

PATULIN - INDUCED CHANGES IN HAEMATOLOGICAL PARAMETERS OF RABBITS FED BY STRAWBERRY LEAVES AFTER 3 WEEKS EXPOSURE

Jana Emrichova^{1*}, Anna Kalafova¹, Katarina Zbynovska¹, Peter Petruska¹, Lubomir Ondruska², Rastislav Jurcik², Lubica Chrastinova², Anton Kovacik¹, Monika Schneidgenova¹, Peter Cupka¹, Marcela Capcarova¹

Address(es): Ing. Jana Emrichova,

¹ Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Tr. A.

Hlinku 2, 949 76 Nitra, Slovak Republic.

² Animal Production Research Centre Nitra, Hlohovecka 2, 949 01 Nitra, Slovak Republic.

*Corresponding author: emrichova@gmail.com

ARTICLE INFO

Received 11. 10. 2013

Revised 22. 11. 2013

Accepted 16. 12. 2013

Published 1. 2. 2014

Regular article



ABSTRACT

The subject of the present study was to determinate the effect of strawberry leaves inclusion into the feed mixture and single dose of patulin on haematological parameters of rabbits: 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). Fifteen rabbits of broiler line Californian were used in this experiment. 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 21 days 2 times a week. The investigation was performed with haematology analyzer Abacus junior VET (Diatron®, Vienna, Austria). Significant decrease in MI% and MID in experimental group E3 in comparison with the control group was found. The values of other parameters (WBC, LYM, LY%, GRA, GR%, RBC, HGB, HCT, MCV, MCH, MCHC, RDWc, PLT, MPV and PDWc) in all groups corresponded with normal haematological values in rabbit's blood.

Keywords: Haematological parameters, strawberry leaves, rabbits, patulin

INTRODUCTION

Plant extracts contain a number of biologically active compounds, which either protect the organism against the toxic effect of various physicochemical agents or enhance treatment of many diseases (Jaganath *et al.*, 2010). Young strawberry leaves contain much more polyphenolic compounds than the fruits and mature leaves (Wang *et al.*, 2000; Hanhineva *et al.*, 2009) and are a rich source of flavonoids and procyanidins (Katalinic *et al.*, 2006). 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; Hung *et al.*, 2004).

Patulin is a frequently found food contaminant mainly produced by the fungi *Aspergillus* and *Penicillium* (Glaser *et al.*, 2012; Malíř *et al.*, 2003; Frisvad and Thrane, 2006; González *et al.*, 2007). Patulin is toxic for animals; mutagenic, carcinogenic and teratogenic, induces intestinal injuries, including epithelial cell degeneration, inflammation, ulceration, and hemorrhages (Mahfoud *et al.*, 2002). The subject of the present study was to determinate the effect of 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 m²). 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 one). Rabbits were fed with a granular feed mixture (Table 1) with strawberry leaves in various doses and all groups received patulin intramuscularly in injectable form at 10 µg.kg⁻¹ for 21 days 2 times a week (Table 2).

Table 1 Chemical composition (g.kg⁻¹) of the experimental diet

Component	Content (g.kg ⁻¹)
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 ⁻¹

Table 2 Concentration of strawberry leaves and patulin of the experimental diet

Group	Concentration of strawberry leaves	Concentration of patulin ($\mu\text{g}\cdot\text{kg}^{-1}$)
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

The results obtained were statistically interpreted using statistical software SIGMA PLOT 11.0 (Jandel, Corte Madera, CA, USA), on the basis of the analysis of variance (ANOVA). The Scheffe test was used for difference estimation at confidence level $P < 0.05$.

RESULTS AND DISCUSSION

The results of blood haematological parameters are summarized in Table 3. Addition of strawberry leaves and patulin influenced some haematological parameters in blood of rabbits. Statistical evaluation showed significant decrease of MID ($P < 0.05$) and MI% in experimental group E3 in comparison with the control group. In contrast with our previous results (Emrichova et al., 2013), the values of MID and MI% showed significant increase after short term (2 weeks) application of patulin in combination with strawberry leaves. This haematological parameter was not influenced after long term (4 weeks) application of patulin in combination with strawberry leaves in our another study (Emrichova et al., 2013). This decrease is probably mediated through enhancement and activation through macrophages or through some cytokine production (Rajkapoor et al., 2003). Parabathina et al. (2011) found the different results in their study with rutin and quercetin on rabbits. Authors observed increase in eosinophils after 28 days of treatment. Slight increase of MI% was found in experiment with pesticide bendiocarbamate applied to rabbits (Capcarova et al., 2010). In another *in vivo* study Keblys et al. (2004) found significant changes in lymphocyte proliferation with lymphocytes from piglets. Cell proliferation was totally suppressed at 0.5 $\text{mg}\cdot\text{l}^{-1}$. A similar response to patulin *in vitro* is also described by Escoula et al. (1988), using spleen lymphocytes isolated from mice and rabbits. In this study the values of other parameters (WBC, LYM, LY%, GRA, GR%, RBC, HGB, HCT, MCV, MCH, MCHC, RDWc, PLT, MPV and PDWc) in all groups corresponded with normal haematological values in rabbit's blood. The values of these haematological parameters were not influenced ($P > 0.05$) by patulin application combined with strawberry leaves. Selected haematological parameters were not influenced by quercetin and T-2 toxin in another study of Petruška and Capcarová (2012).

Table 3 Haematological parameters of rabbits after strawberry leaves and patulin treatment

Parameter	C		E1		E2		E3	
WBC	9.50	± 1.51	10.33	± 1.37	9.08	± 1.39	9.32	± 1.32
LYM	5.67	± 1.44	5.54	± 2.98	5.42	± 0.42	6.79	± 1.47
MID	0.63 ^a	± 0.08	0.49	± 0.04	0.49	± 0.02	0.32 ^b	± 0.17
GRA	3.20	± 0.79	4.30	± 2.73	3.17	± 1.10	2.20	± 1.60
LY %	59.30	± 9.28	53.17	± 24.92	60.30	± 6.66	73.60	± 15.89
MI %	6.70 ^a	± 1.44	4.77	± 0.31	5.43	± 0.78	3.38 ^b	± 1.53
GR %	33.93	± 8.07	42.10	± 24.98	34.23	± 7.40	23.00	± 15.57
RBC	6.31	± 0.29	6.34	± 0.95	6.00	± 0.41	6.20	± 0.57
HGB	146.40	± 7.58	141.72	± 11.74	138.21	± 8.61	141.73	± 9.34
HCT	33.18	± 1.95	32.96	± 2.85	32.19	± 1.94	33.05	± 2.30
MCV	52.57	± 1.01	52.28	± 3.20	53.71	± 0.74	53.36	± 1.24
MCH	23.20	± 0.17	22.50	± 1.47	23.03	± 0.15	22.90	± 0.98
MCHC	441.37	± 6.79	430.04	± 2.95	429.38	± 4.33	428.96	± 10.43
RDWc	18.97	± 0.12	19.07	± 0.75	19.83	± 1.33	19.78	± 1.44
PLT	194.13	± 137.24	200.42	± 133.03	217.13	± 143.86	261.53	± 82.98
PCT	0.12	± 0.08	0.12	± 0.08	0.13	± 0.08	0.16	± 0.05
MPV	6.10	± 0.20	6.00	± 0.52	6.60	± 0.87	6.20	± 0.14
PDWc	31.27	± 1.70	29.10	± 2.39	33.03	± 3.62	31.18	± 1.03

Legend: ^{a,b} – means significant difference at the level $P < 0.05$

WBC - total white blood cell count ($10^9/\text{l}$); LYM - lymphocytes count ($10^9/\text{l}$); MID - medium-size cell count; GRA - granulocytes count ($10^9/\text{l}$); LYM% - lymphocyte percentage; MID% - medium-size cell percentage; GRA% - granulocytes percentage; RBC - red blood cell count ($10^{12}/\text{l}$); 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 ($10^9/\text{l}$); PCT - platelet percentage; MPV - mean platelet volume (fl); PDWc - platelet distribution width (%), C – control group, E1 (0.5 %), E2 (1 %), E3 (1.5 %) – experimental groups. The values shown are the mean ± SD (standard deviation).

CONCLUSION

Significant decrease of MID ($P < 0.05$) and MI% was found after 3 weeks application of patulin in combination with strawberry leaves. The values of other haematological parameters (WBC, LYM, LY%, GRA, GR%, RBC, HGB, HCT, MCV, MCH, MCHC, RDWc, PLT, MPV and PDWc) were not influenced ($P > 0.05$) by patulin application combined with strawberry leaves. Further experimental studies with strawberry leaves are needed to define the specific mechanisms of action.

Acknowledgments: This work was financially supported by VEGA scientific grant 1/0084/12, KEGA grant 030SPU-4/2012, APVV 0304-12 and by European Community under project no 26220220180: Building Research Centre „AgroBioTech“.

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