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Short Communication

ACUTE ORAL TOXICITY OF VETOM 21.77 BASED ON *DUDDINGTONIA FLAGRANS* IN BROILER CHICKENS

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ABSTRACT

A 14-d study was undertaken to test the acute toxicity of a new preparation Vetom 21.77 based on the predacious fungus *Duddingtonia flagrans.* A total of 40 healthy 5-day-old broiler chickens (Hubbard F15, 100 ± 5 g), that had previously gone through a required 5-days adaptation to the environment, were orally dosed with the drug for 5 consecutive days at different doses, after which their health status was assessed daily up to the end of the experiment. According to the results, no substantial changes in the physiological state of the chickens were detected during the experiment. Internal organs weighing revealed no statistically significant differences between the groups, though weight coefficient values of internal organs of treated chickens slightly exceeded those of the control group. Some haematological parameters were significantly higher in the treatment group, without going beyond reference ranges. All chickens used in the experiment survived the study. The preparation has not produced any toxic effect even at a higher dose (4000 µL/kg bw/day). It is concluded that Vetom 21.77 pertains to preparations of IV toxicity class.

Key words: Vetom 21.77, *Duddingtonia flagrans*, acute toxicity, Hubbard F15

INTRODUCTION

Chicken meat is the most popular meat worldwide nowadays. Its global consumption reached about 115 million tons in 2016 (1), and therefore it's a very alarming fact that gastrointestinal diseases in broilers, including necrotic enteritis, viral enteritis, coccidiosis and many others, have become an increasing concern worldwide (2). Meanwhile, investigation of natural supplements for optimization of the intestinal microflora of industrial poultry is quite an active segment in modern scientific research in the field of poultry farming (3). In recent years, fungal substances have been positioned as such agents. For instance, Yudiarti et al. (4) report that the dried culture of *Chrysonilia crassa* added in chickens diet led to duodenal villi development, as well as to reduction of bacteria and fungi in the gastrointestinal tract, although without a positive effect on chicken productivity. A significant characteristic of organic preparations is that their administration does not lead to the drug resistance phenomenon (5).

Fungi are also efficient in the degradation of complex compounds: a number of fungi are able to catalyze the decomposition of the lignocellulosic biomass contained in animal feeds and, consequently, increase the bioavailability of the nutrients (6). Another advantage of fungal substances is also their ability to generate spores; it allows them to maintain a high range of survivability and stability even under adverse conditions (7). In addition, fungi produce β-glucans which activate immune cells (8).

A number of trials have revealed the high predatory efficacy of preparations based on fungi against a wide range of helminths colonizing the gastrointestinal tract of various species, such as the filamentous fungi *Chrysonilia crassa* (9) or *Arthrobotrys oligospora* (10, 11). One of these helminthophags is the predatory fungus *D. flagrans*,

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a spore-mycelial biomass of which is contained in Vetom 21.77 and is supposed to antagonize helminths and pathogenic gut microflora.

Research reports indicate positive results of *D. flagrans* administration in various formulations for biological control of such parasites as *Haemonchus contortus* (12, 13), *Teladorsagia circumcincta* (14), *Angiostrongylus vasorum* (15), *Strongyloidae* (16) and many more. According to some data, *D. flagrans* has demonstrated enzyme activity, producing serine proteases (17, 18). This is an argument in favor of the possibility of fungus to increase the digestibility of farm animal feeds.

However, application rates and possible effects on poultry of preparations based on spore-forming microorganisms of predatory fungi (such as *D. flagrans*) have been neglected in the past, so this study may be regarded as relevant. It was undertaken in ordert to assess the acute toxicity of microbiotic Vetom 21.77 ('Исследовательский центр', Novosibirsk region, Russian Federation) based on *D. flagrans*, to study its influence on the physiological state of the chickens, their growth rate and blood count, as well as to examine the possible side effects of the drug and to assign an acute toxic class to it.

MATERIAL AND METHODS

Animals and experimental design

A 14-d experiment was conducted in October 2017 at the Veterinary Research Laboratory of Agrotechnopark, Shakarim state University (Semey, Kazakhstan) on the basis of OECD Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method (2001). A total of 40 healthy 5-day-old broiler chickens (Hubbard F15, mean weight 100 ± 5 g), that had previously gone through the required 5-days adaptation to the environment, were allocated to 1 control and 1 experimental groups of 20 chickens each. The chickens were housed on poultry bedding. Feed and water were provided ad libitum. The birds were fed with a 'ПК 5-1 Старт' pelleted feed mixture (Крупы Востока, Ust-Kamenogorsk, Kazakhstan), 3150 kCal/kg, containing crude protein (22%), raw fiber (8%) and a number of microelements.

According to the abovementioned method, from day 1 chickens from the test group were administered Vetom 21.77 through the crop for 5 consecutive days at 5, 50, 300, 2000 and 4000 μL/kg bw respectively. The birds were not fed for 4 h before the procedure and 1-2 h after it. Vetom 21.77 is a liquid that contains spore-mycelial biomass (1 x 10^9 CFUs/cm^3) of *D. flagrans* (strain F-882) as a basis.

The drug reactions were observed by a veterinarian for 30 min following the procedure, then every 24 h, for a total of 14 days. Chickens from the control group were not given Vetom 21.77. On day 14, all birds in both groups were humanely slaughtered by decapitation according to GOST 52837-2007 'Slaughter poultry. Specifications' (19) and examined post-mortem with weighing of several internal organs (heart, lungs, kidneys, liver, spleen, intestine, stomach and pancreas) in order to examine the possible adverse effect of the preparation. Effectiveness of the broiler chickens growth was evaluated by European Production Efficiency Factors (EPEF) (20).

$$\text{EPEF} = \frac{\text{LW (kg)} \times \text{LA (\%)}}{\text{SA (days)} \times \text{FCR (kg)}} \times 100,$$

where: LW (kg) = Live weight at the end of the rearing period; LA (%) = Livability (number of birds alive at the end of the rearing period relative to the number of chicks placed); SA (days) = Slaughter age of chicks; FCR (kg) = Cumulative feed intake (kg)/total weight gain (kg).

Criteria for testing the acute toxicity of Vetom 21.77 were the following: physiological state of birds, mortality rate, safety, growth rate, weight and condition of internal organs, haematological and serum biochemical parameters.

All procedures performed in this study were in adherence with the ethical standards of Shakarim State University of Semey city.

Haematological analysis

Blood samples were immediately collected from the hearts of slaughtered chickens. Haematological analysis was carried out using an automatic haematological analyzer PCE 90 vet (HTI, USA). Biochemical parameters were determined via a semi-automatic biochemical analyzer Minitecno (ISE S.r.l., Italy) and a semi-automatic biochemical analyzer Stat Fax 3300 (Awareness Technology, USA).

Statistical analysis

The Mann-Whitney one-tailed U-test was performed to compare the mean values of internal organs weight coefficient, as well as the haematological and biochemical parameters between the groups. All the data were analyzed by means of StatsDirect statistical software, version 3.1.14 (StatsDirect Ltd, UK). Values were presented as mean ± standard deviation. A value of $p < 0.05$ was considered significant.

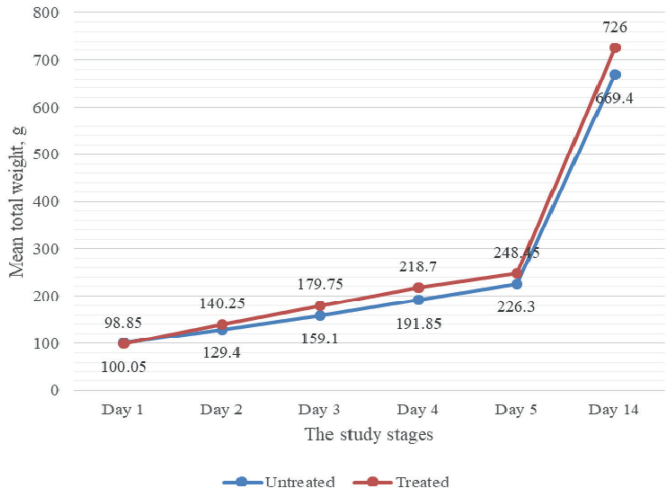

Figure 1. Mean total weight of broiler chickens treated and untreated with Vetom 21.77

RESULTS

Broiler performances and physiological parameters in both groups before the dosing did not show significant differences: the birds were mobile, with sufficient feed and water uptake; their body weight (bw) was determined as normal. No wounds, redness or alopecia were detected. The skin turgor was normal. The plumage was fluffy, light yellow. Chickens conjunctiva, oral mucosa, genital organs and vent were pink, moist, without visible damage. The beak was yellowish, smooth, without damage. Respiratory rate was normal in both groups: 27-28 breaths per min (BPM). The average body temperature (t°) was also within the normal range: 41.7 °C (control) and 42 °C (test). The average body weight in the control group was 100.05 g,, while in the test group – 98.85 g, which is 1.2% lower than the control one.

Within 30 min after the dosing with Vetom 21.77, no significant changes were observed in the physiological state of the birds in both groups. The average respiratory rate was 27 BPM in the groups. The average body t° was still within the norm: 41.9 °C (control) and 41.7 °C (test).

On day 2, the dose was increased to 50 µL/kg bw. The clinical examinations did not reveal any pathological changes in the physiological state of chickens in both groups; the respiratory rate was left unchanged; the mean body t° was the same in the untreated group, while in the test one – 41.8 °C. Treated chickens weighed an average of 140.25 g, controls – 129.4 g. Thus, treated birds' body weight increased 8.4% higher compared with controls (Fig. 1).

On day 3, when the dose was 300 µL/kg bw, test group chickens were more active and their growth intensity was 13% ahead of controls (Fig. 1). There were no changes in the respiratory rate in the control group, whilst in the treated group it was 28 BPM. The body t° was 41.7 °C (control) and 41.8 °C (test).

On day 4 (2000 µL/kg bw), all birds were clinically healthy, while feed intake slightly increased in both groups, indicating satisfactory appetite. The respiratory rate was still physiologically normal: 25 BPM (control), 26 BPM (test). The average body t° was 41.8 °C in both groups. Live weight gain of treated broilers exceeded controls by 14% (Fig. 1).

On day 5 (4000 µL/kg bw), the physiological status of the birds in test and control groups did not differ much. The respiratory rate was 26 BPM in both groups this time. The mean body t° was 41.8 °C (control) and 41.9 °C (test). Live weight gain of test group chickens was 9.8% higher in comparison with controls (Fig. 1).

On day 14, the absolute mass of treated birds was 8.5% higher compared with controls (Fig. 1). The respiratory rate was an average of 27 BPM (control) and 26 BPM (test). The mean body t° was 41.8 °C in both groups. By the end of the experiment, the birds tail feathers have flared.

According to the daily clinical examinations data, the treated birds had adequate appetite; their growth intensity exceeded the control group level. The maximum increase in absolute mass was recorded at 2000 µL/kg bw, day 4 (Fig. 1). No lesions were found in internal organs of the broilers, their weight was slightly more than of those in untreated group (Table 1).

Table 1. The average weight of body and some internal organs of broiler chickens treated and untreated with Vetom 21.77 at the end of the study (day 14)

Parameters	Control group		Test group	
	Weight (g)	Weight coefficient (%)	Weight (g)	Weight coefficient (%)
Body	669,40 ± 34,98	–	726,05 ± 33,33	–
Heart	5,090 ± 0,508	0,737 ± 0,038	5,594 ± 0,519	0,739 ± 0,043
Lungs	2,487 ± 0,247	0,360 ± 0,018	2,737 ± 0,253	0,362 ± 0,029
Liver	22,268 ± 2,212	3,222 ± 0,165	24,384 ± 2,262	3,221 ± 0,191
Spleen	0,666 ± 0,060	0,096 ± 0,005	0,735 ± 0,065	0,097 ± 0,008
Kidneys	3,555 ± 0,353	0,514 ± 0,026	3,906 ± 0,361	0,516 ± 0,040
Intestine	13,812 ± 1,372	1,999 ± 0,102	15,123 ± 1,403	1,998 ± 0,120
Stomach	6,706 ± 0,667	0,970 ± 0,050	7,344 ± 0,681	0,970 ± 0,058
Pancreas	1,310 ± 0,134	0,190 ± 0,010	1,443 ± 0,133	0,191 ± 0,017

Values did not differ significantly between the groups at $P < 0.05$

The weight coefficient of some internal organs of the treated birds slightly exceeded those of the control group: heart – by 0.3%, lungs – by 0.5%, spleen – by 0.8%, kidneys – by 0.3%, pancreas – by 0.6%. However, there were no statistically significant differences between the groups (Table 1). EPEF was 40.46 in the control group and 48.29 in the test group (19.4% ahead of controls). No morbidity or mortality was registered in any of the Vetom-dosed birds during the experimental period. According to the analysis of hematologic and biochemical parameters, they were within the reference values range in both groups. Besides, erythrocytes and hematocrit values were significantly higher in the treated group (Table 2 and 3).

Table 2. Haematological parameters of broiler chickens treated and untreated with Vetom 21.77 at the end of the study (day 14)

Haematological parameters	Control group (n=20)	Test group (n=20)	Reference ranges	P value
Erythrocytes (10^{12}/L)	2.65 ± 0.13	2.72 ± 0.12	2.5-3.5	0.0381
Leukocytes (10^9/L)	22.03 ± 0.53	22.16 ± 0.59	20-40	0.1844
Platelets (g/L)	58.28 ± 4.05	60.07 ± 3.23	32-100	0.0628
Hematocrit (%)	25.95 ± 1.10	26.70 ± 1.45	22-35	0.0452
Hemoglobin (g/L)	99.73 ± 5.16	102.38 ± 6.76	70-130	0.0994
MCH (pg)	37.73 ± 2.58	37.78 ± 3.28	33-47	0.369
Lymphocytes (%)	53.75 ± 1.65	54.55 ± 1.96	52-60	0.0961
Monocytes (%)	5.10 ± 0.72	5.70 ± 1.56	4-10	0.1622

MCH = Mean corpuscular hemoglobin

Table 3. Biochemical parameters of broiler chickens treated and untreated with Vetom 21.77 at the end of the study (day 14)

Biochemical parameters	Control group (n=20)	Test group (n=20)	Reference ranges	P value
Total protein (g/L)	35.4 ± 3.42	37 ± 3.17	30-44	0.0578
Albumin (g/L)	20.0 ± 2.58	21.2 ± 2.24	14-29	0.0698
Urea (mmol/L)	0.75 ± 0.23	0.87 ± 0.26	0.26-2.04	0.0902
ALT (IU/L)	7.2 ± 0.99	7.0 ± 0.92	0.1-14.8	0.7311
AST (IU/L)	145.1 ± 10.49	149.7 ± 11.53	125-269	0.1221
Phosphorus (mmol/L)	1.58 ± 0.19	1.64 ± 0.15	1.22-3.9	0.0827
Calcium (mmol/L)	4.03 ± 0.29	4.18 ± 0.33	3.75-6.75	0.0746
Total cholesterol (mmol/L)	3.86 ± 0.31	3.72 ± 0.51	2.3-5.5	0.851

ALT = Alanine Aminotransferease. AST = Aspartate Aminotransferase

DISCUSSION

Unfortunately, reports on the acute toxicity of fungi-based preparations in any species are very limited. According to the results of an acute oral toxicity study in rats conducted by the European Food Safety Authority (21), *D. flagrans* had a very low oral toxicity with LD50 > 2,000 mg/kg bw.

Another acute oral toxicity study in rats (22) indicated no toxicological effects of the D. *flagrans* strain IAH 1297 (5,000 mg/kg bw). The oral LD50 > 5,000 mg/kg bw was reported. This data is in compliance with the results obtained in the present work. Acute toxicity of some organic feed additives has been studied in broilers, where successful results were stated (23, 24).

As already mentioned, *D. flagrans* is a widespread biocontrol agent of animal parasites. It can produce large numbers of thick-walled chlamydospores and survive even at high temperatures (50-60 °C) and in hostile environments, such as animal digestive tract (25). It has been verified by the work of Campos et al. (26) where different fungal structures of *D. flagrans* were resistant to the digestive process in goats.

Grønvold et al. (27) affirm the absence of interspecific (bacterial isolates) or intraspecific (isolates of the fungal genera) competition on agar plates with *D. flagrans*. According to Fitz-Aranda et al. (28), *D. flagrans* chlamydospores in nutritional pellets maintained their trapping ability against H. contortus larvae regardless of storage time and experimental conditions.

Ahren et al. (29) have found a low level of genetic variation among D. *flagrans* strains. The authors emphasize that this property reduces possible adaptation of the strains to a specific geographical region. Preliminary studies on effectiveness of Vetom 21.77 have proved its non-toxicity and some growth-stimulating effect on mice (30) and hypoallergenicity in rabbits (31).

CONCLUSION

The results of the present study indicate that daily dosing of broiler chickens with Vetom 21.77 caused no physiological alterations that could lead to any damage. According to the daily clinical examinations data, the drug has not produced any toxic effects even at higher dose (4000 µL/kg bw) in the birds, which classifies this product as non-toxic to broiler chickens. The data presented here have implications for the use of Vetom 21.77 as poultry health promoter.

CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

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