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BLOOD SERUM PROTEIN STATUS OF BROILERS EXPOSED TO PROLONGED TREATMENT WITH A LOW DOSE OF OCHRATOXIN A

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The 42 day long trial was performed on Hybro broilers divided into four groups. After a 14 day preexperimental period, the groups were offered feed contaminated by 0.5 ppm ochratoxin A (OA) for 0, 7, 14 or 21 days. Blood samples were taken after the period of toxin addition and the remaining birds from the control and experimental groups were normally fed with mash without added OA until the 42nd day of the trial, when blood samples were taken again.

The total level of serum proteins was not changed during of prolonged treatment with OA, but a significant increase of albumin together with a decrease of the γ-globulin fraction were noted. A/G ratio suggested that globulins were the dominant protein fraction in blood serum samples obtained from all broilers included in this trail. The concentrations of α and β globulins in serum were within the physiological limits. It could be concluded that the low dietary OA level (0.5 ppm) had a possible cumulative, but not acute effect on blood serum protein status in broilers, dependent on the duration of exposure.

Key words: poultry, ochratoxin A, blood serum proteins

INTRODUCTION

Ochratoxins are highly toxic compounds commonly produced as secondary metabolites by two species of fungi: *Penicillium verrucosum Dierckx* and *Aspergillus ochraceus Wilhelm* (Frisvad and Samson, 1991). The toxin is represented by three similar compounds of which ochratoxin A ($C_{20}H_{18}O_6NCl$) is the most toxic and abundant. In recent years ochratoxin A (OA) has received considerable attention because it does not only seriously affect animal performance and health, but it may also have deleterious effects on humans. Of greatest concern in humans (Marquardt and Frohlich, 1992) is its implicated role in an irreversible and fatal kidney disease (Balkan endemic nephropathy).

Ochratoxin A is a natural contaminant of poultry and livestock feedstuffs. Field outbreaks of ochratoxicosis have been documented (Hamilton et al., 1982), and experimental feeding of OA has been shown (Kubena et al., 1988) to have detrimental effects on growing chicks, indicating that OA is a potential hazard to poultry producers. The diagnosis of ochratoxicosis may be difficult. In the absence of specific, well-defined clinical signs (Humphreys, 1988), the most frequent effects usually are poor condition, performance or production. Ochratoxin A exposure in poultry can cause a depression in body weight (Kubena et al., 1984), decreased feed consumption and egg production (Prior and Sisodia, 1978), listlessness, huddling, occasional diarrhoea, ataxia, prostration and death (Prior, Sisodia and O`Neil, 1976), together with atrophy and degeneration of the tubules and interstitial fibrosis in the kidneys (Elling et al., 1975). The biochemical effects of ochratoxin in poultry, among others, include decrease in serum total protein concentration (Prior and Sisodia, 1978), as a major clinical observation.

Many authors (Kubena et al., 1981, 1985; Bailey et al., 1990) have described the negative effect of high dietary OA level (2.5-4.0 ppm) on protein status, protein fractions and A/G ratio in the blood serum of broilers. On the other hand there are limited data about dietary OA levels below 2 ppm or less which are most often detected in feedstufs and feed for animals (Šefer et al., 1998).

The effects of 2 ppm dietary OA on the blood serum protein concentration were described by Kubena et al., (1994). When compared with controls, serum total protein and albumin concentrations, as well as A/G ratio were significantly reduced. These data are consistent with several earlier reports. Thus, Kubena et al., (1989) described a significant decrease of blood serum total protein and albumin, as well as A/G ratio in broilers treated with 2 ppm OA during three weeks. Singh et al. (1990) fed broilers with 0.5 or 2.0 ppm dietary OA during three weeks. Significantly decreased blood serum total protein, albumin and globulin levels were confirmed only after treatment with the higher dose of OA. Similaris, Huff and Ruff (1981) reported a significant decrease of blood plasma total protein only after treatment with OA levels above 2.0 ppm, while lower levels (0.5 or 1.0 ppm) had no significant effects on blood protein concentration.

Ayed et al. (1991) examined the influence of a low dietary OA level (0.5 ppm) on blood protein status. Broilers were fed the experimental diets for 4 weeks after which time the rations were withdrawn and replaced by control finisher diets for a 3 week recovery period. The concentration of total serum protein was significantly decreased in the treated group, but after the recovery period there were no significant differences in total serum protein. This is not in agreement with the results obtained by Chang et al. (1981), who examined the effects of graded levels of dietary OA up to 8 ppm and claimed that total plasma proteins were not altered by any treatment.

Although there has been extensive literature published on changes of blood serum proteins caused by OA, the effects is still poorly understood and not fully explained yet. The present study was, therefore, designed to assess the effect of ment similar Chang et al. (1981). On the other hand, possible association with the inhibition of protein biosynthesis by OA may be related to the changes of protein fractions.

Table 2 Relative blood serum concentrations of albumins, α-, β- andγ-globulins % in control and ochratoxin treated broilers

Group	n	% of Total proteins			
		Albumins	α–globulins	b-globulins	γ-globulins
			21 st day		
K	6	39.11±3.23	11.48±2.96	11.37±2.15	38.04±4.57
A	6	36.89±3.99	13.03±1.59	11.63±2.25	38.45±5.97
			28 th day		
K	6	44.64±1.96^{a}	12.32±1.16^{x}	9.58±1.37	33.46±3.05^{x}
B	6	41.68±1.09^{b}	10.20±0.97^{y}	8.57±0.59	39.47±1.48^{y}
			35 th day		
K	6	43.27±3.51	11.68±1.06^{x}	11.95±1.02^{a}	32.60±2.39
C	6	43.82±2.39	9.39±1.20^{y}	14.26±1.98^{b}	32.53±1.52
			42 nd day		
K	6	33.61±8.02$^{a\,x}$	13.18±1.38^{x}	15.02±2.85^{a}	38.18±6.30$^{a\,x}$
A	6	48.11±3.21^{y}	7.49±1.80$^{a\,y}$	14.16±1.11	30.23±2.69^{b}
B	6	43.17±6.39^{b}	11.63±4.29^{b}	16.04±1.68^{x}	29.16±4.30^{y}
C	6	43.56±3.42^{y}	12.01±0.93^{b}	11.37±2.55$^{b\,y}$	33.05±2.57

*Values expressed as X±Sd
$^{a\,b\,c}$ Mean values within columns with unlike superscript letters were significantly different (p<0.055 LSD test)
$^{x\,y\,z}$ Mean values within columns with unlike superscript letters were significantly different (p<0.01 LSD test)

The serum albumin concentration (Table 1-3) was lower than the globulin concentration and comparing the obtained results with physiologically established values (Pavlović et al., 1978; Stevanović et al., 1990), it can be seen that they range around and under the higher limit. This is in almost complete agreement with the findings of Kubena et al. (1985, 1994) and Bailey et al. (1990), specifying 11.3–14.2 g/l as normal albuminemia. The same authors stated that occurrence of low levels of OA in the diets during a short period of time led to a decrease of serum albumin while the results obtained in this trial are the reverse. In the blood serum of the treated birds an increase of albumin concentration was seen, proportional to the duration of intoxication. Increase of plasma albumin concentration is probably connected with the high affinity of OA for plasma proteins. It could be an essential factor influencing pharmacokinetics and toxicity of OA (Kumagai, 1985; Uchiyama i Saito, 1987), but the relation between high affinity of OA for plasma proteins and toxicity is not fully explained yet (Hagelberg et al. 1989). It was concluded that one of the primary reasons for albumin binding of OA is to retard its elimination by limiting the transfer of OA from the bloodstream

Table 1. Blood serum, concentration of total protein, albumin and globulin *[g/l]* and A/G ratio in control and ochratoxin A treated broilers

Group	n	Concentrations, g/l			A/G
		Totalproteins	Albumins	Globulins	Ratio
			21 st day		
K	6	33.79±1.54	13.19±0.85	20.60±1.79	0.65±0.09
A	6	32.96±2.32	12.19±1.78	20.77±1.54	0.59±0.10
			28 th day		
K	6	31.80±2.76	14.18±1.19	17.62±1.82^{a}	0.80±0.06^{x}
B	6	35.06±2.70	14.60±0.92	20.46±1.84^{b}	0.71±0.03^{y}
			35 th day		
K	6	36.44±1.45	15.94±0.89	20.50±1.39	0.78±0.03
C	6	39.30±4.51	17.28±2.42	22.05±2.41	0.78±0.03
			42 nd day		
K	6	35.12±2.40	11.92±3.47^{x}	23.21±2.21	0.53±0.21^{a,x}
A	6	36.85±1.12	17.71±0.83^{a,y}	19.14±1.66	0.93±0.12^{y}
B	6	36.41±3.58	14.37±1.46^{b}	20.71±3.22	0.78±0.21^{b}
C	6	35.61±2.01	15.49±1.16^{y}	20.12±1.96	0.78±0.10^{b}

* Values expressed as $\overline{X}\pm Sd$
a, b, c Mean values within columns with unlike superscript letters were significantly different ($p<0.05$, LSD test)
x, y, z Mean values within columns with unlike superscript letters were significantly different ($p<0.01$, LSD test)

In the blood serum of the experimental group mild differences possibly influenced by OA treatment were seen, but without statistical significance. These findings did not confirm the results obtained by Kubena et al. (1985; 1989; 1994), who suggested that total blood serum protein concentration was significantly decreased after treatment with OA and Ayed et al. (1991) who claimed that 0.5 ppm dietary OA used through a period of 4 weeks significantly decreased total plasma protein concentration. On the other hand, our results confirm the findings of Huff and Ruff (1981) and Singh et al. (1990), who suggested that total blood serum protein concentration was not significantly decreased after treatment with lower level of dietary OA. Our results may be explained by the lower levels of dietary OA, as well as the shorter duration of intoxication than in the other studies. The target organ in animals is the kidney with resulting failure to reabsorb water and consequent increase of water consumption. Due to the degree of kidney damage, water imbalance affects concentrations of blood constituents. OA has been reported to cause a dehydration-related increase of serum protein concentrations in pigs (Szczech et al., 1973), while decrease of serum proteins without changes of A/G ratio may be connected with blood dilution. Thus, it could be concluded that low levels of OA (0.5 ppm) administered during one to three weeks does not have negative effects on total blood serum proteins, which is in agree-

The concentrations of α- β- and -globulin in blood serum of broilers found in the current study (Table 2-3) are more or less in accordance with literature data (Stevanović et al. 1990; Šefer 1993; Singh et al.1994) Although marked diversity of the globulin fractions was affirmed a decrease of γ-globulin in favor of γ-globulin was not observed. Relative concentrations of α-and β-globulin showed slight variation with almost constant absolute concentration. These data confirmed that treatment with OA did not have any influence on these two globulin fractions However feeding the broilers with OA contaminated feed showed negative effects on γ-globulin concentration both in relative and absolute values especially when administered for a prolonged time.

Increased absolute and relative concentrations of γ-globulins in the blood sera of broilers ($p<0.01$) treated for a shorter period and decreased absolute and relative concentrations of γ-globulins after prolonged treatment may confirm the immunosuppressive effects of OA previously reported by Lea et al. (1989) Although there is no evidence to show that OA inhibits immunoglobulin synthesis particularly IgM (Singh et al. 1990) OA might have decreased the synthesis of immunoglobulin in a similar manner as aflatoxins. No what the exact mechanism is involved it is obvious that OA is able to express immunomodulatory effects earlier described by Hong et al. (1988) and later confirmed by (Singh et al. 1990).

The described differences in concentration and relations between protein fractions could be summarized in the albumiglobulin ratio (A/G) Use of contaminated food for a shortr period of time resulted in a decreased A/G ratio ie increase of globulin on account of albumin concentration with relatively constant concentration of total proteins Prolonged use of contaminated food increased AG ratio due to increase of albumin and decrease of γ-globulin The toxicity of ochratoxin A is based on more than one direct and several indirect effects The primary effect is probably related with impaired protein synthesis due to competitive inhibition of the enzymes involved in phenylalanine metabolism (phenylalanine tRNA synthetase) as a prerequisite for protein synthesis (Meisner and Meisner 1981). Food contaminated with OA exerts negative effects on -globulin concentration both in relative and absolute values especially when administered for a long time. On the other hand duration of treatment with OA was not long enough to decrease total protein synthesis.

Thus the total level of serum proteins was not changed under the influence of prolonged treatment with OA. As significant increase of albumin together with a decrease of the γ-globulin fraction was established The A/G ratio suggested that globulins were the dominant protein fraction and the concentration of α- and β-globulins in serum were within the physiological limits for broilers. It could be concluded that the low level of ochratoxin A (5 ppm) had a possible cumulative but not acute effect on blood serum protein status in broilers. Under naturall circumstances in the field the usuall dietary OA level between (0,2-1) ppm. Therefore changes in total serum protein as well as changes of protein fractions and AG ratio cannot be a diagnostic tool for ochratoxicosis in broilers.

to the hepatic and renal cells. Increased concentration of plasma albumin could be interpreted as a homeostatic mechanism. With the aim of keeping OA in the blood, the liver mayhave increased albumin production which minimized the tissue damage in target organs. Moreover, increasing the albumin concentration, as part of the transport mechanism of nutrients, could be assumed to bea homeostatic mechanism by which the organism is trying to secure enough carriers for optimal transport of essential nutrients.

Observing the concentration of total globulin and globulin fractions in blood serum of chickens the opposite relation could be seen. According to literature data (Stevanović et al 1990; Pavlović et al 1978; Bailey et al 1990; Kubena et al 1985. 1994) globulin concentratiwn prevailed over albumin concentration in blood serum Considering the influence of dietary OA it could be concluded that low amounts of toxin for a short period of time led to the increased concentration of globulin while its consumption during a longer period of time resulted in decreased globulin concentration. Decrease of globulin concentration and increase of albumin concentration were the consequence of protracted intoxication which is contrary to the literature data (Singh et al. 1990. Kubena et al. 1985; 1989; 1994) describing a simultaneous decrease of total protein as well as globulin concentration when higher doses of OA were used.

Table 3. Blood serum concentration of albumins, α-, β- and γ-globulins g/l in control and ochratoxin treated broilers.

Group	n	Protein fractions			
		Albumins	α-globulins	b-globulins	γ-globulins
			21 st day		
K	6	13.19±0.85	3.88±1.00	3.84±0.76	12.87±1.88
A	6	12.19±1.78	4.29±0.64	3.81±0.65	12.66±2.05
			28 th day		
K	6	14.18±1.19	3.93±0.62	3.06±0.62	10.63±1.23^{x}
B	6	14.60±0.92	3.53±0.44	3.00±0.23	13.86±1.47^{y}
			35 th day		
K	6	15.94±0.89	4.25±0.36	4.36±0.46^{a}	11.89±1.15
C	6	17.28±2.42	3.68±0.52	5.57±0.75^{b}	12.80±1.87
			42 nd day		
K	6	11.92±3.47^{x}	4.62±0.49^{x}	5.26±0.98^{a}	13.33±1.84^{a}
A	6	17.71±0.83^{a,y}	2.77±0.72^{a,y}	5.21±0.35	11.15±1.26
B	6	14.37±1.46^{b}	4.18±1.40^{b}	5.60±0.63x	10.70±2.35^{b}
C	6	15.49±1.16^{y}	4.26±0.76^{b}	4.08±1.07^{b,y}	11.77±1.18

*Values expressed as $\overline{X}$±Sd
^{a,b,c} Mean values within columns with unlike superscript letters were significantly different (p.<0.05, LSD test)
^{x,y,z} Mean values within columns with unlike superscript letters were significantly different (p.<0.01, LSD test)

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UTICAJ PRODUŽENOG TRETIRANJA OHRATOKSINOM A NA STATUS PROTEINA KRVNOG SERUMA BROJLERA

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SADRŽAJ

Ogled je izveden na Hybro brojlerima podeljenim u četiri grupe a trajao je 42 dana. Nakon četrnaestodnevnog pripremnog perioda ogledne grupe su hranjene hranom kontaminiranom ohratoksinom A u količini od 5 ppm u toku 7, 14 ili 21 dan. Uzorci krvi za ispitivanje uzimani su nakon perioda ishrane kontaminiranom hranom a preostale jedinke hranjene su hranom bez dodatog toksina do kraja ogleda. Na kraju ogleda uzeti su uzorci krvi za ispitivanje od svih grupa.

Korišćena količina OA u ispitivanom vremenu ekspozicije nema uticaj na koncentraciju ukupnih proteina. Uočeno je signifikantno povećanje albumina zajedno sa smanjenjem γ-globulina. Odnos AG ukazuje da su globulini dominantna frakcija proteina u svim ispitivanim uzorcima. Koncentracija α i β globulina u krvnom serumu kretala se u okvirima fizioloških granica. Može se zaključiti da prisustvo malih količina OA u hrani (5 ppm) poseduje moguće kumulativne ali ne i akutne efekte na proteine krvnog seruma brojlera koji zavise od vremena ekspozicije štetnom dejstvu.

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