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BLOOD SERUM PROTEIN STATUS OF BROILERS TREATED BY T-2 TOXIN

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The influence ofT-2 toxin on blood serum protein status was examined in Hybro broilers in two trials. In the first trial experimental groups were daily treated with T-2 toxin at 0.02 and 0.20 mg/kg BM for 7 days. In the second trial the experimental groups were daily treated with T-2 toxin at 0.02 mg/kg BM, during intervals lasting 7,14 or 21 days. Blood samples were taken after the period of toxin administration in both trials and the remaining animals from the control and experimental groups were normally fed and watered without toxin application until the 42 nd day when blood samples were taken again.

The tested amounts of T-2 toxin had a negative non-significant influence on total protein concentration with an increase of alpha and gamma globulins and a decrease of albumins but a relatively stable concentration of beta globulins. Consequently a closer A/G ratio was found. By comparative analysis of the biochemical results in both trials it could be concluded that the lower amount of T-2 toxin given for a longer period of time showed analogous effects as the higher amount given for a shorter period of time, which indicates a possible cumulative effect of T-2 toxin.

Key words: proteins, blood, T-2 toxin, broilers.

INTRODUCTION

Trichothecenes are a group of toxic secondary metabolities produced mainly by Fusarium species (Bamburg et al. 1968). They are among the more persistent environmental contaminants and are known to cause severe tissue and cell damage (Wyllie and Morehouse, 1978a; Wyllie and Morehouse, 1978b; Ueno, 1977). One ofthe most important trichothecene mycotoxins is the T-2 toxin (3a-hydroxy 4b, 15-diacetoxy-8a-(3-methylbutyryloxy-12, 13-espoxy-trichoec-9-ene). It has been identified in mouldy feed but naturally contaminated feeds generally contain small amounts of T-2 toxin, between 0.17-2.40 ppm (Bata et al., 1984; Duletić and Muntanola, 1989). However, higher amounts (0.5-204 ppm) have been found in barley, com, bran and hay (Gabal et al., 1986).

This toxic sesquiterpenoid is involved worldwide in mycotoxicoses of humans livestock and poultry. T-2 toxin can cause feed refusal, emesis, dermal necrosis, immunological disorders, infertility and other disturbances (Ueno, 1980) along with coma and death (Chi et al., 1977a). Moreover T-2 toxin is a potent inhibitor of protein and DNA synthesis in various animal and biological systems (Ueno, 1984). Inhibiton of protein synthesis has been seen in different tissues characterized by polysome alterations, desegregation or induction of structural alterations but the actual site of inhibition has not been determined yet (Carter and Cannon, 1978). It is well documented (McLaughlin et al., 1977; Ueno, 1977) that trichothecenes inhibit protein synthesis by binding to the 60S subunit of the 80S ribosome, thus blocking polypeptide chain elongation. The possibility of ireversible inhibition of protein synthesis initation causing polysome destruction has also been suggested and it has been showen that T-2 toxin inhibited mitochondrial protein synthesis (Pace et al., 1988).

Because of the health hazards posed by T-2 toxin and especially because of the reports that trichothecene toxins have been used in chemical warfare in South East Asia and Afghanistan (Mirocha et al., 1983; Ciegler, 1986; Kadivar and Adams, 1991), it is important to explore all of its toxic actions. Considering the described effects of T-2 toxin, many authors have investigated the influence of T-2 toxin on blood serum concentrations of proteins and their fractions in an attempt to make the diagnosis of R-2 toxicoses more reliable. Although T-2 toxin has been shown to cause changes in protein status in various animal species, it is difficult to correlate the results from the various studies. Not only different animal species and animals at different stages of maturity have been used, but also the toxin has been administered by different routes at various dosages. The primary objective of this study was to investigate the changes of blood serum protein status of broilers treated with different amounts of T-2 toxin and the time of exposure.

MATERIALS AND METHODS

T-2 toxin produced by Fusarium sporotrichioides kept on liquid media according to Betina (1984) was used for extraction and further refined in the usual way (Bočarov-Stančić and Muntanola-Cvetković, 1988; Bočarov-Stančić et al., 1986). Thin layer chromatography was used for determination of T-2 toxin purity (AOAC, 1980). The degree of purity was 86% compared with the commercial remedy (99,6% pure). According to Chi et al., (1977a) and the purity medium lethal doses (LD50) of T-2 toxin is taken as 5 mg T-2/kg BW.

Two trials on Hybro broilers were under taken in order to investigate the influence of T-2 toxin on blood serum protein status. During the first two weeks of the trial all birds were fed and watered without any treatment when they were randomly grouped according the design of the experiments.

The first trial involved 36 broilers divided into three groups (control and two experimental groups) with 12 broilers in each group. Experimental groups

received T-2 toxin diluted in ethanolin amounts of 0.02 and 0.20 mg/kg BW, and the control group received a mixture of ethanol and physiological saline during a 7 day period.

The second trial was performed on 48 broilers divided into four groups each consisting of 12 broilers. The experimental groups were treated daily with T-2 toxin-at 0.02 mg/kg BM, for 7,14 or 21 days respectively and a mixture of ethanol and physiological saline was administrated to control group chickens.

After the period of toxin administration until the end of the trial (42nd day), animals from all groups in both trials were normally fed and watered. Blood samples were taken by cardiac puncture with a sterile needle over previously disinfected skin, imediately after the end of the toxin administration period. Samples were taken from broilers from each group in both trials. After blood coagulation and separation of the serum the concentration of total proteins was determined by a colorimetric method and their fractions by paper electrophoresis (cit. acc: Majkić-Sing and Spasić, 1982).

All data were statistically processed and an appraisal of the significance of differences in mean values between the groups of broilers was made.

RESULTS AND DISCUSSION

Wide physiological limits of blood serum protein concentrations following strong variation of their fractions were confirmed in our trial. The blood serum concentration of total protein was 26.2-29.7 g/l (Tables 1,3) which is in agreement with data given by Brandt et al (1951) and similar results are cited by Stevanović et al. (1990). Mild differences associated with T-2 toxin treatment were seen in the blood serum of the experimental group. The tested amounts of T-2 toxin had an insignificant negative influence on total protein concentration. Our results were similar to those reported by Chi et al (1977b) and Chi et al. (1981) but not with those given by Kubena et al.(1989a) and Huff et al. (1988) who claimed that one of the primary effects of T-2 toxin is a significant decrease of total protein concentration in blood serum of poultry. The mild decrease of total protein concentration, lower than that cited in the literature, could be explained by the high dietary protein level (NRC, 1994) which is one of the possible ways to prevet deleterious effects of mycotoxicoses (Sinovec et al., 1995).

Table 1. Concentration of total protein and A/G ratioin blood serum from broilers during the first-trial

Grioup day	Total proteins (g/l)		A/G ratio	
	21st	42nd	21st	42nd
K	26.17 ± 1.95	28.83 ± 1.77	1.58 ± 0.29[a]	1.11 ± 0.07
I	25.50 ± 1.50	28.17 ± 2.48	1.38 ± 0.28	1.17 ± 0.62
II	25.58 ± 3.33	28.80 ± 1.66	1.31 ± 0.19[b]	1.09 ± 0.09

*Values expressed as X ± SD
a,b,c, Mean values within columns with unlike superscript are significantly different ($p<0.05$, Student's test)

The relative serum albumin concentration (Tables 2, 4) was higher than the blobulin concentration and, comparing the obtained results with established physiological values, it can be seen that they ranged around and under the higher limit (Pavlović et al., 1978; Stevanović et al., 1990. A decrease of albumin concentration was seen in the blood serum of the treated birds which was proportional to the dosage. The difference was statistically significant with the higher dosage. Using the lower dosage for a longer time led to only a mild insignificant decrease of albumin concentration. Our data are similar to those of Richard et al. (1978) and Huff et al. (1988). Decreasing the concentrations of albumin which forms part of the transport mechanism of nutrients, could be related to the lower digestibility and availibility of feed causing lower performance (Sinovec and Šefer, 1995; Šefer et al., 1997). Moreover, inhibition of protein synthesis could be expressed by lower levels of other proteins such as enzymes, hormones, etc. (Sinovec, 1991; Bratuljević, 1989). The decreased body weights, decreased serum concentrations of total protein and albumin, and decreased activity of serum enzymes are most likely associated with inhibition of protein synthesis by mycoloxins (Kubena et al., 1990; Kubena et al., 1994).

Table 2. Absolute and relative concentrations of ptotein fraction in blood serum from broilers during the first trial.

Group day	Protein fractions (%)		Protein fractions (g/l)	
	21st	42nd	21st	42nd
		Albumins		
K	60.62 ± 4.49[a]	52.67 ± 1.66	15.85 ± 1.58	15.20 ± 1.22
I	57.43 ± 4.77	53.83 ± 1.29	14.70 ± 1.99	15.17 ± 1.48
II	55.79 + 3.85[b]	52.07 ± 2.33	14.21 ± 1.50	14.98 ± 0.86
		α-globulins		
K	23.42 ± 2.63	24.92 ± 1.51	6.14 ± 0.92	7.17 ± 0.48
I	24.63 ± 2.43	25.37 ± 0.85	6.26 ± 0.51	7.14 ± 0.67
II	26.75 ± 2.88	25.50 ± 1.23	6.87 ± 1.29	7.34 ± 0.53
		β-globulins		
K	4.97 ± 0.47	7.00 ± 1.95	1.30 ± 0.20	2.01 ± 0.54
I	5.38 ± 1.03	5.88 ± 1.21	1.36 ± 0.21	1.66 ± 0.36
II	4.72 ± 0.89	7.51 ± 1.61	1.22 ± 0.33	2.17 ± 0.51
		γ-globulins		
K	10.77 ± 2.24	15.42 ± 1.31	2.82 ± 0.64	4.45 ± 0.51
I	12.60 ± 2.18	14.88 ± 1.45	3.18 ± 0.41	4.20 ± 0.49
II	12.71 ± 1.59	14.82 ± 1.87	3.28 ± 0.77	4.27 ± 0.65

*Values expressed as X± SD
a,b,c Mean values within columns with unlike superscript letters are significantly different ($p<0.05$, Student's test)

The mean α-globulin concentrations showed an increasing trent that was obvious in all treated groups in both trials (Tables 2, 4), but wilthout statistical

significance. Similarly, the mean γ-globulin concentrations clearly showed an increasing trend, while only a slight variation could be seen in β-globulin concentrations which is a relatively stable fraction of total protein. Kubena et al. (1987) reported a significant decrease of total protein and globulins in blood sera of treated broilers, but in on other trial (Kubena et al., 1989b) a significant decrease of total protein and albummin was seen. Similar conclusions were made by Richard et al. (1978) and Huff et al. (1988) after using higher amounts of T-2 toxin. Sato et al.(1978) described a decrease of albumin and γ-globulin concentrations with an increase of β-globulin concentrations in blood sera of animals treated with single sublethal doses of T-2 toxin. On the other hand, Chi et al. (1977a; 1977b) considered that the blood serum protein status as not significantly changed in poultry treated with T-2 toxin.

Table 3. Concentration of total protein and A/G ratio in blood serum from broilers during the second trial

Group	Days of trial			
	21st	28th	35th	42nd
	Total proteins (g/l)			
K	26.17 ± 1.95	27.33 ± 1.79	29.67 + 2.49	28.83 ± 1.77
Ia	25.50 ± 1.50	–	–	28.17 ± 2.48
Ib	–	29.00 ± 1.90	–	27.75 ± 1.09
Ic	–	–	30.83 ± 3.44	29.60 ± 1.50
	A/G ratio			
K	1.58 ± 0.29	1.26 ± 0.04	1.17 ± 0.16	1.11 ± 0.07
Ia	1.38 ± 0.27	–	–	1.17 ± 0.06
Ib	–	1.17 ± 0.03	–	1.20 ± 0.11
Ic	–	–	1.11 ± 0.12	1.10 ± 0.09

*Values expressed as X+SD
a,b,c Mean values within columns with unlike superscript letters are significantly different ($p<0.05$, Student's test)

After the treatment period a mild insignificant increase of the total globulin concentration in the blood sera of the treated birds could be seen, so that after the resting period the values obtained were similar to those of control groups. An increasing trend of γ-globulin concentrations was reported earlier by Corrier et al. (1987). Corner and Ziprin (1986) and Ziprin et al. (1990). The authors investigated cell and humoral immunity modulation in animals treated by T-2 toxin and concluded that preinoculation treatment increased, while postinoculation treatment decreased immunity to L. monocytogenes. In addition the authors discussed about different mechanisms (reduction of immunosuppression, indirect or direct b-cell stimulation, and activated macrophages) which are involved in the increase of antibody synthesis in animals treated with T-2 toxin. No matter what the exact mechanism involved, it is obvious that the T-2 toxin was able to stimulate humoral and cellular function (Cooray, 1989; Gyon-

gyossy-Issa and Khachatourians, 1984) causing an increase of resistance to infection if it was given in microdosage for a short time (Corray, 1984).

The A/G ratio summarised all the results obtained and an obvious significant decrease of the ratio was noted (Tables 1, 3) when the dosage was higher and the treatment longer. Comparative analysis of the results from both trials led us to conclude that the lower amount of T-2 toxin given for a long period of time showed analogous effects as the higher amount given for a short period of time, which indicates a possible cumulative effect ot T-2 toxin, especially its effect on blood serum concentrations of proteins and their fractions, but, of course, with full care and attention to possibilities of variation influenced by physiological or any other known reason.

Table 4. Concentration of protein fractions in blood serum from broilers during the second trial.

Group	Unit	Protein fractions			
		Albumins	α-globulins	β-globulins	γ-globulins
			21st day		
K	%	60.62 ± 4.49	23.42 ± 2.63	4.97 ± 0.47	10.77 ± 2.24
	g/l	15.85 ± 1.58	6.14 ± 0.92	1.30 ± 0.20	2.82 ± 0.64
Ia	%	57.43 ± 4.77	24.63 ± 2.43	5.38 ± 1.03	12.60 ± 2.18
	g/l	14.70 ± 1.99	6.26 ± 0.51	1.36 ± 0.21	3.18 ± 0.41
			28th day		
K	%	55.62 ± 1.96	27.25 ± 1.24	5.15 ± 0.43	11.82 ± 1.07
	g/l	15.18 ± 0.76	7.45 ± 0.71	1.41 ± 0.19	3.24 ± 0.44
Ib	%	53.88 ± 0.69	27.98 ± 0.97	5.16 ± 0.33	12.98 ± 1.01
	g/l	15.62 ± 1.02	8.12 ± 0.66	1.49 ± 0.13	3.76 ± 0.37
			35th day		
K	%	53.83 ± 3.75	24.98 ± 2.22	4.77 ± 6.34	16.68 ± 1.98
	g/l	15.91 ± 1.08	7.42 ± 0.97	1.42 ± 0.23	4.99 ± 1.00
Ic	%	52.47 ± 2.59	27.37 ± 2.45	4.45 ± 0.43	15.72 ± 2.32
	g/l	16.15 ± 1.70	8.40 ± 0.88	1.38 ± 0.24	4.90 ± 1.19
			42nd day		
K	%	52.67 ± 1.66	24.92 ± 1.51	7.00 ± 1.95	15.42 ± 1.31
	g/l	15.20 ± 1.22	7.17 ± 0.48	2.01 ± 0.54	4.45 ± 0.51
Ia	%	53.83 ± 1.29	25.37 ± 0.85	5.88 ± 1.21	14.88 ± 1.45
	g/l	15.17 ± 1.48	7.14 ± 0.67	1.66 ± 0.36	4.20 ± 0.49
Ib	%	54.50 ± 2.24	25.00 ± 0.54	6.80 ± 1.68	13.70 ± 1.67
	g/l	15.12 ± 0.84	6.93 ± 0.16	1.89 ± 0.49	3.80 ± 0.53
Ic	%	52.36 ± 2.00	26.00 ± 1.05	6.92 ± 1.20	14.72 ± 1.43
	g/l	15.49 ± 0.83	7.70 ± 0.58	2.06 ± 0.41	4.35 ± 0.44

*Values expressed as X+SD
a,b,c Mean values within columns with unlike superscript letters are significantly different ($p<0.05$, Student's test)

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STATUS PROTEINA KRVNOG SERUMA BROJLERA TRETIRANIH T-2 TOKSINOM

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SADRŽAJ

U cilju ispitivanja uticaja T-2 toksina na koncentraciju proteina u krvnom serumu izvršena su dva ogleda na jednodnevnim Hybro brojlerima. U prvom eksperimentu ogledne grupe su u toku sedam dana tretirane sa po 0.02 i 0.20 mmg T-2/kg TM. U drugom eksperimentu ogledne grupe su svakodnevno tretirane T-2 toksinom u količini od 0.02/kg TM tokom 7,14 ili 21 dan. U oba ogleda uzorci krvi su uzimani nakon tretmana, a preostali brojleri su normalno hranjeni i pojeni bez aplikacije toksina do 42 dana kada su uzorci krvi ponovo uzeti.

Uočeno je negativno nesignifikantno dejstvo korišćenih količina T-2 toksina na koncentraciju ukupnih proteina uz rast alfa i gama globulina na račun albumina uz relativno konstantnu koncentraciju beta globulina sa posledično signifikantno užim A/G odnosom. Komparativnom analizom rezultata oba ogleda može se zaključiti da niske doze date u dužem vremenskom periodu imaju analogne efekte kao visoke doze date u kraćem vremenskom periodu, što ukazuje na moguće kumulativno dejstvo T-2 toksina.