimental diet

Component	
Dry matter	926.26
Crude protein	192.06
Fat	36.08
Fibre	135.79
Non-nitrogen compounds	483.56
Ash	78.78
Organic matter	847.49
Calcium	9.73
Phosphorus	6.84
Magnesium	2.77
Sodium	1.81
Potassium	10.94
Metabolizable energy	12.35 MJ.kg <sup>-1</sup>

#### Results and Discussion

In this study, the effect of long-term exposure of quercetin and one acute dose of T-2 toxin on selected parameters of antioxidant status of rabbits (BR and ALB) was measured from blood serum. The results are presented in figures 1 and 2. Addition of quercetin and T-2 toxin (in the end of the experiment) slightly influenced antioxidant status (BR, ALB, however differences among the groups were insignificant ( $P > 0.05$ )).

Bilirubin has been reported as a member of the antioxidant family and is even known to have toxic effects at high concentration. The combined evidence from animal and human studies indicates that bilirubin is a major physiologic cytoprotectant and might alleviate oxidative stress in the blood (**Sedlak and Snyder, 2004**). In this study we observed increase of concentration of BR in E1 group and decrease in E2 and E3 groups in comparison with the control group, but without significant differences ( $P > 0.05$ ). In our previous study (**Petruška et al., 2013**) long-term application of quercetin caused the increase of concentration of BR. **Capcarova et al. (2011)** found insignificant decrease of content of bilirubin after *Rhus coraria* inclusion to the diet for rabbits. In another study with T-2 toxin-contaminated diet performed on young swine **Friend et al. (1992)** found increase of content of bilirubin in all experimental doses. Based on the literature natural substances could slightly modify the serum bilirubin content and consequently influence antioxidant balance in the organism.

Albumins represent a very abundant and important circulating antioxidant (**Roche et al., 2008**). Study of **Bourdon et al. (1999)** confirmed and extended the idea that serum albumin is an

important protein that presents direct protective effects. In this paper statistical analyses showed insignificant differences ( $P > 0.05$ ) in the content of ALB between the control group and experimental groups (E1, E2 and E3). Lower content of ALB was observed in all experimental groups compared to the control group. Similar results found **Raymond et al. (2003)** in experiment with *Fusarium* mycotoxins added to the feed for horses. In our previous study slight decrease in the content of serum albumins in quercetin groups vs. control group of rabbits was noted, (**Petruška et al., 2013**). In another study, **Bergsjö et al. (1993)** reported significant decrease in serum albumin, in the pigs fed by mycotoxin deoxynivalenol from contaminated oats. In studies with *L. fermentum*, *E. faecium* and *Rhus coriaria* to the feed mixture for chickens and rabbits significant increase in content of ALB in experimental groups in comparison with the control group was measured (**Capcarova et al., 2010; Capcarova et al., 2011**). Several lines of evidence strongly suggest that a reduced serum albumin concentration, although within the normal range, is associated with mortality risk (**Bourdon et al., 1999**).

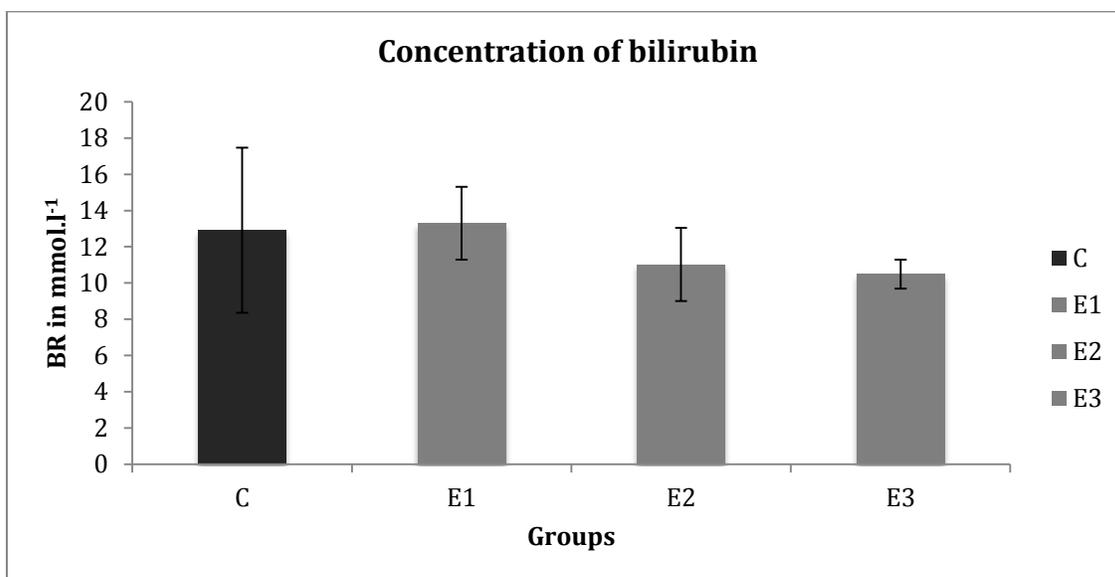


Figure 1.

**The content of bilirubin in rabbits blood after chronic quercetin and acute dose of T-2 toxin application.**

C – control group, E1 - 10  $\mu\text{g.kg}^{-1}$ , E2 - 100  $\mu\text{g.kg}^{-1}$ , E3 - 1000  $\mu\text{g.kg}^{-1}$  of quercetin. T-2 toxin was added to all groups 72h before slaughtered at dose 0,08  $\text{mg.kg}^{-1}$ . Values are means  $\pm$  SD.

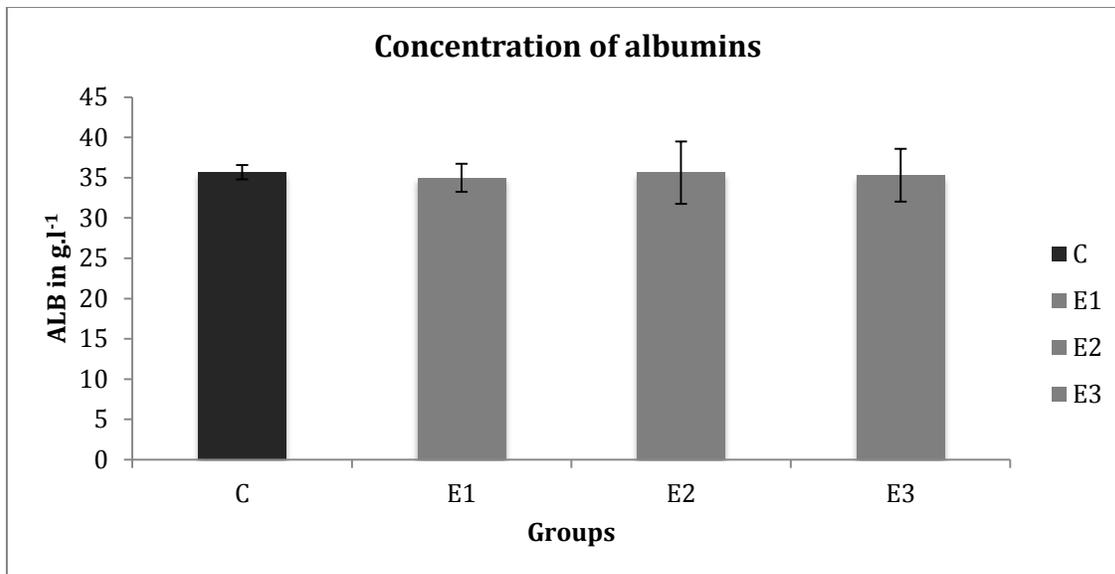


Figure 2.

**The content of albumins in rabbits blood after chronic quercetin and acute dose of T-2 toxin application.**

C – control group, E1 - 10  $\mu\text{g}\cdot\text{kg}^{-1}$ , E2 - 100  $\mu\text{g}\cdot\text{kg}^{-1}$ , E3 - 1000  $\mu\text{g}\cdot\text{kg}^{-1}$  of quercetin. T-2 toxin was added to all groups 72h before slaughtered at dose 0,08  $\text{mg}\cdot\text{kg}^{-1}$ . Values are means  $\pm$  SD.

### Conclusion

The intramuscular application of the quercetin three times a week to the rabbits and addition of T-2 toxin in acute dose 72 hours before slaughtered resulted in some changes in internal milieu of experimental animals. Application of quercetin and T-2 toxin insignificantly changed the content of bilirubin in experimental groups in comparison with the control group. In content of serum albumins we observed decrease in all experimental groups but without significant differences. To our knowledge, there are not a lot of similar studies concerning the effect of intramuscular application of quercetin and addition of T-2 toxin and its effect on antioxidant status of rabbits. Research on the field of quercetin will be worthy of further investigation.

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