Variations in zearalenone activation in avian food species

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Abbreviations:

ZEA : zearalenone; α-ZOL: α-zearalenol ; β-ZOL : β-zearalenol; ZAN : zearalanone; α-ZAL: α-zearalanol; β-ZAL : β-zearalanol.

Abstract

Zearalenone (ZEA), a widely distributed oestrogenic fusariotoxin, constitutes a potential risk for human and animal health. ZEA is metabolised to the main metabolites identified *in vitr*o and *in vivo*: alpha-zearalenol (α-ZOL) and beta-zearalenol (β-ZOL). The efficiency to produce alpha-reduced metabolites appears of particular interest in risk assessment as alpha-reduced metabolites constitute activated forms whereas beta-reduced metabolites are less oestrogenic than ZEA. In this study ZEA activation was compared in avian food species. ZEA and its reduced metabolites were quantified in subcellular fractions of six avian species and rat livers. The α-ZOL/β-ZOL ratio in rats was 19. The various avian food species cannot be considered to be equivalent in terms of ZEA reduction ($P<0.001$). Quails represented high "beta reducers", with α-ZOL/β-ZOL ratio less than two. Weak "beta reducers" included on one part ducks and chickens showing α-ZOL/β-ZOL ratio greater than 3 and up to 5.6 and on a second part geese, showing a lower production of α-ZOL than other poultry. Comparisons of enzyme kinetics in ducks and in quails show that these variations can be explained by the action of various isoforms of dehydrogenases. These results are relevant to food safety, in the context of frequently inevitable contamination of animal feed.

Introduction

Zearalenone (ZEA), one of the most widely distributed fusariotoxin, is common in maize and maize products but also in soybeans and various cereals grains, representing a major component of human food and animal feed. Crop contamination is often inevitable and constitutes a potential risk for human and animal health (EFSA, 2004). Risk assessment of ZEA by the EC Scientific Committee on Food concluded on a temporary tolerable daily intake of 0.2 µg/kg body weight whereas the tolerable daily intake established by JECFA (FAO/WHO Joint Expert Committee on Food additives) was 0.5 µg/kg body weight. Human and animal exposure is mainly due to chronic ingestion of contaminated food, essentially via maize and cereals. Limits for ZEA in maize and other cereals have been defined in several countries worldwide, ranging from 50 to 1000 µg/kg (EFSA, 2004). Levels of ZEA in cereals show very marked variations, notably year-to-year variations of seasonal origin. The levels of occurrence are of the order of ten to several hundred µg/kg, but have been described in the range from a few µg/kg to 8000 µg/kg (Placinta et al., 1999) and may even exceed this range (Zinedine et al., 2007). Although the major route of human exposure is direct via cereals and maize, indirect exposure can also occur via animal products. No limits have been defined for ZEA in these products, despite activation reactions by animals.

ZEA causes oestrogenic syndromes in animals, resulting in reproductive disorders and decreased fertility in a large number of species, with marked variations of sensitivity. Female pigs are considered to be the most sensitive animal species, while poultry and ruminants show a lower responsiveness to ZEA (Gaumy et al., 2001). Absorption and metabolism are implicated in these interspecies variations (Galtier, 1999). Following oral exposure in mammals, ZEA is metabolised in various tissues, particularly in the liver, to the main metabolites identified *in vitr*o and *in vivo*: alpha-zearalenol (α-ZOL) and beta-zearalenol (β-ZOL) (Mirocha et al., 1981; Olsen and Kiessling, 1983; Ueno and Tashiro, 1981; Malekinejad et al., 2005). Further reduced forms have also been identified in mammals (Erasmuson et al., 1994; Miles et al., 1996): zearalanone (ZAN), alpha-zearalanol (α-ZAL) and beta-zearalanol (β-ZAL). Corresponding to the dietary exposure, increasing concentrations of ZEA and α-ZOL were detected in livers and muscle tissues of pigs fed with ZEA-contaminated diet: livers

samples contained predominantly α-ZOL and to a minor extent ZEA and β-ZOL (Doll et al., 2003; Zollner et al., 2002). ZEA and α-ZOL were detected in livers of hens fed for 16 weeks a 1580-µg/kg ZEA contaminated maize at mean concentrations of 2.1 and 3.7 µg/kg respectively (Danicke et al., 2002).The rate of conversion and the α-ZOL/β-ZOL ratio show species variations in mammals and may account for the species differences in terms of sensitivity to ZEA: for example highly sensitive pigs mainly produce α-ZOL whereas less sensitive cattle mainly produce β-ZOL (Danicke et al, 2005; Zöllner et al., 2002; Mirocha et al, 1981). The efficiency to produce α-reduced metabolites appears to be of particular interest as these metabolites are considered to be activated forms, exerting higher oestrogenic effects compared to ZEA according to the sequence: α-ZOL> α-ZAL> ZAN >ZEA> β-ZAL> β-ZOL (Fitzpatrick et al., 1989; Leffers et al, 2001; Ueno et al., 1983). The presence of α-reduced metabolites must also be taken into account when assessing the risk for the consumers of animal or crop products. Only limited information is available concerning ZEA metabolism in poultry species, although exposure is likely to occur via feedstuffs, specially during force-feeding, corresponding to a total intake of approximately 10 kg of maize per duck in 12 days (Tardieu et al., 2004).

The aim of this study was to determine whether there are any differences in the production of α- and β-reduced metabolites between various poultry species. We chose six avian food species: chickens, ducks, quails, geese, guinea-fowls and laying hens. An *in vitro* approach was used to compare the production of zearalenone and reduced metabolites by a simple HPLC method.

2. Materials and Methods

1. Chemicals and reagents

Pure zearalenone (ZEA), NADH and NADPH cofactors, reduced derivatives: β-zearalenol (β-ZOL), α-zearalenol (α-ZOL), zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) were purchased from Sigma Chemical Co (Saint Quentin Fallavier, France). Stock solutions were prepared in acetonitrile (5 mg/ml : ZEA, α- and β-ZOL; 1 mg/ml : ZAN, α- and β-ZAL), before sonication (15 minutes) and they were stored in the dark at -20°C. The following chemicals were used for preparation

of hepatic fractions: KH2PO4 and K2HPO4 from Prolabo, Trisacetate and buthylhydroxytoluene (BHT) from Sigma, KCl, EDTA 2H2O, NaOH, HCL from Merck. Dye reagent concentrate (Biorad) and bovine serum albumine (BSA, 100 µg/ml) were used to assay total proteins.

Distilled deionised water and HPLC grade solvents were used.

2. *Sample preparation*

Subcellular fractions

Two animals from each of six avian food species and two Sprague-Dawley rats (8 weeks old) were used for preparation of subcellular fractions: mallard duck (*Anas platyrhynchos*, 4 weeks old, males); quail (*Coturnix japonica*, 8 weeks old, females); goose (*Anser anser*, 12 weeks old, female and male); guinea-fowl (*Numida meleagridis*, 4 weeks old, females); label chicken (*Gallus gallus*, 12 weeks old, males), standard chicken (*Gallus gallus*, 6 weeks old, males); laying hens (*Gallus gallus* Isa Brown strain, 48 weeks old, females). They were euthanised according to ethical recommendations and in accordance with European Guidelines for the care and use of animals for research purposes. The livers were removed and all subsequent operations were carried out at 0-4°C.

The subcellular fractions were prepared in duplicate for each animal. The livers were cut into small pieces, and 8 g samples were homogenized in 15 ml of ice-cold 0.1M potassium phosphate buffer (pH 7.4; 0.1M Tris acetate; 0.1M KCl; 1 mM EDTA 0.02 mM BHT) in a glass Potter homogenizer with a Teflon pestle. After filtration, the homogenate was centrifuged at 9000×*g* for 30 min in a TGA-65 Kontron ultracentrifuge. The supernatant was stored as 1-ml aliquots at -80°C until use (S9 samples).

The protein concentrations of the subcellular fractions were determined by the method of Bradford, using BSA as standard and the Biorad kit (Microassay procedure).

In vitro metabolism of ZEA and extraction

Previous experiments were conducted in duck fractions to ensure that activities were measured under linear conditions with respect to substrate concentrations. Optimal in vitro metabolism was observed by incubating 5 mg of protein with 16 µM of ZEA (1 µg/mg protein) in an excess of cofactors (Kolf-Clauw et al., 2007). For interspecies comparison assays, 5 mg of protein from the subcellular fractions, 2.4 mM of NADH and NADPH, and 16 µM of ZEA in acetonitrile (50 µL from 100 µg/ml) were

incubated in phosphate buffer for 30 minutes, at 40°C for poultry S9, at 37 °C for rat S9. In order to compare the enzymatic kinetics of the formation of α-ZOL and β-ZOL in ducks and in quails, various concentrations of ZEA were used (62.5, 125, 250, 500, 1000, 2000, 4000, 8000 and 16000 ng/mg protein equivalent to 1, 2, 4, 8, 16, 32, 64, 128, 256 µM). Fluorescence detection was used at ZEA concentrations ≤500 ng/mg protein (8 µM). α-ZOL and β-ZOL formations were modelled according to the Michaelis-Menten equation, based on the hypothesis of two enzymes involved in the reduction of ZEA:

$$y = V1max/(1+K1m/[ZEA]) + V2max/(1+K2m/[ZEA])$$

where "y" represents the metabolite α-ZOL or β-ZOL produced at different [ZEA] concentrations during an incubation time of 30 minutes. Origin Pro 7.05 SRO version 7.5714 software was used to determine the pertinence of the model (R), the affinity constants of the enzymes (K1m and K2m) and the limiting velocity of formation of the metabolites (V1max and V2max).

All samples were extracted twice with 10 ml of extraction solution (diethylether/chloroform, 3:1,v/v) for 30 min and centrifuged at 2000 g for 10 min. The organic phases were collected, evaporated to dryness under nitrogen (50°C, 30 min), and residues were dissolved in 100 µL of acetonitile, and sonicated. Extraction control samples were prepared for each animal and each analysis (ZEA without incubation). This recovery rate was extrapolated to zearalenols, based on previous results in other matrices of similar complexity showing similar recovery rates for ZEA and zearalenols (Songsermsakul et al., 2006; Zöllner et al., 2000). All the results from extraction control samples were used to estimate the mean global rate of ZEA extraction of the assays.

3. HPLC assay of ZEA, ZOLs, ZAN and ZALs

The HPLC apparatus consisted of an isocratic pump (model 2200, ICS) and a 20 µL Rheodyne injection valve (ICS). The reconstituted extract was injected immediately after sonication. The sample was eluted through a Prontosil column 120-5 C18 H (250x4 mm, particle size: 5 µm; Bischoff) with water/acetonitile (55:45, v/v) at a 1ml/mn flow-rate, as previously described (Kolf-Clauw et al., 2007). Briefly, the mobile phase was prepared and degassed before each assay. The column was kept at room temperature and was connected to a guard column (C18, 5µm, Prontosil). Two detectors were used: an

UV detector (Spectra Focus) at 235 nm, and a fluorescence detector (Shimadzu, RF-10AXL) at excitation wavelength λ_{exc} 274 nm/emission wavelength, λ_{em} 440 nm. Chromatograms were integrated with ANAPIC3 software.

The simple HPLC assay allowed the simultaneous determination of ZEA and all of its reduced metabolites using a multidetection mode with clearly distinct peaks for ZEA, α-ZOL and β-ZOL on one part, and ZAN, α-ZAL, β-ZAL on another part. Under these conditions, zearalenone (ZEA), α-zearalenol (α-ZOL) and ß-zearalenol (ß-ZOL) showed separate peaks at retention times of 16-18 minutes, 9-10 minutes, and 6-7 minutes respectively, using UV-detection at 235 nm or fluorescence detection. Zearalanone (ZAN), α-zearalanol and β-zearalanol also showed separate peaks using UV-detection at 218 nm. Elution times were 17-18 minutes, 9-10 minutes, and 6-7 minutes respectively. Limits of detection and quantification were defined as a signal-to-noise ratio greater than or equal to 3 and 10, respectively. The limits of detection using UV were 0.5 to 2 ng/mg proteins and ranged from 0.04 ng to 4 ng/mg proteins with fluorescence detection for all the six metabolites tested. The highest sensitivity was observed for the zearalenols. Fluorescence detection was ten fold more sensitive than UV detection for ZEA and α-ZOL (Kolf-Clauw et al, 2007). A mixture of the standards was injected in parallel to the samples as a control. Metabolites were identified by comparison of retention times with those of standards and quantified by their peak area ratio with standards.

4. Statistical analysis

The results are expressed as means ± SD for 4 incubated samples in one species. Mean differences in reduced metabolites were considered significant at $P<0.05$ by mixed effects ANOVA analysis. Interspecies variations for each metabolite were studied by ANOVA followed by Tukey-Neuman-Keul test in the case of a significant global variation, to identify various types of metabolic profiles for reduction of zearalenone.

3. Results

Metabolites identified

Zearalenols were identified as major metabolites after incubation of S9 samples with ZEA. Mean protein content in S9 samples was 39 ± 3 mg/ml (n=24 measures/species), ranging from 35 mg/ml to 43 mg/ml : 35 in laying hens, 36 in ducks, 38 in quails, 40 in guinea-fowls, 42 in geese and standard chickens, 43 in label chickens. Neither zearalanone nor zearalanols were found in any of the S9 samples. A mean recovery rate of 87 % ± 9 % for ZEA was observed in all the species tested.

Interspecies variations in zearalenols formation

Our results show interspecies variations in the reduction of ZEA, between rat and avian species, and between various avian species.

No species differences were observed for the overall rate of ZEA reduction, expressed as the ratio of zearalenols/sum of ZEA recovered and ZOLs, between any of the species tested including rat, except of geese. In all poultry species tested except for geese, the rate of ZEA reduction was found in the range of 50 % (± 3 %) in ducks to 78 % (± 5%) in quails. This was not significantly different from the rate of reduction of 72 % (± 5%) observed in rats (Fig.1). The goose metabolised one-third of zearalenone (33 ± 4 %) and can be defined as a weakly metabolising species.

All species including rats produced α-ZOL as major metabolite and interspecies differences were observed for α-ZOL ($P<0.001$) and β-ZOL ($P<0.001$). Rats differed from all poultry species by producing the highest quantity of α-ZOL from ZEA ($P<0.05$ to $P<0.001$). The most striking variations were observed between rats and geese ($P<0.001$) and rats and guinea-fowls ($P<0.001$). Our study shows that avian species also produced α-zearalenol as major metabolite but to a lesser extent than rats. Geese differed from all other poultry except guinea-fowls by a lower production of α-ZOL ($P<0.01$). The production of β-ZOL differed significantly between rats and two avian species: quails ($P<0.001$) and hens ($P<0.01$). Among poultry species, quails produced higher quantities of β-ZOL than other avian species except hens ($P<0.01$ to 0.001). The α-ZOL/β-ZOL ratio in rats was 19, while α-zearalenol and β-zearalenol production in avian species allowed to distinguish three groups: high "beta reducers" represented by quails, with a α-ZOL/β-ZOL ratio less than two, and weak "beta reducers". This latter group includes on one part ducks and chickens showing α-ZOL/β-ZOL ratio

greater than 3 and up to 5.6 and on a second part geese, showing a lower production of α-ZOL and α-ZOL/β-ZOL ratio of about 2.

Comparison of *in vitro* metabolism in quails and ducks

Enzyme kinetics were studied in the ducks and quails, representing a "weak β reducer" species and a "high β reducer" species respectively. In quails, both isomers, α-ZOL and β-ZOL, were produced in parallel: a first linear increase up to a ZEA concentration of 4000 ng/mg followed by a plateau. Both isomers were produced to a similar degree in relation to the substrate concentration and the curves overlapped (Fig.3). Two different kinetics for α-ZOL and β-ZOL production were observed in ducks (Fig.3A). As in quails, α-ZOL production reached a plateau from a ZEA concentration of 4000 ng/mg whereas β-ZOL increased linearly in relation to ZEA concentration without saturation at the maximum dose tested (16000 ng/mg protein). These results strongly suggest two different enzymes for the production of α-ZOL and β-ZOL in ducks.

The formation of α-ZOL and β-ZOL was modelled according to a Michaelis-Menten equation based on the hypothesis of two enzymes involved in ZEA reduction. With this model, high coefficients of correlation were obtained in both species: 0.9811 to 0.9983 (table 1). In quails, Km and Vmax for the reduction of ZEA into α-ZOL and β-ZOL showed similar values, suggesting that both reductases produce both metabolites. In contrast, in ducks, different Km and Vmax were obtained for α-ZOL and β-ZOL. For α-ZOL, the kinetic constants resemble those observed in quails. The kinetic constants for β-ZOL production calculated by the model were fairly different and difficult to interpret.

Altogether, these results strongly suggest that different enzymes are involved in α-ZOL and β-ZOL formation in ducks and quails.

4. Discussion

Species-specific variations among poultry in the phase I metabolism of zearalenone (ZEA) were demonstrated for the first time between various avian food species. Our findings are consistent with those of previous studies in other species and in hens, showing that hepatic phase I hydroxylations result in the formation of α-ZOL and β-ZOL, and demonstrate the absence of zearalanols formation. This finding is highly relevant, as it has been suggested that the failure to detect zearalanols in species other than sheep can be attributed to the use of HPLC with fluorescence detection since reduction of the C11-C12 double bound of ZEA leads to loss of fluorescence (Miles *et al*., 1996). The main metabolite of hepatic biotransformation of ZEA in rat liver subcellular fractions was identified as α-ZOL, in agreement with previous studies using S9 and rat liver microsomes (Ueno and Tashiro, 1981; Ueno et al., 1983). In our study, poultry mainly produced α-ZOL *in vitro*, as rat, but at a significantly lower rate than rat. These results are in accordance with *in vivo* results in broilers (Danicke et al, 2003), laying hens (Danicke et al., 2002), turkey poults (Olsen et al, 1986) and ducks (Danicke et al., 2004), but contrast with certain *in vitro* findings in young hens (Malekinejad *et al*, 2006). These authors found higher β-ZOL as compared to α-ZOL in hepatic subcellular fractions of chickens. These differences with some *in vitro* results could be explained by the ZEA concentrations incubated, as it has been previously demonstrated in pigs that the α-ZOL/β-ZOL ratio depends on ZEA concentration (Malekinejad *et al*., 2005). The ZEA concentrations used by Malekinejad *et al*, (2006) were in the range 10 to 2000 µM, while a concentration of 16 µM was used in our study for interspecies comparison. This concentration was representative of the liver concentrations observed after natural exposure to this mycotoxin, as a carryover factor of 0.005 was calculated from the (ZEA + α-ZOL)/ZEA concentration ratio in the diet of hens (Danicke et al., 2002). A similar factor can be calculated from a previous study in turkeys whith a 800-fold higher dietary ZEA concentration (Olsen al., 1986). On the basis of these results, it can be estimated that 1000 µg ZEA /kg of feed would result in hepatic concentrations of 5 µg ZEA/kg liver. This estimated exposure shows that our incubation conditions of 16 µM (1µg/mg protein), equivalent to 12 µg/kg liver would be realistic. Furthermore, the mean α-ZOL/ β-ZOL ratio of four in the bile of Pekin ducks fed with a Fusarium-toxin-

contaminated wheat (Danicke et al., 2004) was similar to the *in vitro* α-ZOL/ β-ZOL ratio observed in mallard ducks in our study, suggesting that our incubation conditions reflect physiological conditions and allow interspecies comparisons.

The major result of this comparative study concerns the identification of three groups of poultry among avian food species based on α-ZOL and β-ZOL production: quails were identified as high beta-reducers, guinea-fowls, ducks and chickens as weak beta-reducers, whereas geese were identified weak alpha- and beta-reducers. The species-specific variation in β-ZOL formation has been demonstrated in S9 fractions from various mammals and chickens (Malekinejad et al., 2006). Although enzyme polymorphism is known to exist in poultry and in mammals, species-specific variations of metabolism have rarely been investigated in poultry. In our study, species-specific variations were quantitative variations, in accordance with two recent *in vitro* studies , using midazolam in chickens, turkeys, pheasants and bobwhite quails or the mycotoxin aflatoxin B1 in chickens, ducks, quails and turkeys (Cortright and Craigmill, 2006; Lozano and Diaz, 2006).

Our hypothesis to explain the variation of α-ZOL/ β-ZOL formation between various poultry species is that at least two types of ZEA reductases are involved, as described in mammals. In mammals, previous results have suggested that at least two types of ZEA reductases are involved, differing in terms of optimum pH, kinetic parameters, enzyme localization (Ueno et al., 1983, Olsen and Kiessling, 1983; Malekinejad et al., 2006). Our hypothesis is supported by enzyme kinetics. Reduction of ZEA resembles that of steroid metabolism catalyzed by steroid deshydogenases (HSD) in humans, with three types of 3α-HSD catalyzing the formation of 3 α-hydroxysteroids (Matsunaga et al., 2006), or 3β-HSD catalyzing the formation of 3 β-hydroxysteroids. This last 3β-HSD occurs in two distinct forms, and catalyzes a deshydrogenase activity involving NADH as co-factor (Thomas et al, 2002). There are no published reports on these enzymes in animal tissues, but previous investigations have shown that the distribution patterns differed between species and were also coenzyme-dependent. Hens formed α-ZOL almost entirely in microsomal fraction and β-ZOL only in the cytosolic fraction and only with NADPH as coenzyme (Olsen and Kiessling, 1983). Further findings suggest that the enzymatic activity of 3 α-HSD and 3β-HSD varies in different subcellular fractions, i.e occurring as microsomal and cytosolic forms, and depends on the organ and animal species investigated

(Malekinejad, 2005). S9 samples therefore appear to be the most appropriate fractions for *in vitro* interspecies comparison of α-ZOL and β-ZOL production and to demonstrate species-specific variations of the α-ZOL/ β-ZOL ratio.

Species-specific variations of metabolism based on variations of enzyme profiles imply that the various avian food species cannot be considered as equivalent in terms of the activation/inactivation balance and for risk assessment. Conversion of ZEA to α-ZOL can be regarded as a bioactivation, whereas conversion to β-ZOL constitutes an inactivation reaction (Fitzpatrick et al., 1989; Leffers et al, 2001). Activated metabolites are determinants in xenobiotic toxicity in domestic animals (Nebbia, 2001), but are also involved in risk assessment for humans, due to the presence of residues. This process is of major concern for a food contaminant such as zearalenone, which exerts a limited direct toxicity in poultry, but raises potential problems of residues in livestock. ZEA is not usually considered to be a major food safety concern, although it may be involved in human cervical cancer (Hsieh, 1989) and in premature thelarche (Saenz de Rodriguez et al., 1984). In Puerto Rico, residues of oestrogenic compounds in red meat and poultry remain two of the most likely causes of premature thelarche (Saenz de Rodriguez and Toro-Sola, 1982), although the implication of ZEA remains controversial. Exposure to ZEA and its metabolites via the food chain following animal feed contamination would more probably result in more subtle long-term effects. These estrogenic effects through diet should be considered together with those of other environmental sources of endocrine disrupters in risk assessment for human health (Harvey and Everett, 2006). These effects include not only reproductive development, but immune, neurobehavioural development, and cancer susceptibility (Mantovani, 2006). Estimated human dietary exposure to ZEA in various European countries might range from 1 ng/kg bw/d to 420 ng/kg bw/d (EFSA, 2004). This last estimation is more than twice the SCF t-TDI of 0.2µg/kg bw/d. Although the main sources of human exposure to ZEA are bread and cereal products, animal products could be considered to be a source of potential residues in a context of high animal feed contamination. There is now overwhelming evidence of worldwide contamination of cereals with ZEA, mainly maize in Europe (for review, see Zinedine et al., 2007). Raw maize was the highest contaminated food in Europe, with reported contamination rates of 14% of maize (with levels> 0.2 mg/kg) with the highest level of 6492 mg/kg (Scoop, 2003). Maize from Africa was found

to be contaminated at levels as high as 9.8 to 38.4 mg/kg (El-Maghraby et al., 1995). Because poultry are highly resistant to the effects of ZEA compared to other livestock, there is a risk that contaminated animal products could be marketed. Residues of ZEA and α-ZOL have been identified in the livers of chickens and hens following ZEA exposure (Mirocha et al., 1982; Danicke et al., 2002). European regulation does not consider residues from animal products in the assessment of ZEA exposure for humans, assuming that "secondary human exposure resulting from meat, milk and eggs is expected to be low, contributing only marginally to the daily intake" (EFSA, 2004). However, quantification of this secondary exposure should include the most relevant animal species. A weak beta-reducer species should be chosen for avian food species and ZEA risk assessment, for example chickens or ducks.

In conclusion, the present results show that 1) zearalanols are not produced in poultry, 2) avian food species cannot be considered to be equivalent in terms of ZEA reduction and α-ZOL/β-ZOL ratio, ie in terms of activation/inactivation balance. Interspecies differences of Phase I reactions in poultry can be explained by the involvement of several isoforms of dehydrogenases, with different cosubstrate and kinetic parameters, as described in humans and mammals.

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TABLE AND FIGURE LEGENDS

Figure 1

Rate of metabolism of ZEA (zearalenone) by subcellular fractions (S9) of livers from avian food species and from rats, after 30 min of incubation (16 µM and 5 mg S9 proteins): means of 4 independent measures/species (m ±SD). * significantly different from other species ($p<0.05$)

Figure 2

Interspecies variations in α-ZOL (alpha-zearalenol) and β-ZOL (beta-zearalenol): $P<0.001$ respectively (means of 4 independent assays/species: m ±SD). Poultry and rat S9 fractions (5mg proteins) were incubated with 16 µM zearalenone for 30 min (40°C for avian food species S9, and 37°C for rat S9). Species with the same letter are not statistically different. α-ZOL: a>b>c; β-ZOL: x>y>z.

Figure 3

Enzymatic kinetics in ducks (A) and quails (B): Incubation for 30 minutes at 40°C, with 2.4 mM of NADH and 2.4 mM of NADPH. ▲: α-ZOL; **X** : β-ZOL.

Table 1

Kinetic constants for conversion of ZEA into α-ZOL and β-ZOL by S9 fractions from ducks and quails (30 minutes of incubation at 40°C, with 2.4 mM of NADH and 2.4 mM of NADPH): Michaelis-Menten equation model: $y= V1max/(1+K1m/[ZEA]) + V2max/(1+K2m/[ZEA])$

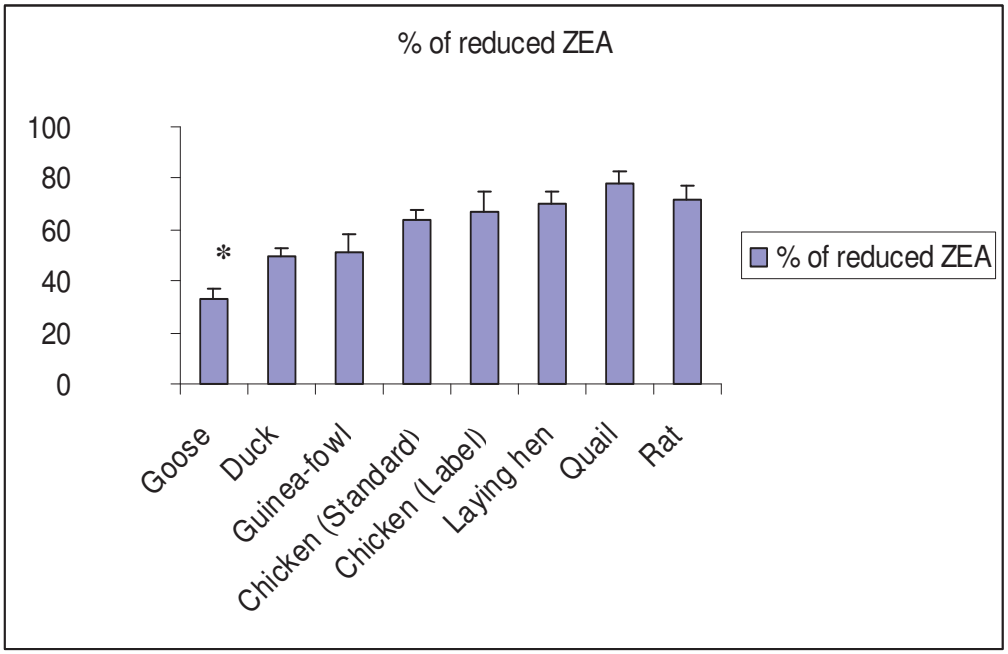


Fig.1

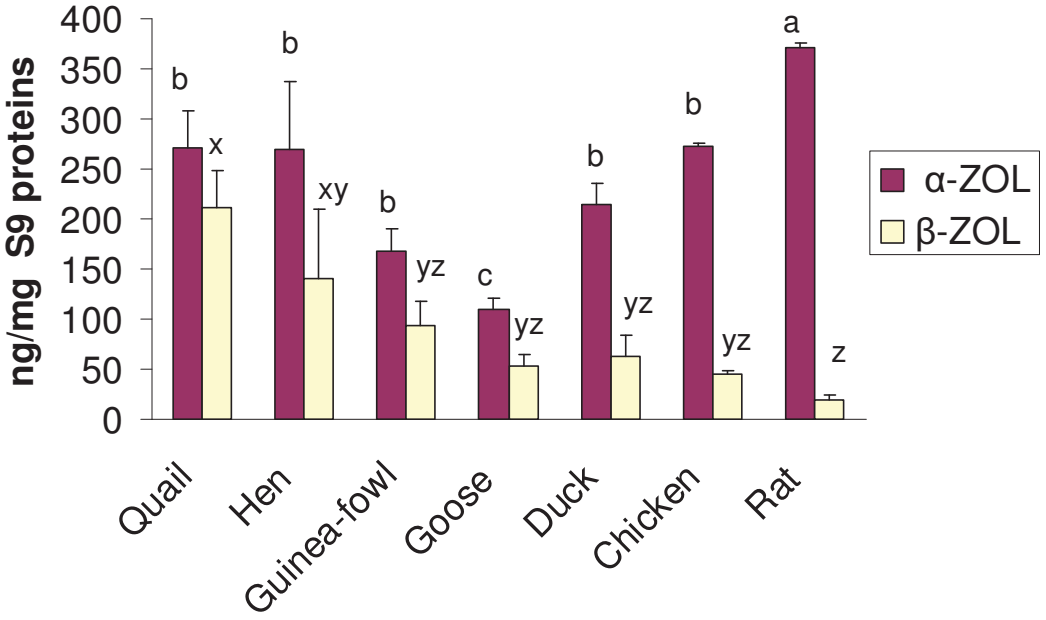
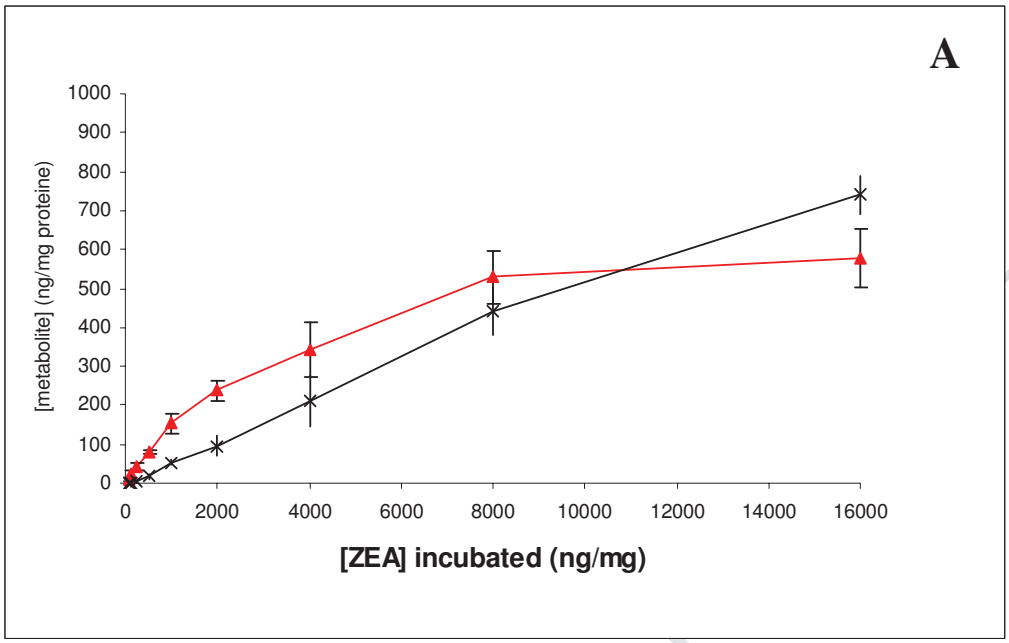


Fig.2

Fig.3A et 3B



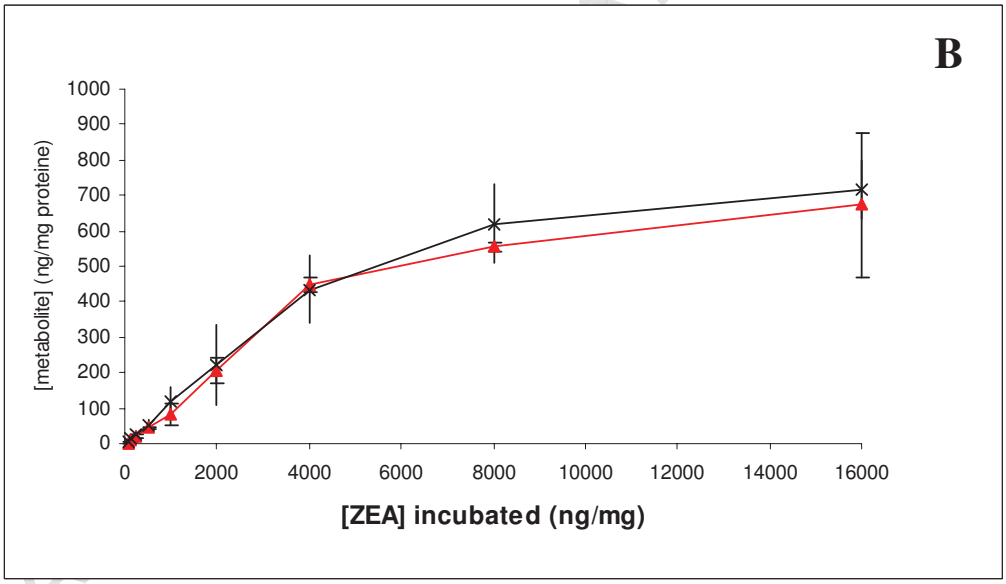


Table 1

	Enzyme 1		Enzyme 2		
	V1max (ng/mg /min)	K1m (ng/mg protein)	V2max (ng/mg /min)	K2 m (ng/mg protein)	R
Quail					
α-zearalenol	2256	28918	2458	28969	0.9811
β-zearalenol	2474	29717	2605	29462	0.9909
Duck					
α-zearalenol	1662	20711	2060	20683	0.9945
β-zearalenol	- 4824	$-6.61.10^{6}$	17303	298088	0.9984