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## Effects of Ochratoxin A on heavy pig production

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**Abstract** – Ochratoxin A (OTA) is a widespread mycotoxin mainly produced by *Aspergillus ochraceus* and *Penicillium verrucosum* which can affect cereals and which occurs in meat and meat products. It has been demonstrated that OTA is nephrotoxic, hepatotoxic and carcinogenic in all mammalian species; pigs and poultry are the species most exposed to OTA toxicity. The aim of this research was to evaluate the effects of OTA added to the growing pigs' diet, with regards to growing parameters and the amount of OTA present in animal meat. Sixty-four pigs, divided into 2 homogeneous groups, were reared for 119 days, from live weight of 41 kg to slaughtering. The control group was fed a commercial pig feed and the treated group was given the same feed with the addition of 25  $\mu\text{g}\cdot\text{kg}^{-1}$  of OTA. No significant differences were observed regarding growing parameters after 62 days but at the end of the trial, OTA supplementation decreased final live body weight (163.4 kg vs. 170.9 kg,  $P<0.05$ ), average daily gain (1030 g vs. 1094 g,  $P<0.01$ ) and feed efficiency (about -7%,  $P<0.05$ ) whilst feed intake did not differ. A higher amount of OTA was observed in the kidneys. There was also a much higher amount of OTA in the liver and semimembranosus muscle than the maximum limit of 1  $\mu\text{g}\cdot\text{kg}^{-1}$  established by the European Union for human consumption food.

### ochratoxin A / pigs / growth parameters / meat

**Résumé** – Effets de l'Ochratoxine A sur la production du porc lourd. L'Ochratoxine A (OTA) est une mycotoxine largement répandue, produite principalement par *Aspergillus Ochraceus* et *Penicillium verrucosum*. On la retrouve dans les céréales, mais aussi dans la viande et ses dérivés. Il a été démontré que l'OTA était néphrotoxique, hépatotoxique et cancérigène pour tous les mammifères, les porcs et les volailles étant les espèces les plus exposées à sa toxicité. L'objectif de cette étude était d'évaluer les effets de l'OTA introduite dans l'alimentation des porcs sur les performances de croissance et sur la présence de résidus d'OTA dans l'animal. Soixante-quatre porcs, répartis en deux groupes homogènes, ont été élevés pendant 119 jours, de 41 kg de poids vif jusqu'à l'abattage. Le groupe témoin a été nourri avec un aliment commercial et le groupe expérimental a reçu le même aliment additionné de 25  $\mu\text{g}\cdot\text{kg}^{-1}$  d'OTA. Si aucun paramètre de croissance n'a été affecté par le traitement jusqu'à 62 jours de mesure ; à la fin de l'expérience, le poids vif final (170,9 kg vs. 163,4 kg,  $P<0,05$ ), le gain moyen de poids quotidien (1094 vs. 1030 g,  $P<0,01$ ) et l'efficacité alimentaire (-7 %,  $P<0,05$ ), des porcs consommant l'OTA, ont été diminués ; tandis que l'ingestion alimentaire est restée inchangée. La teneur la plus élevée en OTA a été trouvée dans les reins. Dans le foie et dans le muscle *semimembranosus*, elle a été largement supérieure à la limite maximale de 1  $\mu\text{g}\cdot\text{kg}^{-1}$  fixée par l'Union Européenne pour les denrées destinées à l'alimentation humaine.

### ochratoxin A / porcs / performances de croissance / viande

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## 1. INTRODUCTION

The contamination of animal feed and the potential contamination of animal meat by mycotoxins could present a serious hazard to humans and animals. Ochratoxin A (OTA) is one of the most common and dangerous mycotoxins. OTA is a naturally occurring mycotoxin produced by *Aspergillus ochraceus*, mainly in tropical regions, and *Penicillium verrucosum* mainly in temperate areas [18].

Interest in OTA increased in 1994, when the International Agency for Research on Cancer classified OTA as a possible human carcinogen, based on sufficient evidence of carcinogenicity in experimental animal studies [7]. OTA has a potent toxicity and its nephrotoxic, hepatotoxic and carcinogenic effects have been demonstrated in all mammalian species [10]. The products naturally contaminated with OTA include cereal grains, green coffee beans, peanuts, sorghum, olives, poultry and pig meats, cheese, dried fruit, wine and grape juice [8].

The main effects of OTA are related to the reduction of protein, DNA and RNA synthesis due to the properties of OTA which is a powerful competitive inhibitor of phenylalanine-tRNA ligase [20]. Interest in OTA in animal production is limited to monogastric species because the activity of symbiotic microorganisms in ruminants can hydrolyse the peptide bond of OTA into phenylalanine and ochratoxin  $\alpha$  which is generally considered to be non-toxic [10]. The target organs of toxicity in monogastric species are mainly the kidneys, but also the liver, which has a detoxification function [4]. In Italy, heavy pigs are reared for more than 9 months and slaughtered at 160 kg live weight to produce dry-cured products like Parma and San Daniele hams. There is, then, a real risk of using OTA contaminated feed during the long rearing period. OTA can also reduce feed efficiency with subsequent lower growth; it also weakens the immune system.

The aim of this work was to evaluate the effects of OTA in the growing pigs' diet on growth parameters and to calculate the amount of OTA in the animal body and urine.

## 2. MATERIALS AND METHODS

### 2.1. Animals and controls

Sixty-four Large White  $\times$  Landrace pigs, 50% castrated males and 50% females, from 8 litters, at mean live weight of 41 kg, were divided into 2 homogenous groups of 32 animals each and housed in pens holding 4 animals. The animals used in this study were reared and treated in compliance with the Directive 86/609/EEC relating to the protection of animals used for experimental or other scientific purposes, according to the Italian Legislation with Legislative Decree January 27, 1992 No. 116. The growth period was divided into two phases, which corresponded to different nutrient supplies: the first phase was from the beginning of the trial to the 62nd day (about 100 kg BW) and the second phase from the 62nd day to the end of the trial (119th day, about 170 kg BW). The control group was given a commercial pig feed for growing pigs (Control diet). The main ingredients of commercial feed were cereals and soybean. According to the Consortium of Parma Ham regulation, cereals in the growing pig diet must be at least 55% of total dry matter and soybean must not exceed 15%; wheat bran, molasses, animal fat and minerals were the other ingredients. The chemical composition of the feed during the first phase was the following: crude protein 15.9%, crude fibre 4.1%, ether extract 4.6%, ash 5.6%, Net Energy content 2300 kcal $\cdot$ kg $^{-1}$ . In the second phase, the chemical composition was the following: crude protein 17.6%, crude fibre 3.8%, ether extract 5.4%, ash 6.2%, Net Energy content 2360 kcal $\cdot$ kg $^{-1}$ . The natural contamination of feed by OTA was approximately 0.3  $\mu$ g $\cdot$ kg $^{-1}$ .

For the treated group, the same feed was used with the addition of 25  $\mu\text{g}\cdot\text{kg}^{-1}$  of Ochratoxin A from *Aspergillus ochraceus* (OTA 25  $\mu\text{g}\cdot\text{kg}^{-1}$  diet), which was obtained from Sigma-Aldrich s.r.l. Milano. A total of 25 mg of OTA, in a powder state, was dissolved in a methanol-water solution, then it was progressively added to 25 kg of feed and mechanically stirred; this feed amount was introduced in an industrial ribbon mixer and was blended for 3 hours.

The diets were fed ad libitum and the animals had free access to drinking water.

All pigs were individually weighed at the start of the trial, after 62 and 119 days, just before slaughtering. Live body weight (BW) and average daily weight gain (ADG) were recorded. Feed Intake (FI) of each pen was recorded during the trial to calculate Feed Efficiency (FE). At slaughter, the kidneys, liver and 50 g of semimembranosus muscle of 4 pigs from each group were collected and immediately stored at  $-20\text{ }^{\circ}\text{C}$ .

The meat from 4 pigs of each group was taken to make seasoned sausages; analyses were carried out to determine OTA in fresh sausages, after 40 and 100 days of seasoning. One sample of urine from each group was collected at the beginning of the trial, after 15, 45, 75, 105 and 119 days and immediately stored at  $-20\text{ }^{\circ}\text{C}$ . A sample of urine was manually collected from 4 females, for each group, housed in the same pen.

Ochratoxin A in the kidneys, liver and meat was detected by using an immunoaffinity step followed by HPLC with fluorimetric detection according to Jorgensen and Petersen [9]. Analyses for OTA in urine were carried out using the method described by Pascale and Visconti [15].

## 2.2. Statistical analysis

All data were analysed by ANOVA according to the General Linear Model of the S.A.S. package [16] in order to evaluate the effects of the diet. BW and ADG recorded during the study were covaried

with the initial BW. The statistical model employed for BW and ADG was the following:

$$Y_{ijk} = \mu + \alpha_i + \beta_j (x_{ij} - \bar{x}) + \gamma_k + \varepsilon_{ijk}$$

where  $\mu$  was the general average,  $\alpha$  the effect of the diet,  $\beta$  the correction factor for the covariate,  $\bar{x}$  the average of the observations,  $\gamma$  the effect of the pen and  $\varepsilon$  the error.

A statistical model without the effect of the pen and without a covariate was employed for FE, FI and OTA contents in semimembranosus muscle, liver and kidneys.

For OTA content in sausage meat, the following model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijk}$$

where  $\mu$  was the general average,  $\alpha$  the effect of the diet,  $\beta$  the effect of seasoning,  $(\alpha \times \beta)$  the interaction between the main effects and  $\varepsilon$  the error.

Since only one value of urine for each diet was available, no statistical analysis was possible for OTA content in urine.

## 3. RESULTS AND DISCUSSION

Growth parameters of pigs are reported in Table I. All animals were apparently in good health before and throughout the trial. Firm faeces were observed throughout the study with no occurrence of diarrhoea. No significant differences between the groups were observed that were attributable to the pen effect. Initial BW, BW after 62 days, and ADG in the first period of the trial did not differ between the two groups. FI was not significantly affected during the experimental period; the pigs fed the control diet showed heavier final weight: 170.9 kg vs. 163.4 kg ( $P < 0.01$ ) after 119 days, and better ADG: 1094 g vs. 1030 g. As a result of the lower ADG of the treated group, FE at the end of the trial was higher for the control

**Table I.** Effects of OTA in feed on growing parameters of pigs.

	Control	OTA	SE	Significance
Body weights (kg)				
Initial <sup>1</sup>	40.7	41.2	0.9	NS
At 62 d <sup>1</sup>	102.1	103.7	2.0	NS
At 119 d <sup>1</sup>	170.9	163.4	2.6	*
Period 1 (0 to 62 days)				
ADG <sup>1</sup> (g·d <sup>-1</sup> )	990	1015	32	NS
Feed Intake <sup>2</sup> (kg·d <sup>-1</sup> )	3.365	3.313	0.090	NS
Feed Efficiency <sup>2</sup> (kg gain per kg feed)	0.294	0.306	0.020	NS
Period 2 (62 to 119 days)				
ADG <sup>1</sup> (g·d <sup>-1</sup> )	1207	1048	37	**
Feed Intake <sup>2</sup> (kg·d <sup>-1</sup> )	4.418	4.428	0.125	NS
Feed Efficiency <sup>2</sup> (kg gain per kg feed)	0.273	0.233	0.017	*
Total trial (0 to 119 days)				
ADG <sup>1</sup> (g·d <sup>-1</sup> )	1094	1030	22	**
Feed Intake <sup>2</sup> (kg·d <sup>-1</sup> )	3.869	3.881	0.115	NS
Feed Efficiency <sup>2</sup> (kg gain per kg feed)	0.283	0.265	0.016	*

NS:  $P > 0.05$ , \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

<sup>1</sup> Data computed from each animal (n=32 per treatment).

<sup>2</sup> Data computed from each pen (n=8 per treatment).

group (0.283 vs. 0.265 kg gain per kg feed,  $P < 0.05$ ).

The FE values observed were consistent with data obtained by many authors on heavy pigs fed ad libitum [1, 14]. The decrease of ADG and FE confirms the conclusions of Harvey et al. [6] and Madsen et al. [12] who suggested that OTA can affect growth performance and serum biochemical and haematological values. During the first period (from 40 kg to 100 kg), no significant differences were observed regarding ADG and FE. Only in the second period of the trial did the OTA effect become evident, significantly reducing ADG (1207 g vs. 1048 g); it can be concluded, therefore, that two months of ingestion of contaminated feed, at the tested dose, is not long enough to reduce the growth parameters and a reduction occurs only towards the end of the experimental period.

Feed intake did not decrease in the second phase (62–119 days, from 100 kg to

170 kg), whereas FE was considerably reduced (0.233 vs. 0.273,  $P < 0.05$ ). This loss of FE is probably connected to an alteration of protein synthesis caused by OTA, as demonstrated by many authors [2, 20], which determines a reduction in growth.

Table II shows OTA in the kidneys, liver and meat. Due to natural contamination of commercial feed used in the trial, OTA has also been found in the control group. The effect of OTA in the kidneys and liver after 4 months of contaminated ingestion is manifest; at slaughtering, OTA content in the treated group was much higher in the kidneys (69 vs. 10.5  $\mu\text{g}\cdot\text{kg}^{-1}$ ,  $P < 0.001$ ), liver (52 vs. 3.5  $\mu\text{g}\cdot\text{kg}^{-1}$ ,  $P < 0.001$ ) and in semi-membranosus muscle (6.1 vs. 0.88  $\mu\text{g}\cdot\text{kg}^{-1}$ ,  $P < 0.001$ ).

OTA content in the control diet was much lower than 5  $\mu\text{g}\cdot\text{kg}^{-1}$ , being the maximum limit of tolerance in cereals fixed by the EEC regulation 472/2002 [3] for human feed, however it was enough to determine,

**Table II.** Effect of feed OTA on Ochratoxin A in meat.

	Control	OTA	SE	Significance
Semimembranosus muscle ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	0.88	6.10	0.20	***
Kidneys ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	10.50	69.00	0.54	***
Liver ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	3.50	52.00	0.74	***
Sausage meat 0 days ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	0.70 a	5.00 b	0.16	
Sausage meat 40 days ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	0.90 a	4.65 b		
Sausage meat 100 days ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	0.95 a	5.25 b		

NS:  $P>0.05$ , \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ .

Means followed by different letters differ significantly at  $P<0.05$ .

after lengthy exposure, a final detectable amount in the kidneys, liver and muscle. On examination, the kidneys and liver of the treated group presented visible lesions, soft consistency and a serious haemorrhagic condition.

Recorded data confirm the conclusions of many authors who suggest that the kidneys and liver are the target organs for OTA and, according to van Egmond and Speijers [19], the highest content was found in the kidneys. OTA content in the semimembranosus muscle was lower than in the kidneys and liver, as suggested by Gareis [4] and by Jorgensen [8]. Madsen et al. [13] also found a higher level of OTA in the kidneys than in meat, however, this difference was lower than that found by the other authors.

OTA content was lower in sausage meat than in *semimembranosus* muscle, as shown in Table II. The values observed for the treated group were consistent with the results of previous studies [5, 11]. Lard is added to fresh meat to produce sausages and, according to Gareis [4], OTA accumulates more in muscle than in adipose tissue; as a consequence, OTA content in sausages is lower.

Statistically significant differences, regarding OTA content in sausages, were observed between groups, whereas no differences were found between the different times of seasoning ( $0.70 \mu\text{g}\cdot\text{kg}^{-1}$ ,  $0.90 \mu\text{g}\cdot\text{kg}^{-1}$ ,  $0.95 \mu\text{g}\cdot\text{kg}^{-1}$  and  $5.00 \mu\text{g}\cdot\text{kg}^{-1}$ ,  $4.65 \mu\text{g}\cdot\text{kg}^{-1}$ ,  $5.25 \mu\text{g}\cdot\text{kg}^{-1}$  for the control and treated

group respectively after 0, 40 and 100 days of seasoning). These data confirm the conclusions of many authors [11, 17] who suggested that production procedures such as seasoning and storage have no effects on the reduction of OTA levels in meat products.

OTA content in urine was determined at the beginning of the trial, after 15, 45, 75, 105 and 119 days. In the control group, OTA was never found, during the whole experimental period; in the treated group, OTA was absent at the beginning of the trial and it was detected at  $3.10$ ,  $3.50$ ,  $3.60$ ,  $4.35$  and  $3.70 \mu\text{g}\cdot\text{kg}^{-1}$ . According to Pascale and Visconti [15], there is a clear correlation between OTA intake and OTA concentration in urine, so the determination of OTA in urine could be used as a rapid and non-invasive tool to verify OTA contamination in piggeries, therefore avoiding the need to analyse all feeds used throughout the growing period.

#### 4. CONCLUSIONS

The results of this experiment indicate that the addition of  $25 \mu\text{g}\cdot\text{kg}^{-1}$  of Ochratoxin A to the diet of growing swine can determine decreased growth rate and feed efficiency whilst feed intake is not affected. The decrease of growth rate becomes evident from the third month of intake of contaminated feed. The final amount of ochratoxin A was very high in the kidneys and

liver and, it probably reduced the functionality of these organs causing lower growth. The final OTA content in meat was much higher than the maximum limit of  $1 \mu\text{g}\cdot\text{kg}^{-1}$  established by the European Union for human consumption food; seasoning and storage do not seem to have any effect on the reduction of the levels of ochratoxin A in meat products. OTA content in urine could be used as a rapid and non-invasive tool in monitoring OTA contamination in piggeries.

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