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Original article

The influence of the feeding Flour Beetle *Tribolium confusum*-infested fodder on the selected indices of the health status of rats

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Abstract

The present experiment was designed to demonstrate differences in the degree of fodder contamination with benzoquinones at various *Tribolium confusum* levels, the impact of infested feed on the beetle population and the impact of infested feed on the health status of rats.

The feeding studies were done on female rats divided into 3 groups: a control group and two experimental groups. Experimental groups were fed with a fodder infested by 150 individuals of *T. confusum* per kg (group D1) and 300 individuals of *T. confusum* per kg (group D2). The insects were grown in the fodder for 5 months and the contaminated fodder was given to the laboratory animals for 8 weeks. After that period the rats were sacrificed, blood was drawn for morphological, biochemical and immunological analyses, as well as the samples of internal organs were taken for histopathology.

Regardless of initial degree of infestation, after 5 months incubation period the content of benzoquinones in fodder reached the maximum level that reduced beetle population.

The resulting concentration to benzoquinones had no effect upon feed intake nor growth of rate, whereas caused the presence of these substances in feces, urine and also in tissues which was indicated by pathological lesions observed in the study.

The results obtained point to the possibility of the benzoquinones accumulation in the organisms of farm animals fed fodder containing pests.

Key words: Flour Beetle, infested fodder, benzoquinones, rats

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Introduction

Flour Beetle *Tribolium confusum*, belonging to the order *Coleoptera*, family *Tenebrionidae*, is a rusty-brown beetle, 2.6-5.0 mm of body length, being the most frequent silage pest in the world. Adult forms live up to 3.5 years. They most often feed on the damaged grain, grain products or grain fodder (Olejarski and Ignatowicz 2010, Olejarski at al. 2013).

Beetles possess the defense glands which excrete quinones, such as 2-methyl-p-benzoquinone, 2-ethyl-p-benzoquinone, hydroquinone (Alexander and Barton 1943, Loconti and Roth 1953, Ladisch and Suter 1967, Howard 1987) commonly referred to as benzoquinones. Benzoquinones are a group of compounds showing toxic, carcinogenic and enterotoxic properties, which also give the quinone-contaminated products a persistent acrid odor and a heather-like color (Loconti and Roth 1953). The amount of quinones excreted by one individual into the substrate (corn, corn meal, corn flakes) is in the range from several micrograms to 0.5 mg/insect, but due to the number of individuals in the infested food, being hundreds to thousands individuals, it gives the high concentration of quinones (Yezerski et al. 2000, Yezerski et al. 2004, Lis and Bakuła 2011).

Aim

The aim of the study was to assess the degree of fodder contamination with benzoquinines produced by the flour beetle (*Trifolium confusum*) at different extensiveness of the infestation as well as the influence on the rats health status measured by the weight gain, the morphological, biochemical and immunological indices of the blood, as well as histopathological lesions of organs.

Materials and Methods

The study was preceded by establishing the laboratory culture of the insects at the Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University od Warmia and Mazury in Olsztyn, Poland. The individuals were selected for culture according to criteria by Halstead (1963).

The insects were fed on the full ration fodder for laboratory animals LABOFEED H (for mice and rats).

After 6 month growing adult individuals were chosen for infestation of the experimental fodder. The fodder was infested with 150 individuals per kg of the fodder in the experimental group D1 and with 300 individuals per kg of fodder in the experimental group D2. The insects were grown in the fodder for 5 months.

After the pest growing was finished (after 5 months) the number of insects per kg of fodder was established and the level of bemzoquinones (MBQ – 2-metylo-1,4-benzochinon) was assayed with the high performance gas chromatography coupled with GC-MS detector according to the paper by Hodges et al. (1996) with the internal standard (2-(1-naphtyl)-cyclopentanone). The apparatus was Thermo Scientific TRACE Ultra GC with Thermo ISQ mass detector. The column was 15 m, DB-5 type (5% silicone phase). The sample was 5 microliter splits. Temperature 200°C. The thermal profile was 0-5 min.- 50°C, 5-25 min.- 50-280°C, 25-30 min. -280°C.

For feeding studies 23 8-weeks-old female rats of Wistar/Hannover strain were used. The study was done on 3 groups: 1 control (n=7) and two experimental (n=8 in each group). The rats were kept individually in metabolic cages and were fed *ad libitum* with the fodder consumption control. The experiment lasted 8 weeks. After that time the blood was drawn for biochemical and immunological studies and after sacrifice the internal organs, heart, lungs, kidneys, spleen, pancreas, liver, stomach, duodenum, jejunum and colon, were taken for histopathological examination.

The blood biochemistry was done in RT-1804 VET Reaction Tube system (Rayto Electronics Inc.). The blood was centrifuged and in the obtained plasma alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase ALT, creatinine (Cr) and urea were assayed.

The activity of immunological system was assessed by assaying ceruloplasmin, total proteins, gamma globulins, IL-1B, IL-6 and IL-10 in the blood.

The results obtained were shown as a mean and standard deviation, and were analyzed with a Fisher's LSD test (based on Student t-test).

The histopathological examinations were done on samples of the above-mentioned organs fixed in 10% neutralized formalin. The samples were embedded in paraffin blocks which were cut with microtome. The sections were stained with hematoxylin and eosine.

All experimental procedures involving animals were conducted according to the Polish legal regulations concerning experiments on animals (following a decision issued by the Local Ethical Committee for Experiments on Animals in Olsztyn No. 19/2010).

Table 1. Fodder infestation level after finishing the pest growing (after 5 months).

	After growing phase		
	Alive	Dead	Together, mean per kg
D1	2536 per 8 kg (317/1kg)	2688 per 8 kg (336 /1kg)	653/kg
D2	1616 per 8 kg (202/1kg)	13712 per 8 kg (1714/1kg)	1916/kg

Table 2. Benzoquinones levels in the urine and stool of experimental rats.

	D1				D2			
	Urine		Stool		Urine		Stool	
Compound	I	II	I	II	I	II	I	II
MBQ (μg/g)	0.494	0.434	0.884	2.246	0.358	0.520	2.347	9.259

I – after feeding rats for 5 weeks ; II – after feeding rats for 8 weeks.

Table 3. Mean body weight and weight gains during the experiment (g), internal organs weights (g) and their weight percentages relative to body weight (%).

Parameter		K (n=7)	D1 (n=8)	D2 (n=8)
Mean body weight of rats on the day of samples collection	s	212.84	199.67	209.35
	±	7.38	15.01	8.65
Mean body weight gain during the experiment	g	31.22	30.6	29.89
Heart	s	0.74	0.71	0.72
	±	0.05	0.04	0.06
	%	0.35	0.35	0.34
Lungs	s	1.27	1.17	1.38
	±	0.06	0.20	0.14
	%	0.60	0.58	0.66
Kidneys	s	1.55	1.41[ab]	1.57
	±	0.11	0.11	0.06
	%	0.73	0.71	0.74
Liver	s	5.69	5.45	5.86
	±	0.27	0.30	0.48
	%	2.67	2.73	2.8
Spleen	s	0.69	0.66	0.77
	±	0.15	0.08	0.09
	%	0.32	0.33	0.37
Stomach	s	1.88	1.58[ab]	1.79
	±	0.31	0.12	0.24
	%	0.88	0.79	0.85
Small intestine	s	8.10	4.69[ab]	7.31
	±	0.80	1.07	0.52
	%	3.8	2.35	3.5
Large intestine	s	5.17	1.99[ab]	4.45
	±	1.08	1.08	0.46
	%	2.43	1.0	2.12

K – control group, D1, D2 – experimental groups
s – mean ± Standard Deviation
a – statistically significant differences between K and D1 or D2 at $p \leq 0.05$
b – statistically significant differences between D1 and D2 at $p \leq 0.05$

Table 4. Histopathological changes in the sections of the studied organs.

Laesion	K (n=7)	D1 (n=8)	D2 (n=8)
1	3	3	4
Heart			
Hyperaemia	1	3	1
Parenchymatous degeneration of cardiomyocythes	–	1	1
Focal vacuolar degeneration of cardiolyocythes	1	–	–
Subtotal	**2**	**4**	**2**
Lungs			
Acute alveolar emphysema	1	2	2
Focal atelectasis	–	1	1
Thickened walls of alveoli	1	2	1
Hyperaemia	2	4	6
Lymphocytic, plasmatic and granulocytic infiltration	1	1	3
Numerous macrophages filled with lipids	–	–	1
Excessive desquamation	–	1	–
Mucosal epithelium hyperplasia	–	1	–
Catarrhal bronchitis	–	1	–
Catarrhal bronchiolitis	–	2	2
Interstitial pneumonia	–	–	1
Subtotal	**5**	**15**	**17**
Liver			
Parenchymatous degeneration	1	3	2
Portal hyperaemia	1	3	–
Lymphocytary infiltration	–	1	–
Vacuolar degeneration of hepathocytes around portal space, hepatic trabecular and cellular dissociation around central veins	–	–	1
Cysts filled with amorphic, weakly eosinophilic material	–	1	–
Subtotal	**2**	**8**	**3**
Kidneys			
Parenchymatous degeneration of tubular epithelium	1	2	2
Hyalinic degeneration of tubular epithelium	–	1	1
Vacuolar degeneration of tubular epithelium	–	1	–
Protein masses in contortous tubule	–	1	–
Glomerular hyperaemia	1	2	2
Glomerular loop lobulization	–	1	–
Lymphocytary inflammation of renal pelvis	–	1	–
Subtotal	**2**	**9**	**5**
Spleen			
Lienar follicles enlargement	1	–	3
Increased haemosyderosis	1	2	1
Splenocytic hyperplasia	–	3	1
Subtotal	**2**	**5**	**4**
Pancreas			
Moderate hyperaemia of endocrine pancreas	–	1	–
Increased acidophilia of endocrine cells	–	–	1
Subtotal	**–**	**1**	**1**
Stomach			
Glandular cells desquamation	–	1	–
Duodenum			
Enlarged lymphatic follicles in villi	–	2	1
Hyperplasia of crypt cells	–	–	1
Deformed villi	1	4	3
Apical necrosis of villi	1	1	1
Decreased number of lymphocytes in *lamina propria*	–	2	2

cont. Table 4

1	2	3	4
Increased number of lymphocytes in villi	1	–	1
Increased number of eosinophiles in *lamina propria*	–	1	–
Subtotal	**3**	**10**	**9**
Jejunum			
Deformed villi	2	–	4
Catarrhal inflammation	1	–	3
Superficial villi necrosis	1	–	–
Enlarged lymphatic follicles	–	–	1
Lyp\mphoidal cell infiltration	1	–	–
Decreased number of lymphocytes in *lamina propria*	–	1	–
Subtotal	**5**	**1**	**8**
Large intestine			
Enlarged lymphatic follicles	–	–	2
Superficial mucosal necrosis	–	1	–
Cellular infiltration in mucosa	–	1	–
Thickened mucus layer on the mucosa	–	1	–
Subtotal	**–**	**3**	**2**

Results

A significant difference in the level of initial fodder infestation with flour beetle (150 vs. 300/kg) did not cause any difference in the benzoquinone content (the content of 2-methyl-benzoquinone in both fodders was in the narrow range 1.11-1.18 microgram/g), but the number of dead insects was almost 5-fold higher where the initial infestation was more intense (1714 vs. 336/kg) (Table 1).

During the feeding of rats with the infested fodder the content of 2-methyl-benzoquinone in the stool of fed rats was rising from 0.884 to 2.347 mcg/g in D1 group and from 2.246 to 9.259 mcg/g in D2 group, while the content of 2-methyl-benzoquinoines in the urine was at the similar level, independently from the big differences of the compound in feces (Table 2).

Morphometric examination of internal organs, namely heart, lungs, liver and spleen did not show any differences between groups. However, statistically significant differences ($p=0.05$) were found as regards the mean weight of kidneys, stomach, small and large intestines between groups D1 and D2, as well as between these groups and control group (Table 3). The number of morphological changes in the test organs is shown in Table 4. Histopathological examination showed the most changes in the lungs. An example of the changes can be lymphocytic, plasmatic and granulocytic infiltration (Fig. 1).

Histopathological changes occurred also in the kidneys, Histopathological changes occurred also in the kidneys, an example can be parenchymatous degeneration of the tubular epithelium (Fig. 2).

View changes have also been observed in different sections of the gastrointestinal tract, especially in the duodenum and jejunum, and especially within the intestinal villi (Fig. 3).

The results of serum biochemical analysis showed statistically significant differences in the activity of ALP and AST, and Cr concentration between the experimental and control group (Table 5).

Results of immunological assays in the blood showed a reduction in the value of gamma globulin in the Group D1 which was a statistically significant difference, while other indicator levels did not differ statistically (Table 6).

Discussion

The level of pollution by benzoquinones depends on the number of insects, and the ground and time of infestation. It can be assumed that a large number of flour beetles introduced into food for rats has resulted in the rapid growth of their population, and this has led to increased concentrations of benzoquinones in the fodder, which may have an impact on the increasing mortality rate and eventually on the radical decline of the population. The reason of population decline can also be cannibalism (Nawrot 2002).

Similar contents of 2-methyl benzoquinoines in the urine in both research groups, and at the same time, large differences in the level of this compound in feces may indicate two possible situations – the restricted absorption of benzoquinones from the alimentary tract (among others due to the presence of

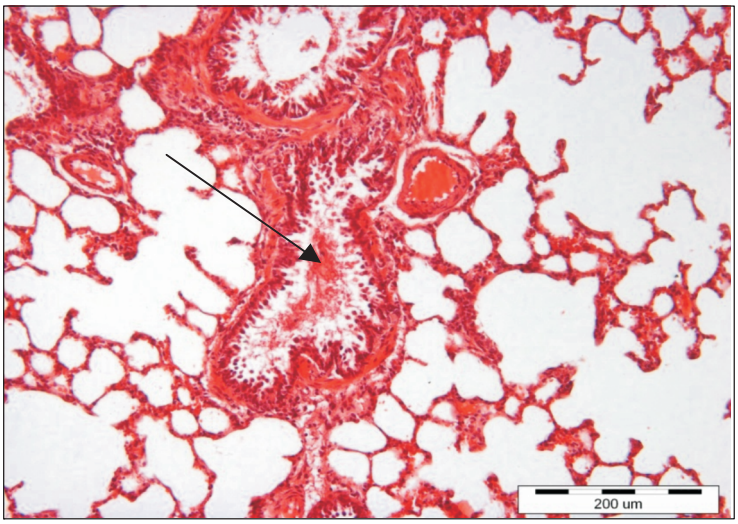

Fig. 1. Lungs. Lymphocytic, plasmatic and granulocytic infiltration D2.

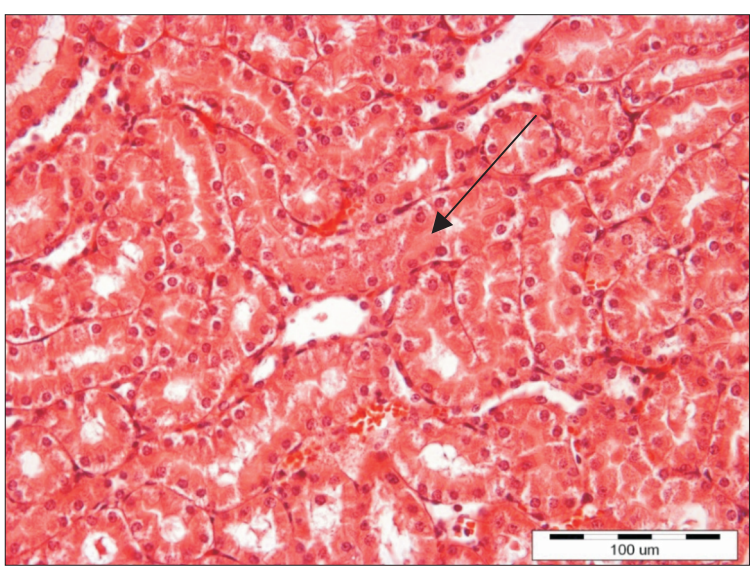

Fig. 2. Kidneys**.** Parenchymatous degeneration of the tubular epithelium D2.

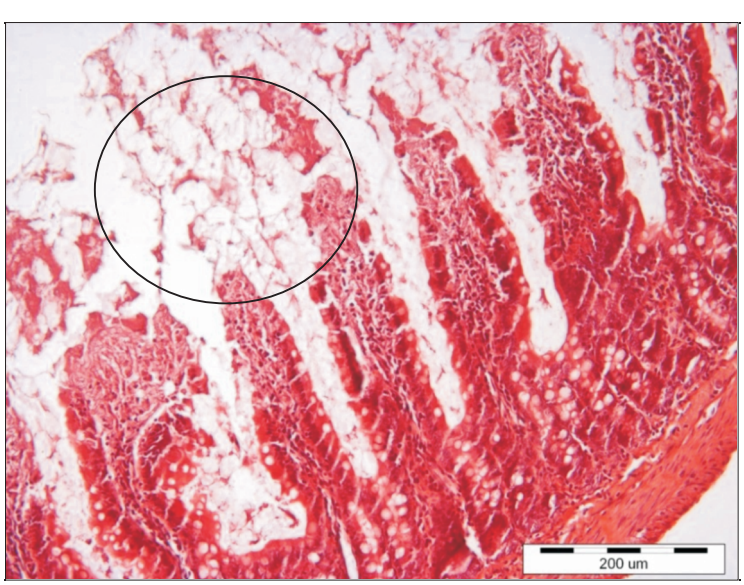

Fig. 3. Duodenum. Deformed villi, apical necrosis of villi D2.

Table 5. Results of blood biochemistry analysis.

Parameter		K (n=7)	D1 (n=8)	D2 (n=8)
ALP (U/l)	s	167.76	55.90[a]	83.35[a]
	±	48.77	27.97	22.45
AST (U/l)	s	175.83	233.53[a]	118.83
	±	29.06	58.56	44.26
ALT (U/l)	s	52.66	50.86	36.60
	±	19.15	8.40	10.09
Cr (mg/dl)	s	0.49	0.69[a]	0.62[a]
	±	0.04	0.09	0.13
URINE (mg/dl)	s	37.00	48.81	40.28
	±	18.82	11.48	7.89

Description as in Table 3

Table 6. Results of immunological assays in the blood.

Parameter		K (n=7)	D1 (n=8)	D2 (n=8)
Lysozyme mg/l	s	5.80	4.80	7.09
	±	1.97	2.49	3.29
Ceruloplazmin IU	s	129.57	122.09	126.00
	±	12.68	8.93	6.58
Total proteins g/l	s	67.04	65.10	63.69
	±	2.87	3.25	4.00
Gammaglobulins g/l	s	9.70	6.74[a]	8.80
	±	2.10	2.15	1.38
IL-1B pg/ml	s	31.73	32.54	34.86
	±	1.05	1.55	5.36
IL-6 pg/ml	s	23.23	23.11	23.66
	±	1.73	1.48	1.62
IL-10 pg/ml	s	65.17	65.81	66.84
	±	1.97	2.13	2.33

Description as in Table 3

these compounds in the bodies of digested insects) and/or the limited capacity of kidneys in excretion of these compounds, what may result in the increased retention in the organism.

The decreased mean mass of the organs is reflected by the increased number of morphological lesions. The lungs were the most sensitive organ of rats to the experimental factor. There have been many progressive changes in the lung tissue mirrors. These changes may be indicative of the sensitivity of the lung tissue, which may be related to volatile benzoquinones (Roth and Howland 1941).

Changes in the kidneys can confirm the fact that quinones or metabolites of benzene coupled with glucuronide are excreted by these organs, among other things, causing the parenchymatous degeneration of the tubular epithelium.

Regards to the damage in the gastrointestinal tract, most numerous changes occurred in the duodenum and jejunum, and especially within the intestinal villi changes. These changes are correlated with levels of benzoquinones in feed and feces collected during the experiment.

Significant differences in the activity of AST and Cr level may be the effect of the metabolic and pathological changes in kidneys. The differences of the studied indices between the groups correlate with the histopathological picture of kidneys. Elevated AST may suggest damage to liver cells. While an increase in the Cr occurs with reduced excretion (kidney failure, drug side effect, or during food poisoning with organic and inorganic compounds.

A decrease in immunoglobulin concentration may be associated with an inhibiting effect of benzoquinones. However, other indices, including interleukins, remain ad at the same level without statistically significant differences, what may suggest that

benzoquinones did not stimulate nor suppress the rat immunological system.

A lack of significant differences in body weight gains may suggest that the pest-infested fodder is not harmful for fed animals. Breeders may disregard the presence of the pest in the fodder for their animals. However, the results of urine and stool analysis for excreted benzoquinones as well as the histopathological changes in the internal organs confirm the presence of benzoquinones in the organisms of experimental animals, the possibility of their accumulation and the negative health effect.

Conclusions

Regardless of initial degree of infestation, after 5 months incubation period the content of benzoquinones in fodder reached the maximum level that reduced beetle population.

The resulting concentration to benzoquinones had no effect upon feed intake nor growth of rate, whereas caused the presence of these substances in feces, urine and also in tissues which was indicated by pathological lesions observed in the study.

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