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PATULIN – INDUCED CHANGES IN HAEMATOLOGICAL PARAMETERS OF RABBITS FED BY STRAWBERRY LEAVES AFTER CHRONIC EXPOSURE

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INTRODUCTION

The flavonoids are a large group of naturally occurring compounds that are found in plants and are frequently consumed as part of the human diet. Flavonoids seem to play an important role in human health and to possess beneficial effects in the prevention of human diseases (*Deepshikha et al.,* 2008; *Mareš et al.,* 2008). Strawberry leaves have higher oxygen radical absorbance capacity (ORAC) than fruits (*Shiow et al*., 2000). This leaves as phytogenic feed additive can improve welfare (*Mareš et al.,* 2008), restrict growth of fungi (*Marcinčák et al.,* 2010).

LITERATURE SURVEY

The strawberry leaves contain a wide range of phenolic compound (*Hanhineva et al.,* 2009), quercitrin, quercetin (*Dreyer et al.,* 2010; *Oberbeil et al.,* 2005), myricetin, kaempferol and epikatechin (*Minárik,* 2009).

Phytogenic feed additives are defined as a herbal substances included in the feed mixure for the purpose of enhancing production performance, improving performance feed and increase of quality animal products (*Václavková et al.,* 2010). Natural substances contained in these phytogenic feed additives affect (can improve) the sensory quality of feed (*Mareš et al.,* 2008). The use of plants in feed production can be restrict growth of fungi and production of mycotoxins in animals feed. They reduce the need of use drugs. Their feeding does not require of adherence to any withdrawal periods (*Marcinčák et al.,* 2010).

Mycotoxins are secondary metabolites of fungal origin (*Painter et al.,* 2003; *Šimůnek,* 2004; *Serra et al.,* 2005; *Frisvad et al.,* 2006). Patulin is produced by various species of *Aspergillus* and *Penicillium* (*Polster,* 1984; *Betina,* 1990; *Malíř et al.*, 2003; *Frisvad et al.,* 2007; *González et al.,* 2007). Patulin has bacteriostatic, bactericidal and fungicidal effects. It is toxic to plants and animals cells (*Toman et al.,* 2003; *Sabater-Vilar,* 2004), exhibits carcinogenic (*Herzig,* 2002; *Sabater-Vilar,* 2004), mutagenic and teratogenic activity (*Sugiyanto et al.,* 1993; *Schumacher et al.,* 2005), activate gastrointestinal disorders, anaemia, swelling and haemorrhage of various organs (*Jesenská,* 1987; *Rimárová*, 2002; *Sabater-Vilar,* 2004).

The aim of present study was to determinate the effect strawberry leaves inclusion to the feed mixture and single dose of patulin on haematological parameters of rabbits.

MATERIAL AND METHODS

Animals and diet

Fifteen adult male rabbits of Californian broiler line were used in experiment. Rabbits were obtained from an experimental farm of the Animal Production Research Centre in Nitra, Slovak Republic. Rabbits (in the age of 4 months, weighing 3.5 – 4.0 kg) were housed in individual flat-deck wire cages (area 0.34 m^2). The animals were healthy and their condition was judged as good at the commencement of the experiment. Animals were kept in cages, at standard conditions (temperature

20 – 22°C, 14 h light period). Drinking water and feeding mixture for all animals was provided on an *ad libitum* basis. Animals were divided into four groups, one control group C (n =3) and three experimental groups E1, E2 and E3 (n = 4 in each group). Rabbits were fed with a granular feed mixture (FM) with strawberry leaves in various doses and all groups received patulin in injectable form at 10 μg.kg^{-1} for 28 days 2 times a week (Table1).

Table 1.

Concentration of strawberry leaves and patulin of the experimental diet

Group	Concentration of strawberry leaves	Concentration of patulin (μg.kg^{-1})
Control (n = 3)	0 %	10
E1 (n = 4)	0.5 %	10
E2 (n = 4)	1.0 %	10
E3 (n = 4)	1.5 %	10

Blood sampling and analyses

Blood samples from *vena auricularis* were taken from all animals. In whole blood, selected haematological parameters as total white blood cell count (WBC), lymphocytes count (LYM), medium size cell count (MID), granulocytes count (GRA), lymphocyte percentage (LYM%), medium size cell percentage (MI%), granulocytes percentage (GRA%), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc) were measured using haematology analyzer Abacus junior VET (Diatron®, Vienna, Austria).

Statistical analyses

To compare the results, one-way ANOVA test was applied to calculate basic statistic characteristics and to determine significant differences between 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 $P < 0.05$.

RESULTS AND DISCUSSION

The results of blood haematological parameters are summarized in Table 2. Addition of strawberry leaves and patulin influenced some haematological parameters in blood of rabbits. Statistical evaluation showed significant decrease of MCHC ($P < 0.05$) in E2 and E3 group in comparison with the experimental group E1. We observed significant lower values in HGB in E3 group in comparison with the control group. In contrast with results observed by *Petruška et al.* (2012), the values of HGB and MCHC were not influenced ($P > 0.05$) after quercetin/T-2 toxin treatment. Treatment with phytoadditives had no significant effect on HGB of male laboratory mice (*Singh et al.,* 2008) and rats (*Babayi et al.,* 2007; *Chinnadurai et al.,* 2013).

Decrease of HGB and MCHC in our study can related with absence of iron and starting of anaemia (*Rolinec et al.,* 2010; *Bačovský,* 2013). Patulin caused the anaemia in study of *Camguilhem et al.* (1976) in sheep**.** The results of our study showed no significant differences in LYM, MID, RBC, HGB, HTC, MCH, PLT, PTC and MPV after long term application of patulin in combination with strawberry leaves. Selected haematological parameters were not influenced by quercetin and T-2 toxin in another study of *Petruška et al.* (2012).

Table 2.

Haematological parameters of rabbits after strawberry leaves and patulin treatment

Parameter	C	E1	E2	E3
WBC	12.97±1.21	13.53±2.58	13.61±2.25	10.39±2.28
LYM	8.22±2.52	7.39±4.72	7.48±1.98	6.82±1.3
MID	0.58±0.15	0.44±0.27	0.50±0.18	0.54±0.29
GRA	4.17±1.92	5.71±2.45	5.63±0.80	3.03±1.79
LY%	63.03±16.02	51.93±22.37	54.25±6.83	66.27±9.64
MI%	4.53±1.42	3.48±2.32	3.65±1.06	5.33±3.14
GR%	32.47±14.74	44.58±21.40	42.05±7.70	28.43±12.79
RBC	6.39±0.39	6.15±0.58	6.15±0.28	5.91±0.41
HGB	154.94±5.75[a]	148.22±4.78	146.07±6.21	138.17±8.76[b]
HCT	34.29±1.91	32.48±1.34	33.16±1.57	31.59±2.19
MCV	53.72±0.30	53.97±2.74	53.88±0.90	53.46±0.83
MCH	24.27±0.60	24.2±1.42	23.73±0.51	23.37±0.47
MCHC	452.13±8.80	456.46±4.35[a]	440.7±12.67[b]	437.53±3.54[b]
RDWc	18.47±0.57	18.60±0.67	19.2±0.88	18.93±1.10
PLT	256.6±67.09	209.9±68.45	185.9±138.31	183.49±40.79
PCT	0.19±0.05	0.13±0.05	0.12±0.08	0.12±0.03
MPV	7.37±1.15	6.08±0.39	6.98±1.24	6.77±0.70
PDWc	33.27±0.61	30.08±2.10	33.85±4.87	32.57±3.27

[a,b] – means in the same line with the different letters are different at the level $P < 0.05$

WBC - total white blood cell count (10^9/l); LYM - lymphocytes count (10^9/l); MID - medium-size cell count; GRA - granulocytes count (10^9/l); LYM% - lymphocyte percentage; MID% - medium-size cell percentage; GRA% - granulocytes percentage; RBC - red blood cell count (10^{12}/l); HGB - haemoglobin (g/l); HCT - haematocrit (%); MCV - mean corpuscular volume (fl); MCH - mean corpuscular haemoglobin (pg); MCHC - mean corpuscular haemoglobin concentration (g/l); RDWc - red cell distribution width (%); PLT - platelet count (10^9/l); PCT - platelet percentage; MPV - mean platelet volume (fl); PDWc - platelet distribution width (%), C – control group, E1 , E2, E3 – experimental groups. The values shown are the mean ± SD (standard deviation).

CONCLUSIONS

Significant decrease of HGB and MCHC was probably caused by long term exposure of patulin, which can lead to anaemia. Selected concentrations of strawberry leaves in individual doses did not cause protection of the homeostasis. Higher concentrations could act as an antioxidant as we expected. Further experimental studies with strawberry leaves are needed to define the specific mechanisms of action.

Keywords: haematological parameters, strawberry leaves, patulin, rabbit, chronic exposure

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