

Natural exposure of Martina Franca jennies and their foals to Ochratoxin A

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Introduction

Ochratoxin A (OTA) is a worldwide occurring mycotoxin produced by *Penicillium* and *Aspergillus* fungi during unfavourable storage of cereals, with nephrotoxic, immunotoxic, teratogenic and reprotoxic activity. Monogastric animals have high sensitivity to OTA, and swine and horses are naturally exposed (Pozzo et al 2010; Minervini et al 2013). To date, no data are available on natural exposure of Martina Franca jennies and their foals to this mycotoxin during late pregnancy and after delivery.

Materials & Methods

Sampling of Feed & Blood

Feed and blood samples were collected from January to September 2018.

Feed samples (n=53) were collected every 15 days, two months before and three months after delivery of jennies according to the sampling procedures of the EU Regulation (CE) N. 152/2009.

Blood samples (n=101) from jennies followed the same time sampling, and the blood samples from foals were collected after delivery.

Determination of OTA in feed samples

Feed samples were homogenized and analysed by the AOAC Official method No. 2000.03 (Entwisle et al, 2000) for the determination of OTA in barley based on immunoaffinity column clean up of extracts and HPLC/FLD detection, with minor modifications.

Chromatographic conditions

HPLC	Agilent 1260 Series
Fluorescence Detector	Agilent 1260 Series
Wavelengths	λ_{ex} 333 - λ_{em} 460
Column	Zorbax SB-C18 5 μ m 4,6 x 150mm (Agilent)
Flow rate	1 mL/min
Mobile phase	H ₂ O:CH ₃ CN:CH ₃ COOH (99:99:2)
Injected amount	100 μ L

Determination of OTA in blood samples

OTA determination in blood samples (n= 101) was performed by ELISA (RIDASCREEN® Ochratoxin A) according to the protocol provided by the manufacturer (r-Biopharm AG, Darmstadt, Germany).

Results

Out of 53 tested feed samples, 68% showed OTA levels lower than the quantification limit, 26% had levels ranging from 0.3 to 0.7 ng/g and 6% ranging from 1.4 to 2.7 ng/g.

The analysis of each cereal component present in the feed showed similar low OTA levels (0.15-0.18 ng/g) in bran, corn flake and soy, whereas barley and oat resulted uncontaminated (detection limit of 0.1 ng/g). Toxin concentrations were far below the guidance values of OTA in feed materials reported by the EU Recommendation No. 2016/1319.

Ochratoxin A concentration in feed samples

Feed samples	Number of feed samples with OTA concentration		
	< 0.3	from 0.3 to 1	From 1 to 3
Contamination ranges (ng/g)			
N = 53	36	14	3
Incidence of contamination	68%	26%	6%
Range of OTA level (ng/g)	-	0.3-0.7	1.4-2.7

Limit of Quantification (LOQ): 0.3 ng/g

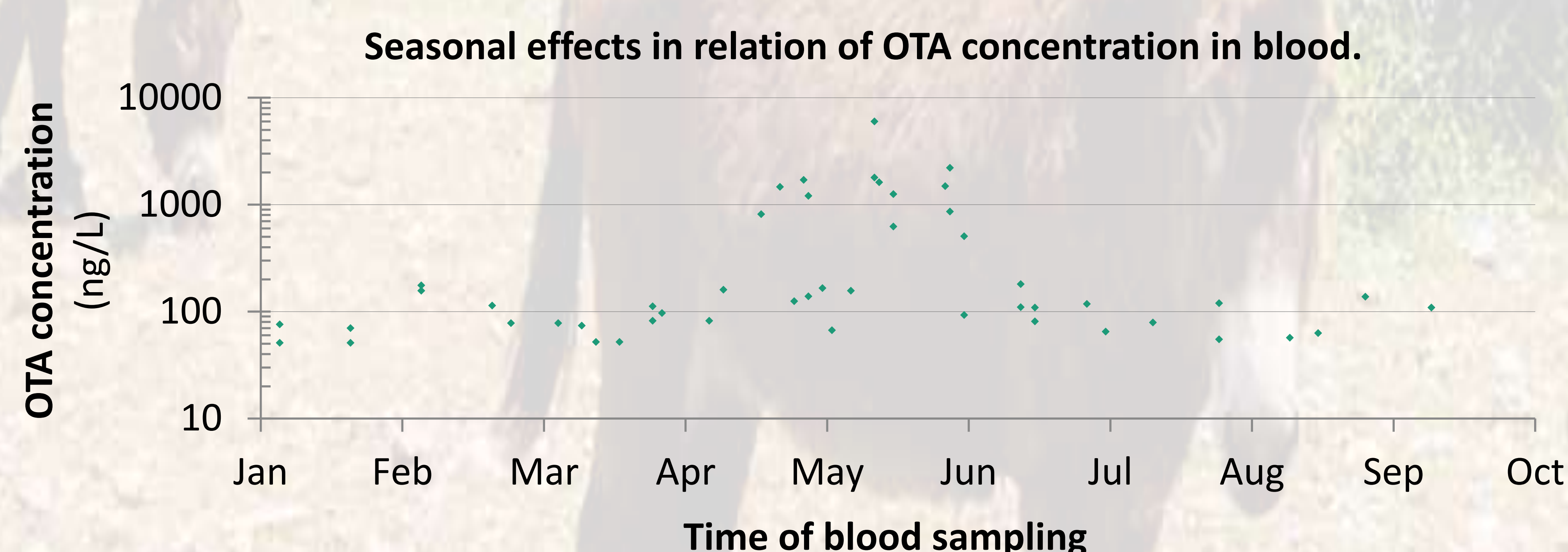
The OTA concentrations found in feed samples ranged from 0.3 to 2.7 ng/g and were below the guidance OTA values in feed materials (0.25 μ g/g) and feeds for pigs, poultry, cats and dogs (0.01-0.1 μ g/g) reported by Commission Recommendation (EU) 2016/1319.

In jennies, the OTA incidence rate of positive blood samples (with OTA levels higher than the detection limit) was 73%, with median value of 114 ng/L and range from 51 to 6,000 ng/L. No significant differences of OTA serum levels were found among jennies, but a season-effect on the levels of OTA in the blood was found with significant increases from 10 to 60 times in 75% of the positive samples collected from May to September, probably due to possible contamination of the hay. The median value and the incidence of OTA levels in blood samples collected from jennies were lower than those found by Minervini et al. (2013) in horses (121.4 ng/L and 83%, respectively).

Ochratoxin A concentration (ng/L) in blood samples collected from jennies

Jennies name (age)	Positive/total samples	Incidence (%)	Median (ng/L)	Range	
				Minimum	Maximum
Adelaide (11)	7/11	64	76	52	1796
Fransisca (6)	5/9	56	1489	97	6000
Gaia (5)	4/8	50	101	63	624
Etiopia (7)	10/11	91	132	74	1620
Antica (11)	8/9	89	98	51	1467
Falaria (6)	5/9	56	110	52	2215
Eriteria (7)	10/10	100	115	57	1255
Total 7	49/67	73	114	51	6000

Level of detection (LOD): 50 ng/L



Concerning foals, the number of positive blood samples were 17 with the incidence rate of 50%. Median value of OTA in positive samples was 134 ng/L, ranging from 79 to 4040 ng/L.

No placental transfer of OTA was observed in all tested jennies and no influence on pregnancy length and health of foals was observed. On the contrary, in horses the placental transfer was observed in 50% of neighbors and this disagreement should be linked to the different placental structure of the donkey and the mare.

References

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