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Biochemical Changes in Pig Serum After Ochratoxin A Exposure

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Abstract The objective of this study was to investigate the effect of ochratoxin A (OTA) on serum biochemical parameters of pigs during subchronic treatment with 300 μg OTA/kg of feed for 30 days. OTA treatment resulted in significantly higher ($p < 0.05$) serum levels of creatinine, urea, potassium and alkaline phosphatase, and significantly lower levels of glucose and total protein. These changes in serum biochemical parameters in treated pigs were indicative of impaired liver and kidney function caused by OTA exposure.

Keywords Ochratoxin A · Pig serum · Biochemical parameters · Subchronic treatment

Ochratoxins, of which ochratoxin A (OTA) is the most prevalent, are secondary fungal metabolites of some toxigenic species of *Aspergillus* and *Penicillium* that can be found in various feed ingredients (Höhler et al. 1999). Several studies have shown that OTA is nephrotoxic, causing both acute and chronic lesions of kidneys, and that it is hepatotoxic, carcinogenic, teratogenic and immunotoxic to several animal species (Stormer and Lea 1995; Pfohl-Leszkowicz and Manderville 2007; Denli and Perez 2010). Immunosuppression occurs with low concentrations of OTA, while high concentrations lead to kidney toxicity. The mechanism of action of OTA until has not yet been fully clarified but it is known that OTA poses a risk for human and animal health when ingested through contaminated food or feed. The International Agency for Research on Cancer (1993) has classified OTA in group 2B as a possible carcinogen to humans.

In animals, long-term exposure to OTA typically results in increased mortality, poor feed conversion, poor growth rates and feed refusal (Marquardt and Frohlich 1992; Denli and Perez 2010). In pigs that consumed OTA (25 μg/kg of feed), daily body weight gain and final body weight were decreased (Malagutti et al. 2005). Among farmed animals, pigs are particularly sensitive to OTA, while ruminants are less sensitive to OTA because they are able to biotransform the toxin in the rumen (Hult et al. 1976). In pigs, OTA is mainly distributed to the kidneys, with lower concentrations in the liver, muscle and fat, and its disappearance from blood is slower than from tissues (Walker and Larsen 2005).

Studies demonstrate that highest amounts of OTA were found in the blood (Gareis and Scheuer 2000). Serum half-life of this toxin in pigs is in the range of 72–120 h (Galtier et al. 1981). The slow elimination of OTA due to its high binding affinity to serum proteins such as albumin and smaller molecular weight fractions (Walker and Larsen 2005) and enterohepatic recirculation substantially contribute to the development of its chronic effects (Fuchs and Hult 1992; Dietrich et al. 2005). OTA was found to be immunosuppressive in humans and carcinogenic and teratogenic in laboratory animals (Lioi et al. 2004). Also, Kane et al. (1986) have shown that the DNA lesions induced by OTA in vivo were no longer repaired in case of repeated exposure. These effects manifest especially in a dose-dependent and dose-time related fashion (Marquardt and Frohlich 1992). Earlier investigations showed changes in

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blood biochemical parameters in spontaneous cases of ochratoxicosis in pigs (Stoev et al. 1997, 2001).

OTA has been primarily recognized as a nephrotoxic mycotoxin that induces significant changes in serum parameters after ingestion in different animal species (Elaroussi et al. 2008; Kumar et al. 2007). In basic toxicology research and in preclinical toxicity testing, liver damage is usually evaluated by serum biochemical parameters prior to confirmation by histopathology. Spontaneous cases of mycotoxic nephropathy (ochratoxicosis) in pigs revealed increased serum levels of potassium, sodium, aspartate-aminotransferase, glutamate dehydrogenase, creatinine and urea (Stoev et al. 1997). The increase in the levels of creatinine and urea is also related to nephrotoxicity (Mir and Dwivedi 2010). Liver function tests that can detect loss of liver synthetic capacity include glucose, albumin and urea nitrogen. Alterations in these blood parameters usually require significant loss of liver function (>70 %–80 %) since liver has a tremendous reserve capacity for the synthesis of these analytes (Meyer and Harvey 2004). Investigations in various animal species showed changes in the values of blood parameters to be observed mostly within 1–2 months of OTA exposure, and to depend on the dose applied and length of treatment (Marquardt and Frohlich 1992; Mir and Dwivedi 2010).

The present study was undertaken to investigate the effect of OTA on serum biochemical changes in pigs, caused by 30-day subchronic treatment with the most frequent natural level of contamination.

Materials and Methods

The experiment was carried out in 8 male pigs, Zegers hybrid type, aged 90 days, body mass 70 kg, farm-bred, and kept under the same zoohygienic conditions. Animals were divided into two groups: 4 pigs were treated with OTA and 4 pigs were left untreated and served as control animals. Ochratoxin A was obtained from Acros Organics (Geel, Belgium). The experimental group was treated with feed contaminated with 300 μg OTA/kg (300 ppb). OTA as a pure chemical (standard) was mixed with lactose and distributed in gelatin capsules that were applied directly into pig mouth. Experimental animals were treated with OTA for 30 days. In all study animals, blood samples for biochemical studies were obtained from cranial vena cava using Venoject® vacutainer test tube. Blood samples were taken on day 1, 10, 20 and 30 of treatment. Upon sampling, they were gently shaken and left to stay for several hours at room temperature. Serum was separated after centrifugation at 2,000 rpm for 5 min at room temperature and stored at −20°C until analysis of biochemical parameters. The experimental protocol was designed in accordance with current regulations and standards issued by the Ministry of Agriculture, Fishery and Rural Development. We used a relatively small number of animals to prevent animal suffering due to OTA toxicity and cost of the experiment. The levels of serum biochemical parameters in pig serum were determined by an autoanalyzer (Abaxis, Vetscan, USA). The data were presented as mean (±SD) of 4 representative values.

Statistical analyses were performed using Statistica Ver. 7 software (StatSoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) was performed to determine the significance among various days of treatment while significance between two groups were determined by students' t test. A p value of <0.05 was considered significant.

Results and Discussion

Certain biochemical changes were recorded in pig serum during the experiment. Experimental group showed statistically significant changes in the levels of glucose, creatinine, urea and total protein (Table 1). There were no statistically significant differences between groups in albumin and globulin concentrations.

Table 1 Mean values ± SD of serum parameters of control and OTA treated pigs

Parameter	Control group	Treated group			
		Day 1	Day 10	Day 20	Day 30
Glucose (mmol/L)	5.15 ± 0.19	5.21 ± 0.22	4.65 ± 0.14*	4.70 ± 0.21*	4.53 ± 0.15*
Creatinine (μmol/L)	110.6 ± 8.33	115.7 ± 9.86	121.3 ± 15.90	118.0 ± 13.33	142.3 ± 16.80*
Urea (mmol/L)	4.10 ± 0.08	4.19 ± 0.16	4.45 ± 0.07*	5.10 ± 0.14*	4.70 ± 0.28*
Total protein (g/L)	70.3 ± 1.41	69.4 ± 3.11	67.7 ± 4.16	70.0 ± 2.08	58.5 ± 9.19*
Albumin (g/L)	40.0 ± 1.15	38.7 ± 3.09	36.3 ± 6.60	36.0 ± 4.36	34.3 ± 0.58
Globulin (g/L)	36.8 ± 4.5	37.2 ± 5.04	33.0 ± 4.97	31.33 ± 5.51	29.0 ± 6.83
Bilirubin (μmol/L)	3.25 ± 0.5	3.30 ± 0.20	3.0 ± 0.0	2.5 ± 0.58	3.25 ± 0.5

* Significantly different ($p < 0.05$) between experimental and control group

Table 2 Mean values ± SD of serum enzyme activities of control and OTA treated pigs

Parameter	Control group	Treated group			
		Day 1	Day 10	Day 20	Day 30
Alkaline phosphatase (U/L)	123.50 ± 2.65	125.3 ± 5.56	140.7 ± 17.95	159.3 ± 8.62*	179.3 ± 10.69*
Alanine aminotransferase (U/L)	45.5 ± 1.15	47.3 ± 2.18	42.2 ± 2.82	47.3 ± 9.90	50.5 ± 9.76
Alfa-amylase (U/L)	1,239 ± 11	1,252 ± 45	1,208 ± 132	1,193 ± 187	1,273 ± 259

* Significantly different ($p < 0.05$) between experimental and control group

Table 3 Mean values ± SD of macroelements in serum of control and OTA treated pigs

Parameter	Control group	Treated group			
		Day 1	Day 10	Day 20	Day 30
Sodium (mmol/L)	135.6 ± 0.82	131.2 ± 5.16	137.5 ± 4.12	138.3 ± 3.21	139.5 ± 4.95
Potassium (mmol/L)	6.80 ± 0.11	6.61 ± 0.19	7.03 ± 0.14	8.45 ± 0.14*	7.20 ± 0.21
Calcium (mmol/L)	2.59 ± 0.05	2.52 ± 0.11	2.64 ± 0.24	2.55 ± 0.11	2.63 ± 0.45
Phosphorus (mmol/L)	2.75 ± 0.04	2.80 ± 0.18	2.92 ± 0.28	3.05 ± 0.20	2.92 ± 0.26

* Significantly different ($p < 0.05$) between experimental and control group

Although the values of ALP in both experimental and control group were within the physiological limits, significantly higher values were observed in experimental group on days 20 and 30 (Table 2).

The concentration of ALT was slightly higher on the same days of experiment, however, the difference did not reach statistical significance.

The minerals assessed in our experiment were within the physiological range and only potassium concentration showed significant changes in experimental pigs (Table 3).

In Croatia, natural OTA concentrations in feed have been reported to vary greatly in the last decades, being significantly higher in rainy years (Domijan et al. 2005; Pepeljnjak et al. 2008). In our study, we investigated changes in serum biochemical parameters in samples obtained from pigs that received 300 μg OTA/kg of feed for 30 days. This concentration was chosen because previous studies revealed a similar OTA concentration in feed to be quite frequently recorded in our region (Pepeljnjak et al. 2008).

The results of our study pointed to a significant decrease ($p < 0.05$) in serum glucose levels on days 10, 20 and 30 of treatment compared to control group. A statistically significant decrease in glucose concentrations observed on day 10 of treatment pointed to early kidney damage in experimental pigs. While OTA in kidney mainly impairs proximal tubular functions and couses glucosuria (Anzai et al. 2010), and mobilization of glucose from liver and muscle is reduced because OTA inhibits the protein kinase (Pohland et al. 1992) as a consequence and the low serum glucose levels are expected. Earlier studies showed that ochratoxicosis induced hypoglycemia in pigs (Stoev et al. 1997) and other species (Purchase and Theron 1968; Raina et al. 1991). Schaefer and Hamilton (1986) report that OTA feeding caused accumulation of glycogen in the muscle and liver, and that the resulting stores of glycogen were not readily hydrolyzed to glucose. Decrease in serum glucose levels may be attributed to either decreased absorption due to damage to alimentary mucosa and diarrhea or liver damage, leading to disturbed carbohydrate metabolism or nephrosis causing extensive loss of glucose as well as interference with resorption by damaged proximal convoluted tubules (Mir and Dwivedi 2010). Some contradictory results point to the increased level of serum glucose, which was explained by compensatory hyperglycemia in later stages of mycotoxic porcine nephropathy, while hypoglycemia in some of the early stages is most likely a consequence of impaired reabsorption of glucose in proximal tubules.

Studies have shown that serum creatinine is critical indicator of impaired kidney function and an increase in the levels of creatinine and urea is reflective of nephrotoxicity (Mir and Dwivedi 2010; Stoev et al. 2011). In our study, creatinine level increased throughout the treatment and was significantly increased ($p < 0.05$) on day 30 in comparison to the control group. Exposure to OTA also resulted in significantly increased concentrations of urea on all days of measurement. Urea and creatinine, which depend on glomerular filtration for their excretion, accumulate almost in proportion to the number of nephrons that have been destroyed, and hence directly reflect the functional status of the kidneys. Hepatic insufficiency can also result in a reduced serum urea nitrogen and raised plasma ammonia, since the urea cycle in the liver is the major pathway for

conversion of intestine-derived ammonia to urea nitrogen (Ramaiah 2007). The increased urea concentrations can be attributed to amino acid catabolism, which occurs due to the low glucose concentration and consequently higher levels of GDP and ADP that activates enzymes in the amino acid degradation pathway (Strayer 1981).

Serum urea, creatinine and glucose levels observed in our study were similar to the results obtained with 0.5 mg OTA/kg of feed, reported by Stoev et al. (2011), and those obtained with 2.5 mg OTA/kg of feed as reported by Raja et al. (2008). Contamination with 32.2 μg OTA/kg of feed did not cause increase in urea and creatinine levels in gilts after 14-day feeding (Jarczyk et al. 2008). The observed differences may be related to the high activity of creatine kinase in serum obtained in a previous study where pigs received a similar dose of OTA in feed (Jarczyk et al. 1998).

OTA intoxication also resulted in a significantly reduced ($p < 0.05$) total serum protein level on day 30 of treatment and nonsignificant reduction of albumin levels. Our study results are comparable with earlier investigations of OTA intoxication in different animal species, which also reported decreased serum protein levels (Raina et al. 1991; Stoev et al. 1997, 2011; Kumar et al. 2007; Elaroussi et al. 2008) and decreased albumin levels (Lippold et al. 1992; Mir and Dwivedi 2010). The decrease in total protein could be explained by the fact that OTA produces hepatotoxicity and due to hepatotoxicity, the decrease in secreted protein from liver occurs. The reduced serum protein level may also be due to nephrotoxicity related proteinuria. In addition, reduction in serum albumin level could be due to inflammation which may be related to type I acute phase response (APR I). In APR I, the level of serum albumins is decrease (Ramaiah 2007).

OTA treatment resulted in a significantly higher ($p < 0.05$) level of potassium on day 20. During the experiment, sodium levels were non-significantly increased in the experimental group, which is in accordance with the results obtained with 32.2 ng OTA/kg of feed (Jarczyk et al. 2008).

OTA treatment had no statistically significant effect ($p > 0.05$) on calcium and phosphorus levels in pig serum. Our results are consistent with those reported by Jarczyk et al. (2008). However, literature data show that OTA may induce hypercalcemia in pigs, attributing it to the balance of phosphorus in blood serum and the action of parathyroid hormone (Finco 1980). In our study, the mean concentration of calcium was low in both experimental and control group of pigs, but it also was in the physiological range and so was the concentration of phosphorus. Harvey et al. (1989) report that 2 mg of OTA/kg of feed caused calcium decrease after 28-day exposure. These results can be explained by differences in breed susceptibility, age of pigs and higher dose of OTA in comparison to our experiment.

Bilirubin, which is usually used in liver toxicity studies, is a parameter that defines the ability of liver of excretion of endogenous compounds, biochemical uptake and detoxification.

In our study, there was no significant difference between groups ($p > 0.05$) in the levels of bilirubin, which could be explained due to the short animal exposure to OTA.

Reduced serum ALT activity below the reference range is occasionally observed in toxicology studies, making it insensitive parameter of liver damage (Ramaiah 2007). ALP is a nonspecific enzyme and its serum level is indicative of liver damage (Ramaiah 2007). The concentration of OTA used in our study caused no changes in ALT activity. However, higher OTA concentration (0.5 mg OTA/kg of feed) caused a statistically significant increase of ALT 35 and 49 days after exposure (Stoev et al. 2011). Although the values of ALP were within the normal limits (92–294 U/L; Winnicka 2003), OTA treatment in our study resulted in significantly higher ($p < 0.05$) ALP levels on day 20 and 30 of treatment. OTA treatment had no effect on amylase activities and there were no significant differences between groups ($p > 0.05$) on this parameter.

Mir and Dwivedi (2010) reported on significantly higher levels of all biochemical parameters investigated as compared with our study, which could be explained by the higher OTA dose (1,000 and 2,000 μg OTA/kg of feed) and longer period of treatment (8 weeks).

In this study subchronic treatment of pigs with OTA induced an increase in serum levels of creatinine, urea, potassium and ALP, whereas the levels of glucose and total protein were decreased. On day 1 of treatment, the levels of biochemical parameters were similar to those measured in control group. However, changes of biochemical parameters indicating kidney damage (urea) and liver damage (ALP) were observed as early as 10 and 20 days of subchronic OTA dose administration, respectively.

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