Residues of aflatoxins B1 and M1 in different biological matrices of swine orally administered aflatoxin B1 and *Saccharomyces cerevisiae*

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RIASSUNTO – Residui di aflatossine B_1 e M_1 in differenti matrici biologiche di suino alimentato con diete contenenti aflatossina B_1 e *Saccharomyces cerevisiae. – Le micotossine, e la aflatossina B_1 (AFB_1) in particolare, rappresentano da sempre un problema di sanità pubblica a livello mondiale di difficile risoluzione. Scopo del presente lavoro è stato quello di valutare le quantità di aflatossine B_1 e M_1, in fegato, rene, muscolo e grasso di suini che hanno assunto per 5 settimane diete sperimentalmente contaminate con 280 μg/kg di AFB_1. E' stata valutata anche la capacità adsorbente del Saccharomyces cerevisiae nei confronti di AFB_1, somministrando la micotossina (280 ppb) insieme ad una dieta contenente lo 0.2% del lievito. Le quote residuali delle due aflatossine sono state determinate con metodo HPLC in fluorescenza, previa purificazione con estrazione solida (SPE). Muscolo e grasso sono risultati costantemente negativi per entrambe le micotossine, mentre i livelli rilevati in fegato e rene appaiono molto bassi per la AFB_1 (valori compresi tra 0.03-0.12 ppb) e leggermente superiori per la AFM_1 (valori compresi tra 0.39-0.72 ppb).*

Key words: aflatoxins B_1 and M_1, swine, residues.

INTRODUCTION – Mycotoxins are acutely toxic, carcinogenic, mutagenic, and oestrogenic secondary metabolites produced by moulds, mostly of the genera *Aspergillus*, *Penicillum* and *Fusarium*. Aflatoxins (AFs) are dangerous molecules to animals and humans produced mostly by *Aspergillus flavus* and *A. parasiticus* (Marin *et al.*, 2002). Aflatoxin B_1 (AFB_1) is the most potent of all aflatoxins and is of great concern because of its adverse effects to the health of humans and domestic animals, including also teratogenic and immunosuppressive effects (Eaton and Callagher, 1994). Aflatoxin M_1 (AFM_1) is a hydroxilated metabolite of AFB_1 characterized by a rapid elimination through the milk and the urine, and by a lower toxicity than its parent compound. These mycotoxins can be detected in meat, visceral organs, milk and eggs. Residues of AFB_1 and AFM_1, when present in animal products, represent a threat to human health, and their levels should be constantly monitored and controlled. In pigs, acute toxicity following consumption of high doses of aflatoxin, is characterized by feed refusal, reduced weight gain, changes in haematological and biochemical parameters, and liver and kidney lesions (Miller *et al.*, 1981; Harvey *et al.*, 1988). One of the methods for controlling mycotoxin hazards in animal husbandry is based on the use of specific yeast cultures, such as *Saccharomyces cerevisiae* strains, that are able to adsorb mycotoxins, thus limiting their gastrointestinal bioavailability (Yiannikouris *et al.*, 2003). To check the efficacy of toxin adsorption by *Saccharomices cerevisiae* and to evaluate the carry-

over of AFB_1 and AFM_1 in tissues of heavy pigs, the Authors verified the presence of the two mycotoxins in samples of liver, kidney, muscle and fat of swine administered AFB_1 in the diet.

MATERIAL AND METHODS – Fourty pigs (145 kg mean body weight) were housed in floored indoor pens. The pigs were fed on a basal diet and randomly assigned to one of the four experimental groups: a 280 μg AFB_1/kg feed group, without adsorbent (group 0-AF) and a 280 μg AFB_1/kg feed group, supplemented with 0.2% isolated and autoclaved *Saccharomices cerevisiae* (group S-AF). Further two groups of animals (ten pigs each) were as controls (group 0-0 basal diet; group S-0 basal diet supplemented with 0.2% Saccharomices cerevisiae). The diets were experimentally contaminated with AFB_1 (Sigma Aldrich s.r.l. - Milano - Italia). Before any supplementation, the basal diet was tested by HPLC to ensure that it contained no residual mycotoxins. The different diets were administered to all pigs for 5 weeks, the animals were given *ad libitum* access to water and to the feed, assuring that the animals of the groups 0-AF and S-AF daily ingested 0.952 mg of AFB_1. At the end of the experiment, the animals were slaughtered and samples of kidney, liver, muscle, and adipose tissue were collected and stored at –20°C pending analysis. The levels of AFB_1 and AFM_1 in the considered tissues were determined by extraction (dichloromethane and citric acid 20%) and purification by a solid phase (Isolute, SI-silica columns [Stepbio, Bologna-Italy]), followed by HPLC analysis (column: Phenomenex Luna C18, 250x4.6 mm [Chemtek, Bologna-Italy]; mobile phase: water-isopropylic alcohol-acetic acid 1%-acetonitrile (79:7:7:7) [0.8 ml/min]) with flourescence detection (365 nm excitation and 418 nm emission wavelenghts) (Cirilli *et al.* 1986; Simonella *et al.* 1990). Animal care and experimental procedures were conducted according to Directive 86/609/EEC (1986). Differences between treatments were compared using the tests of Student's t and the Mann-Whitney'U. A difference with $P < 0.05$ was considered to be statistically significant. The study was performed according to ISO 9001:2000 requirements.

RESULTS AND CONCLUSIONS – The HPLC method was characterized by accuracy, good specificity and linearity in the considered range of concentrations. With the described chromatographic conditions, the limits of detection (LOD) for AFB_1 and AFM_1 in all the considered matrices ranged from 0.05 ppb to 0.4 ppb, whereas the limits of quantification in the same matrices ranged from 0.25 ppb to 1 ppb. Recoveries from the tissues spiked with the two AFs were in the range 50%-70%. During the experimental period, all pigs in every group were healthy, and the mycotoxin and *Saccharomyces* had no apparent effects on feed intake, and weight gain. These results are in contrast with the observations of Marin *et al.* (2002) in piglets fed 280 ppb AF-contaminated diet (70% AFB_1) for 4 weeks. No AFB_1 or AFM_1 was found in muscle and in fat of 0-AF and S-AF animals. As reported in Table 1, data related to the levels of both mycotoxins in the liver and in the kidneys showed very low amounts in both experimental groups. These data confirm the poor capacity of AFB_1 and AFM_1 (a polar metabolite of AFB_1) to store in tissues (Rizzi *et al.* 2003; Zaghini *et al.* 2005, unpublished data). The absence of AFB_1 in muscle and in fat, and the values of the aflatoxin found in liver and in kidney agree with the records of Stubblefield *et al.* (1991) and of Bonomi (1999); in addition Bonomi (1999) showed a dose-dependent effect between the contamination levels in the tissues and the dose administered to the animals. The same Table 1 underlines the higher quantities and percentage of positiveness of AFM_1 *versus* AFB_1 according to the kinetics of the two mycotoxins (Galtier, 1998; Neal, 1998). The lack of statistical differences ($P>0.05$) between the experimental groups suggests a scanty ability of *Saccharomyces cerevisiae* to adsorb AFB_1 at the administered dosages. Moreover in both 0-AF and S-AF groups the differences in the values of the two AFs did not have statistical significance ($P>0.05$); this might be due to the high standard deviations of the mean values, indicating the relevant individual variability. Although the levels of AFB_1 present in the diet administered to the animals are high compared to the limit authorized by EU legislation (50 ppb), they are well within the range for animal feeds usually employed in farm. Our results suggest firstly the lower susceptibility of swine to aflatoxins compared to other animal species like rainbow trout, duckling, turkey poult, chicken and rabbit (Giambrone *et al.* 1985; Smith and Ross 1991). Secondly, in relation to Afs, food of animal origin can be usually considered safe to humans due to the ability of farm animals to biotransform and to excrete these mycotoxins to a large extent.

Table 1. Levels (ppb) of AFB_1 and AFM_1 in liver and kidneys. Values are expressed as mean value±standard deviation.

Experimental groups	0-AF		S-AF	
Biological matrices	AFB_1	AFM_1	AFB_1	AFM_1
Liver	0.08±0.03	0.39±0.10	0.12±0.04	0.54±0.29
	80%	*100%*	*44%*	*100%*
Kidneys	0.08±0.08	0.72±0. 32	0.03±0.01	0.48±0.16
	40%	*100%*	*33%*	*100%*

Italic numbers refer to percentages of positiveness.

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