INVESTIGATION OF THE DISTRIBUTION OF ZEARALENONE AND ITS METABOLITES IN THE PIGS FED WITH FEED CONTAMINATED BY ZEARALENONE

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Abstract

Male pigs with an initial body weight of 60 kg were fed with fodder containing 15 ppm zearalenone. After two weeks the zearalenone resp. zearalenol content was measured by capillary gaschromatography. It was found that the zearalenone resp. zearalenol content of different muscle tissues is lower than 10 μg/kg.

Zearalenone was detected in faeces, urine, liver and kidneys in a quantity of 3700, 450, 70 and 30 μg/kg resp. Zearalenol was also detected in faeces, urine, liver and kidney, the amount of alfa-zearalenol was always higher than that of beta-stereoisomer.

Introduction

As result of the very intensive research work in last two decades (since discovery of aflatoxins) it is generally known that many species of fungi common in various feeds are capable to produce mycotoxins. Under conditions in Hungary and many other countries having similar climatic and agricultural conditions the mycotoxins produced by Fusarium fungi represent the most important danger. As Fusarium is a ubiquitous fungus it easily contaminates crops in the fields or in stores and can, therefore constitute a constant health risk to domestic animals.

The active component produced by Fusaria was isolated from cultures of *Gibberella zeae (Fusarium roseum)* by STOB et al. (1962) and named zearalenone (URRY et al. 1966). The chemical structure of zearalenone (ES)-2,4-dihydroxy-6-(6'-oxo-10'-hydroxy-1-undecenyl)-benzoic-acid lactone and his most important metabolites (α- and β-zearalenol) are shown in Fig. 1. The results of earlier research on zearalenone were summarized by MIROCHA et al. (1971). Since the discovery of this mycotoxins more than hundred derivatives of zearalenone were synthetized chemically. A good survey relating to this topic was given by SHIPSHANDLER (1975) and PATHRE and MIROCHA (1976). A few of these compounds have oestrogenic properties more potent than that of zearalenone itself.

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Two aspects of zearalenone contamination are of particular interest to agriculture resp. food industry: (a) zearalenone ingestion by livestock may cause losses in terms of poor performance and health of animals, (b) residues of zearalenone and its metabolites in plant and animal food products may be of concern in terms of food safety. In addition, in some countries use of oestrogenic additives (such as zeranol, RALGRO, 17-β-oestradiol) is allowed resulting in an increase of muscle tissue production in pigs (BRÜGGEMANN and RICHTER, 1976).

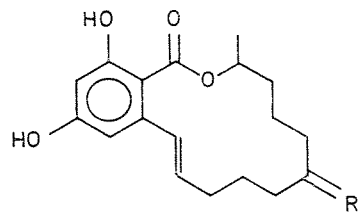

Fig. 1. Structure of zearalenone and zearalenol

Name	R
Zearalenone	=O
Alfa-zearalenol	<OH H
Beta-zearalenol	<H OH

To fully understand the toxic manifestations of zearalenone, it is necessary to study its metabolic fate in experimental animals and livestock. The results of such studies will help to have an adequate information concerning residue level of these compounds in meat and meat products and estimate the eventually health hasards.Although some very interesting and valuable results were published in this field and in development of more sensitive analytical methods intensive further research is needed to elucidate all the questions.

The mechanism of action of zearalenone can be explained by the fact that zearalenone binds to oestrogenic receptors (KIANG et al. 1978), although an action through a disturbance of the steroid metabolism cannot be excluded.

The metabolism of zearalenone in rat liver was investigated by several authors. KIESSLING and PETTERSSON (1978) found that zearalenone was metabolized along two principal pathways, conjugation with glucuronic acid and reduction to zearalenol. This reduction was postulated to be catalysed

by a hydroxysteroid dehydrogenase. In a newer work, OLSEN et al. (1981) reported that the zearalenone reducing enzyme was identified as 3α-hydroxysteroid dehydrogenase. At least two multiple forms occur of the enzyme with different subcellular locations and pH-optima. The activity was localized in the microsomes with NADH as coenzyme and in both microsomes and cytosol with NADPH. More recently, TASHIRO et al. (1982) isolated a zearalenone reductase from microsomes of rat liver. On the basis of experiments it was stated that this enzyme is not identical with 3α-hydroxysteroid dehydrogenase, and has a unique character.

Less data are available about the metabolism of zearalenone in domestic animals. Zearalenone levels in tissues of treated animals were reported by MIROCHA et al. (1977). Crystalline zearalenone was administered to young female pigs at levels of 0, 3.5, 7.5 and 11.5 mg zearalenone per kg body weight by FARNWORTH and TRENHOLM (1981). All animals receiving the mycotoxin exhibited vulva vaginitis and had enlarged reproductive tracts, 1 week after dosing. Free zearalenon was found in the blood, faeces and urine of treated animals. The highest zearalenone level detected was 2.61 ng/ml from a pig that received a dosage of 7.5 mg/kg. After 24 hours the collected faeces contained an average up to 308 ng zearalenone per gramm of dried faeces. Zearalenone levels of up to 59 mg/ml and α-zearalenol levels of up to 155 ng/ml urine were found. β-zearalenon was also detected in the urine. Metabolism of zearalenone in pregnant saws was studied by VÁNYI et al. (1983). From analytical point of view developing of very sensitive HPLC and fluorimetric methods for detection and quantitative determination of zearalenone and zearalenol (TRENHOLM et al. 1981) resp. zeranol and zearalanone (FRISCHKORN et al. 1978, STAN and HOHLS, 1978) and stereoisomers of zearalenol (VÁNYI et al. 1983) may be mentioned.

In this paper some results of the investigation about the distribution of zearalenone and its metabolites in pigs fed with feed contaminated by zearalenone are reported.

Experimental

Animals, housing, diet

Two male KAHYB pigs from the minimal disease herd maintained at State Research Animal Farm weighing 60 kg were fed with a standard corn-wheat-soybean diet contaminated with 15 ppm zearalenone. After 14 days the pigs were slaughtered and different parts of their body were investigated. The zearalenone and zearalenol content of faeces and urine was also controlled at the 13-th day of experimental feeding.

Analytical work

Materials

Zearalenone Makor Chemicals (Jerusalem, Israel) 0.1 mg/cm^3 in acetone. Alfa- and beta-zearalenol: From University of Minnesota (Minnesota, USA) 0.1 mg/cm^3 in acetone, by the courtesy of Prof. Dr. G. J. Mirocha. BSTFA reagent Pierce (Rockford, Ill., USA). Sephadex LH-20 Pharmacia Fine Chemicals AB (Uppsala, Sweden). Kieselgel 60 mesh Merck (Darmstadt, FRG). Glucuronidase enzyme Sigma Chem. Co. (St. Louis, USA). Organic solvents Reanal (Budapest, Hungary) distilled before use.

Apparatus

The GLC was carried out on a Packard chromatograph equipped with a flame-ionization detector (Model 427) and HP 3390 A integrator. Wall-coated open-tubular capillary column (15 m, 0.25 mm i.d.) was used. The column was prepared according to the method of GROB et al. (1977, 1978). The capillary was drowen from Pyrex glass having a 8 mm i.d. and 3 mm i.d. on a Hupe Busk Hewlett Packard machine. The inner surface of the capillary was coated with $BaCO_3$ which was then deactivated with PEG 1000. The coation was carried out with a SE 52 stationary phase, static method. The peak symmetry and resolution was satisfactory for all components of GROB et al. test mixture (1977, 1978). The theoretical plate number was 2000/m. The temperatures of the injection port and the detector were 240 °C and 240 °C, respectively; column temperature was programmed from 180 °C to 260 °C at 3 °C/min. The carrier gas was H_2 (hydrogen). Split ration 1 : 20.

Sample preparation

To 20 cm^3 resp. 20 gr of samples 40 cm^3 of acetone was added. After thorough mixing to the mixture 2 cm^3 of a 1% solution of glucuronidase enzyme in water were added. The mixture was incubated at 37 °C for two hours. After incubation the mixture was heated to 55—60 °C, then it was allowed to stand for two hours.

The precipitate was filtered on a Machery-Nagel filter paper. The precipitate remained on the filter paper, was washed with 10 cm^3 of acetone. The acetone was distilled off on a vacuum "Rotadest" apparatus and the remaining aqueous part was extracted with 20 cm^3 of ethyl acetate. (The extraction with ethyl acetate was carried out in the following manner: the

first part of the solvent was added to the distillation flask which was rinsed thoroughly and the mixture obtained was then poured into a separation funnel.)

The organic phase was dried over anhydrous Na_2SO_4 and was evaporated on a vacuum "Rotadest" apparatus to dryness. The dry residue was dissolved in 10 cm^3 of 1 v/v% methanol in benzene. The solution was evaporated to 2 cm^3 and poured on a Sephadex LH 20 (10+1 cm) column. The column was eluted with 20 cm^3 of benzene, then the zearalenone and its derivatives were eluted with 20 cm^3 of a benzene-methanol mixture (9 : 1). The eluate containing the zearalenone was evaporated to 1 cm^3 which was then poured on a Kieselgel 60 column. The column was eluted 10 cm^3 of benzene, then the desired substances were obtained by elution with 20 cm^3 of a benzene-acetone mixture (9 : 1).

The benzene-acetone fraction was evaporated to dryness. An aliquot of the extract was silylated with BSTFA reagent (15 minutes, 60 °C) and 1 mm^3 was injected into the gas chromatograph.

Results and discussion

The efficiency and standard deviation of the purification method was determined at 20 μg/kg and 1000 μg/kg concentration levels. The samples containing the standards (i.e. zearalenone, alfa-zearalenol and beta-zearalenol) in a concentration value of 20 μg/kg were prepared from swine muscle, whereas samples with a concentration value of 1000 μg/kg were prepared from urine.

Nine and eleven parallel tests were carried out with the muscle and urine, respectively. The results are summarized in the Table 1.

Table 1

Recovery and standard deviation of the method used

Tested substance	Added quantity (μ/kg)	Recovery %	Standard deviation
Zearalenone	20	78	21
Alfa-zearalenol	20	74	26
Beta-zearalenol	20	71	18
Zearalenone	1000	81	14
Alfa-zearalenol	1000	79	12
Beta-zearalenol	1000	80	14

The average content of zearalenone and its metabolites in different organs and tissues of the experimental animals and also urine and faeces is summarized in Table 2. Zearalenone was detected in faeces, urine, liver and

Table 2

Zearalenone resp. zearalenol content of samples

Sample	Zearalenone	α-zearalenol	β-zearalenol
		µg/kg	
Faeces	3710	15750	4140
Urine	450	1270	620
Liver	70	160	28
Kidney	30	220	85
Feromal muscle		< 10 µg/kg	
Shoulder blade muscle		< 10 µg/kg	
Heart		< 10 µg/kg	
Spleen	~ 10	25	~ 10
Lymphatic gland		10 µg/kg	
Pancrease		10 µg/kg	
Adipose tissues		10 µg/kg	
Fat around kidney	< 10	15	< 10
Blood serum	< 10	10	< 10

kidneys. Very low concentration was measured in the blood plasma. Muscle tissue does not contain detectable amounts of zearalenone. The content of zearalenols is also the highest in urine and faeces. Considerable amount was found in some inner organs. The muscle tissue and also adipose tissues of pig contain very small quantities of these metabolites. The amount of α-zearalenol is in all samples much higher than that of β-stereoisomer. In the samples originating from control animals fed with normal and not contaminated diet there were not detectable amounts either of zearalenone nor of zearalenols. The most important finding — from practical point of view — is the fact that the toxin resp. metabolite content of meat (muscles) is in every case lower than 10 µg/kg.

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