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EFFECT OF COMBINED SORBENT PREPARATION ON GROWTH, PERFORMANCE AND HAEMATOBIOCHEMICAL ALTERATIONS IN BROILER CHICKENS UNDER THE INFLUENCE OF T-2 TOXIN AND DEOXYNIVALENOL MIXED TOXICOSIS

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ABSTRACT

The combined sorbent preparation consists of anthracite, saponite and inactivated yeasts. To determine the detoxification activity of the combined sorbent preparation on the mixed chickens' mycotoxicosis, thirty, two-weeks-old chickens cross "Ross 308" were divided into three groups: A (control); B (T-2 toxin and deoxynivalenol); C (T-2 toxin, deoxynivalenol and the combined sorbent preparation). Chickens were weighed every week, hematological and serum biochemical investigations were provided at 28-th and 42-nd day of chicken`s age. Applying of the combined sorbent preparation in T-2 toxin and deoxynivalenol mixed chickens toxicosis at 3 % by weight of the feed, neutralizes the negative effects of mycotoxins on the bird. It manifests high yield carcass weight and lowers the feed conversion, with almost no variations in hematological and serum biochemical parameters of blood.

Key words: Broiler chickens combined sorbent preparation, T-2 toxin, deoxynivalenol, haematobiochemical parameters

INTRODUCTION

There are more than 40 trichothecen mycotoxins (fusariotoxins), secondary metabolites of various representatives of microscopic fungi Fusarium known T-2 toxin and deoxynivalenol (vomitoxin, DON) are the most common trichothecenes in Ukraine, the content of which often exceeds the maximum permissible levels in the animal feedstuff. Feedstuff, contaminated by trichothecenes, causes poor feed efficiency, reduced growth and impaired immune status of broiler, increasing their death.

A characteristic feature of T-2 toxin is dermonecrotic action while DON causes vomiting. Both of toxins have embryotoxic, teratogenic, mutagenic, carcinogenic action (Tutelyan and Kravchenko, 1985).

Specific treatment of mycotoxicosis is not developed. The treatment includes such arrangements as exclusion suspect feed from the diet, absolute diet and gastric lavage with 3 % solution of sodium bicarbonate, bulk cathartics (Achmetov *et al.,* 2001; Malinin *et al.,* 2002). Feed additives and premixes, which include enterosorbents, are commonly used to prevent mycotoxicosis too (Beaver *et al.,* 1989; Lohov *et al.,* 2008). There is a wide range of proposed sorbents on the market of veterinary drugs: inorganic, organic, and combined (Gorcovenko *et al.,* 2006; Berezovskiy, 2009; Kotsyumbas *et al.,* 2009). Most sorbents are ineffective against fusariotoxins (Ivanov *et al.,* 2008; Grigorenko, 2011), so the development of new combined sorbent is promising and relevant.

MATERIALS AND METHODS

Site and animals

This study was conducted in the Pharmacology and Toxicology Laboratory and vivarium of Department of Veterinary medicine, Nation University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine. This experiment had been approved by the institution's Ethics Committee. Thirty two-weeks-old chickens cross "Ross 308" were used. They were kept in bird-cages at room temperature with ventilation and under natural light and dark periods, and they received water and animal feed ad *libitum*. Mycotoxins-free feed was procured from local feed manufacturer.

Experimental design

Cultures of *Fusarium sporotrichiella* v. *poae* strain 407/4 and *Fusarium macroceras* strain 108270 were used for the production of T-2 toxin and DON respectively.

The combined sorbent preparation was developed on the basis of sorbtion capacity of different minerals. Carbon sorbents (anthracite, birch activated carbon), lignin and saponite show the highest sorption capacity of T-2 toxin and deoxynivalenol. The combined sorbent preparation consists of anthracite 70 %, saponite 20 % and inactivated yeasts 10 %. All components have meshed not more than 0.2 mm.

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Two-weeks-old chickens cross "Ross 308" were divided randomly into three groups of 10 birds each. Dietary levels of T-2 toxin and DON in experimental diets were adjusted by adding calculated amount of mouldy wheat to the diet. The experimental diets containing graded concentration of T-2 toxin and DON were fed at 3.2 ppm and 0.14 ppm respectively in group B and C from 15-th to 42-nd day of age. Group C additionally received the combined sorbent preparation at 3.0 % dietary level. Group A birds did not receive T-2 toxin, DON and sorbent and served as control.

To study the effect of T-2 toxin on growth, experimental chickens were weighed individually every week and weekly feed intake was also recorded to calculate feed conversion ratio (FCR) of each treatment group.

Haematological and serum biochemical analysis

Blood samples were collected from jugular vein using heparin solution anticoagulant from five birds of each group at 28-th and 42-nd day of age. Serum was separated and stored at 4°C. Individual whole blood samples were analyzed for haemoglobin (Hb) value, red cell count, white cell count, erythrocyte sedimentation rate using common methods. Individual serum samples were analyzed for serum total protein, calcium and phosphorus, creatinine, uric acid, total bilirubin values, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) activity, thymol test using common methods.

RESULTS AND DISCUSSION

Body weight investigations

Significant reduction in body weight was observed in group B starting from 4-th week; group C from 5-th week compared to group A (Table 1). The highest FCR was recorded in group B whereas lowest FCR was recorded in group C. The reduction in body weight of chickens in the present study due to T-2 and DON induced toxicosis was treatment dependent that is similar as described by Pande (2006). It may due to inflammation, contact erosion and irritation of alimentary tract resulting into decrease in feed consumption and consequently decrease in body weight of toxicated birds. However, small difference in body weights of chickens group C in comparison to group A confirms positive effect of sorbent preparation.

Haematological analysis

At 28-th and 42-nd days of age Hb values of chickens group B were significantly ($P < 0.05$) lower compared to control (Table 2). The reduction in Hb in the present study recorded may be due to decreased protein synthesis in toxicated chickens. The results of red cell count of experimental chickens at both the period of observations indicated non-significant effect of graded levels of dietary T-2 toxin and DON.

There was leucopenia elucidated in the blood of chickens group B and C, upon that white cell count in the blood of chickens group B was to 28% less at 28-th day of age and to 27% less at 42-nd day compared to control group. White cell count in the blood of chickens group C was 8% less at 28-th day of age, but was not significantly different from control at 42-nd day. The significant effect of dietary mycotoxins at eosinophil count was observed in the blood of chickens group B and C during present experiment. Eosinophilia may be due to the reactions of hypersensitivity of the body. There was significant ($p < 0.01$) rise in erythrocyte sedimentation rate of chickens group B (approx. 1.7 times) compared to control. However, the difference in erythrocyte sedimentation rate among experimental groups A and C was not significant.

Serum biochemical analysis

Total serum protein in chickens was significantly influenced by the experimental treatments. The total protein value in group B was significantly ($p < 0.001$) lowered at both the period of observations (approx. 1.5 times) in contrast to group A (Table 3). On comparison of serum calcium and phosphorus values of the experimental groups, it was observed that phosphorus value was significantly ($p < 0.05$) reduced in group B as compared to other groups (A and C) during experiment. However, the difference in serum calcium values among all experimental was not significant. At 28-th and 42-nd days of age serum creatinine values of chickens group B increased approx. 1.5 times and 1.3 times compared to control respectively.

The rise in serum creatinine values may be due to the renal insufficiency. There was significant reduction in serum uric acid of chickens group B at 28-th day ($p<0.01$) and 42-nd day ($p<0.001$) of age compared to control group. There was significant ($p< 0.001$) rise in serum total bilirubin values of chickens group B compared to group A during all experiment. Upon that serum total bilirubin values of all experimental groups raised in 2.5 times from 28-th day to 42-nd day of age.

Table 1. Mean weekly body weights (g) of experimental chickens from various treatment groups, M±SD, n=5

Age (weeks)	Treatment		
	A	B	C
2-nd	419.1±8.2	434.2±8.7	424.3±15.5
3-rd	849.7±15.2	798.4±18.1	848.7±8.9
4-th	1272.0±28.3	1043.1±19.8[c]	1195.7±25.5
5-th	1984.8±100.6	1410.2±48.9[c]	1854.6±71.7
6-th	2574.8±148.4	1698.2±60.3[c]	2375.8±76.0
FCR	2.06	2.39	1.89

A – control group; B – T-2 toxin and DON 3.2 ppm and 0.14 ppm dietary level respectively; C – T-2 toxin, DON and combined sorbent preparation 3.2 ppm, 0.14 ppm and 3.0 % dietary level respectively. FCR – feed conversion ratio. [c]Significant difference between A and B ($p < 0.01$).

Table 2. Haematological parameters of broiler chickens after 4 weeks of the experiment, M±SD, n=5

Parameter	Treatment		
	A	B	C
Haemoglobin, g/L	87.00±1.05	82.80±1.46[b]	85.80±1.59
Red cell, trillion cells/L	2.70±0.10	2.94±0.05	2.76±0.05
White cell, billion cells/L	15.24±0.45	11.08±0.85[c]	14.52±0.91
Erythrocyte sedimentation rate, mm/hr	5.40±0.24	9.60±0.93[c]	5.00±0.32

A – control group; B – T-2 toxin and DON 3.2 ppm and 0.14 ppm dietary level respectively; C – T-2 toxin, DON and combined sorbent preparation 3.2 ppm, 0.14 ppm and 3.0 % dietary level respectively.
[b]Significant difference between A and B ($p < 0.05$), [c]Significant difference between A and B ($p < 0.01$).

On comparison of ALT activity of the experimental groups, it has been observed that ALT activity was significantly reduced in group B compared to other groups (A and C) at 28-th day ($p < 0.001$) and 42-nd day ($p < 0.05$) of age. The results of AST activity of experimental chickens at both the period of observations indicated non-significant effect of graded levels of dietary mycotoxins. There was significant ($p < 0.01$) rise in ALP activity of chickens group B compared to control group during all experiment. At 28-th day of age there was significant ($p < 0.05$) rise in ALP activity of chickens group C but at 42-nd day of age there was no difference compared to control group. There was positive thymol test in group B at both the period of observations in contrast to group A and C. Reduction in ALT activity, rise in ALP activity and positive thymol test may be due to hepatotoxic effect of T-2 toxin and DON. On the contrary, increase in ALT has been reported in broiler chicks fed 4 ppm T-2 toxin from 7 to 55 days (Raina *et al.,* 1991). However, a decrease in ALT level was reported in broiler chickens after 35 days of feeding with 0.3 ppm AF and 3 ppm T2 toxin (Raju *et al.,* 2000).

Hence, it may be concluded that T-2 toxin at 3.2 ppm and DON at 0.14 ppm level could adversely affect the health of broiler chickens when fed from 28-th day of age to 42-nd day of age. The study also indicated the potential of T-2 toxin to interact significantly with DON in altering haematological and serum biochemical constituents like Hb value, white cell count, erythrocyte sedimentation rate, total protein, phosphorus, creatinine, uric acid, total bilirubin values, ALT and ALP activity. Application of the combined sorbent preparation in experimental mixed T-2 and DON toxicosis of chickens at 3 % by weight of the feed neutralizes the negative effects of mycotoxins on the adsorbents to animal feed provides versatile tools of preventing bird. It manifests high yield carcass weight and lower rates of feed conversion, with almost no variations in hematological and serum biochemical parameters of blood. This research confirms that the addition of different adsorbents or of very promising derivatized mycotoxicosis (Huwig *et al.,* 2001).

Table 3. Serum biochemical parameters of broiler chickens after 4 weeks of the experiment, M±SD, n=5

Parameter	Treatment		
	A	B	C
Total protein, g/L	36.80±0.73	23.20±1.07[d]	35.80±0.97
Total bilirubin, μmol/L	10.40±0.68	15.04±0.57[d]	8.90±0.81
Thymol test, units	0.25±0	0.40±0.04[c]	0.25±0
Uric acid, mmol/L	2.14±0.07	1.64±0.02[d]	2.06±0.07
Creatinine, mmol/L	0.03±0.01	0.04±0.01[d]	0.03±0.01
Calcium, mmol/L	2.45±0.07	2.61±0.02	2.66±0.06
Phosphorus, mmol/L	2.36±0.12	2.04±0.04[b]	2.35±0.08
ALT, U/L	3.10±0.20	2.37±0.12[b]	3.00±0.12
AST, U/L	27.33±0.65	27.80±0.88	27.93±0.48
ALP, U/L	320.69±12.6	454.12±16.9[d]	348.36±24.0

A – control group; B – T-2 toxin and DON 3.2 ppm and 0.14 ppm dietary level respectively; C – T-2 toxin, DON and combined sorbent preparation 3.2 ppm, 0.14 ppm and 3.0 % dietary level respectively.
[b]Significant difference between A and B ($p < 0.05$), [c]Significant difference between A and B ($p < 0.01$).
[d]Significant difference between A and B ($p < 0.001$).

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