Mutagenic activation and detoxification of benzo[*a*]pyrene *in vitro* by hepatic cytochrome P450 1A1 and phase II enzymes in three meat-producing animals

Darwish W. S.[a,b], Ikenaka Y.[a], Eldaly E. A.[b] and Ishizuka M.[a,*]

[a] *Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18, W9, Kita-ku, Sapporo 060-0818, Japan*

[b] *Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt*

***Corresponding Author:** Ishizuka Mayumi, Associate Prof., PhD,

Laboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18, W9, Kita-ku, Sapporo 060-0818, Japan

Phone: +81-11-706-6949

Fax: +81-11-706-5105

E-mail address: ishizum@vetmed.hokudai.ac.jp

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Abbreviations:

Benzo[*a*]pyrene (B[*a*]P), bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), cytochrome P450 (CYP), ethoxyresorufin O-deethylase (EROD), glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PDH), glutathione reduced form (GSH), glutathione-S-transferase (GST), polycyclic aromatic hydrocarbons (PAHs), UDP-glucuronic acid (UGA), UDP glucoronosyl transferase (UGT).

Abstract

The mutagenic activation activity of hepatic microsomes from three meat-producing animals (cattle, deer and horses) was compared with those of rats as a reference species. In the Ames *Salmonella typhimurium* TA98 assay, the liver microsomes of all examined animals mutagenically activated benzo[*a*]pyrene, an ideal promutagens, in terms of production of histidine-independent revertant colonies. The microsomes of horses had the highest ability to produce revertant colonies of the examined animals under both low and high substrate concentrations. Inhibition of this mutagenic activity using α-naphthoflavone, anti-rat CYP1A1, CYP3A2 and CYP2E1 antibodies suggests that this activity was mainly because of CYP1A1 in these animals as well as in rats. The addition of co-factors for two phase II enzymes, microsomal UDP glucoronosyl transferase and cytosolic glutathione-S-transferase, reduced the production of the revertant colonies in a concentration-dependent manner. Interestingly, horses had the highest reduction rate among the examined animals, suggesting that phase II enzymes play a great role in producing a state of balance between the bioactivation and detoxification of xenobiotics in these meat-producing animals. This report is the first to investigate the mutagenic activation activity of the hepatic microsomes and the role of phase II enzymes against this activity in meat-producing animals.

Key words: Mutagenesis, CYP1A1, Phase II enzymes, Meat-producing animals

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (B[a]P) are commonly formed by the incomplete combustion of organic matter (Baird et al., 2005; Shimada et al., 2007). A number of polycyclic aromatic hydrocarbons, including B[a]P, are mutagenic and carcinogenic, and are widely believed to make a substantial contribution to the overall burden of cancer in humans and animals (Phillips, 2002; Shoket, 1999). B[a]P requires metabolic activation by phase I enzymes, especially cytochrome P450 (CYP) 1A, prior to reaction with DNA, to exert its genotoxic effects (Phillips, 2005; Shimada, 2006; Volker et al., 2008).

Meat-producing animals like cattle, deer and horses are in danger of exposure to promutagenic and procarcinogenic polycyclic aromatic hydrocarbons such as B[a]P, and the principle route of exposure is via their diet (Phillips, 1999; Phillips, 2002). Moreover, results obtained by several researchers showed that these meat-producing animals have drastically higher CYP1A-dependent ethoxyresorufin-O-deethylase (EROD) activities than rats (Machala et al., 2003; Nebbia et al., 2003; Sivapathasundaram et al., 2001; Darwish et al., 2010). This ungulate-specific phenomenon suggests the probability that these animals are exposed to large amounts of metabolically-activated mutagens and carcinogens. However, there is no clear information about the mechanism of protection in meat-producing animals against the adverse effects of such exposure to these promutagens.

Living organisms have developed enzyme systems that metabolize xenobiotics to hydrophilic and readily excretable metabolites. Such metabolism usually proceeds through distinct stages: phase I metabolism introduces a functional group into the molecule, and phase II metabolism involves conjugation of the phase I metabolites

through the functional group, with substrates such as glutathiones and glucuronic acids (Ioannides, 2002; Nebert and Dalton, 2006; Sivapathasundaram et al., 2003b). The phase II metabolism is catalyzed by a number of microsomal and cytosolic enzyme systems such as the UDP-glucoronosyl transferases (UGT), and glutathione-S-transferases (GST). These enzyme systems are very important to produce a state of equilibrium between the bioactivation and bioinactivation of foreign chemicals in the bodies of the living organisms (Sivapathasundaram et al., 2003b). Thus, this study was performed to investigate the ability of the hepatic microsomes of cattle, deer and horses to mutagenically activate B[*a*]P compared to rats in a comparative way. In addition, the role of phase I and II enzymes in that mutagenic activation, and the mechanism of protection from this mutagenic activity were investigated in this group of animals. Rats were used as reference species in this study because the role of their phase I and II enzymes in the mutagenic bioactivation and detoxification of B[*a*]P is well documented (Fujita et al., 1988).

2. Materials and Methods

2.1. Chemicals and reagents

All test reagents used were of reagent grade. Benzo[*a*]pyrene, α-naphthoflavone, ρ-nitrophenol, 1-chloro-2,4-dinitrobenzene (CDNB), glutathione reduced form (GSH), UDP-glucuronic acid (UGA) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). NADPH, glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PDH), and co-factor S9 were from Oriental Yeast (Tokyo, Japan). Polyclonal rabbit anti-rat CYP1A1, CYP3A2, and CYP2E1 antibodies (Daiichi Pure Chemical Co., Ltd., Tokyo, Japan), and rabbit IgG were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-rat CYP1A1 is reported to cross-react with the CYP1A2 of rat microsomes. All other reagents were analytical grade or the highest quality available and purchased from Wako Pure Chemical Industries (Tokyo, Japan).

2.2. Animals

All experiments using animals were performed according to the guidelines of the Hokkaido University Institutional Animal Care and Use Committee. Liver samples were collected from five adult females of each species. Samples from Holstein cattle (*Bos taurus*) between the ages of four and five years (4.67±0.58 year old) were purchased from Hokkaido University cattle farm. Samples from thoroughbred horses (*Equus caballus*) between the ages of four and six years (5.33±1.15 year old) were kindly gifted from JRA (Japan Racing Association, Japan). Ezo shika deer (*Cervus hortulorum yesoensis*) were hunted from wild life (Hokkaido, Japan) during winter season, and their

ages were estimated by tooth eruption and tooth wear patterns (2.00±0.71 year old). The livers of these ungulates were excised immediately after slaughter or hunting and were transferred to liquid nitrogen tanks. Nine week old Wistar female rats (SLC Hamamatsu, Japan) were housed at 24±1°C with 12 hr light and 12 hr dark cycles, and given laboratory feed and water ad libitum. Rats were anaesthetized and sacrificed with carbon dioxide. The rat livers, used as controls in this study, were removed and perfused with cold 1.15% KCl which removed the blood.

2.3. Preparation of liver microsomes

Liver microsomes from the animals were prepared by the method described by Omura and Sato (1964) with slight modifications. Livers were minced and homogenized in three volumes of ice-cold 1.15% KCl solution using a Teflon homogenizer. Homogenized samples were centrifuged at 9,000 x g at 4°C for 20 min. The supernatant fraction was centrifuged at 105,000 x g at 4°C for 70 min to obtain a mitochondria-free microsomal pellet. The washed microsomes were then suspended in 0.1 M potassium phosphate buffer (KPB), pH 7.4, divided among 1.5 ml tubes, snap-frozen in liquid nitrogen, and kept at -80°C until use. The protein concentrations of the microsomal, cytosolic, and S9 fractions were determined according to the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard.

2.4. Ames mutagenicity assay

A mutagenicity assay was performed according to the method described by Ames et al. (1975) with slight modifications. Briefly, *Salmonalla typhimurium* strain TA98, which is sensitive to frameshift mutations, was used. One milliliter of the reaction mixture containing 10 mg of protein, 10 mM G-6-P, 1 or 10 μM B[a]P in dimethyl sulfoxide (Fujita et al., 1988), 1 mg co-factor S9 mixture. The reaction was started by adding 20 μL of a mixture of 50 mM NADPH and 200 U/mL of G-6-PDH. After incubation for 20 min at 37°C, the reaction was terminated by adding of top agar (maintained at 45°C). The tubes were then immediately plated onto minimum glucose plates (2% glucose, 15% agar) in duplicate and incubated at 37°C for 48 h. Histidine-independent mutants were scored manually using a colony counter. Background colony formation was consistently between 28 and 35 colonies/plate.

2.4. Inhibition of mutagenic activation of B[a]P

To inhibit B[*a*]P mutagenic activation, α-naphthoflavone, a well-known chemical inhibitor of the CYP1 subfamily (Ioannides, 2006; Sivapathasundaram et al., 2003a), was added at concentrations of 2.5, 5, 10, or 20 μM to the reaction mixture using 10 μM B[a]P. Immunoinhibition was performed in this experiment using a rabbit anti-rat CYP1A1 antibody that recognizes both isoforms of the CYP1A subfamily. To investigate the contribution of other CYP subfamilies in this activity, anti-rat CYP3A2 and anti-rat CYP2E1 antibodies were used. The used anti-rat CYP antibodies were reported to cross-react with CYP subfamily proteins from other species (Sivapathasundaram et al., 2001; Nebbia et al., 2003). Antibodies were preincubated with microsomes in a concentration range of 2.5-10 μg/μL for 30 min based on the

manufacturer's instructions and the method recommended by Fukuhara et al. (1999). The assays were subsequently completed as described previously with the use of 10 μM B[a]P. At the same time, rabbit IgG was used as a negative control with the same concentrations as the antibody. The effect of the inhibitors on the bacterial growth was also examined.

2.5. Effect of phase II enzymes on mutagenic activation of B[a]P

To investigate the effect of phase II enzymes on the mutagenic activation of B[*a*]P, UGA, a co-factor for UGT, was added in different concentrations (0.2, 1, and 10 mM) or GSH (10, 20, and 30 mM) in 50 mM phosphate buffer (pH 7.5) to the reaction mixture using 10 μM B[a]P and incubated it at 37°C for 20 min prior to addition of the top agar as described by Fujita et al. (1988). In the case of GSH, the liver S9 fraction was used instead of liver microsomes because of the higher concentration of GST in the cytosol than in the microsomes. The effect of the co-factors on the bacterial cell viability was also examined.

2.6. UGT activity in meat-producing animals and rats

An assay was performed using ρ-nitrophenol as a substrate according to the method described by Fujita et al. (1988) with minor modifications. Briefly, the reaction mixture was prepared in an optical cuvette containing 0.5 mg/mL microsomal fraction, 0.02% Triton X-100, 5 mM $MgCl_2$, 0.25 mM ρ-nitrophenol, and 1.5 mM of UGA in 0.1 M tris-HCl buffer (pH 7.0) in the final solution. The reaction was started by the addition of UGA. The decrease in absorbance at 400 nm was continuously measured using a

Hitachi U3300 spectrophotometer. The reaction was linear with time for at least 2 min. Further linearity of the reaction was obtained by increasing UGA concentrations.

2.7. GST activity in meat-producing animals and rats

CDNB was used as a substrate to determine of GST activity, according to the method described by Habig et al. (1974) with minor modifications. In short, the reaction mixture contained final concentrations of 25 μg/mL cytosolic fraction, 0.5 mM CDNB, and 0.5 mM GSH in 0.1 M potassium phosphate buffer (pH 7.0). The mixture was preincubated at 37°C for 2 min, and the reaction was started by the addition of GSH. Incubation was carried out in a shaking water bath for 3 min and was terminated by the addition of 33% trichloroacetic acid. The mixture was centrifuged at 1500 x g for 10 min. The absorbance of the supernatant was determined at a wavelength of 340 nm. The same procedure was applied to the assay mixture without the cytosolic fraction to assess nonenzymatic conjugation. The amount of enzymatic CDNB-GSH conjugate formed was calculated by subtracting the non-enzymatic conjugate formation from the total conjugate formation using an extinction coefficient of 9.6 mM^{-1} cm^{-1} for the GSH conjugate of CDNB.

2.8. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical significances were evaluated by Tukey's Kramer HSD difference test using JMP (SAS Institute, Cary, NC, USA). $P<0.05$ was considered to be significant.

3. Results

3.1. Mutagenic activation of B[a]P by liver microsomes of meat-producing animals and rats

The liver microsomes of all animals had the ability to mutagenically activate B[*a*]P in the Ames mutagenicity assay. Horse CYP1A had the highest ability to produce revertant colonies through B[*a*]P hydroxylation among the species examined in the two concentrations examined. The mean values of the revertant colonies produced by horse, cattle, deer and rat liver microsomes were 71±7, 48±4, 49±6 and 49±2 at 1 μM B[a]P, and 138±12, 100±10, 70±1 and 82±13 at 10 μM B[a]P (Fig. 1A, B). In this assay, *Salmonalla typhimurium* strain TA100 was also used, but we did not find significant difference between the two strains (Data are not shown).

Addition of α-naphthoflavone, a chemical inhibitor of the CYP1A subfamily, significantly inhibited this mutagenic activity in a concentration-dependent manner. This inhibition was especially noticeable at 20 μM, when the inhibition percentages were 50%, 51%, 57%, and 52% in rats deer, cattle, and horses respectively (Fig. 2A). In a trial to inhibit the mutagenic activity by immunoinhibition, after incubation for 30 min with the microsomes, all animals examined showed cross reactivity with the anti-rat CYP1A1 antibody used in this study. Anti-rat CYP1A1 antibody inhibited the B[*a*]P mutagenic activation in all animals in a concentration-dependent manner. Significant inhibition was clear when the concentration of the antibody was 10 μg because the inhibition percentages were 83%, 80%, 70%, and 83% in rats, deer, cattle and horses respectively (Fig. 2B). To investigate the contribution of other cytochrome P450 subfamilies in this activity, the liver microsomes were incubated with anti-rat CYP3A2

and anti-rat CYP2E1 antibodies for 30 min prior to the reaction; however, we did not find any significant inhibition in all species examined, as shown in Figures 3A and 3B, suggesting that these subfamilies have no role in this metabolic activity under these conditions. The inhibitors did not show any significant effect on the cell viability in the absence of the liver microsomes.

3.2. Role of phase II enzymes against B[*a*]P mutagenic activation in meat producing animals and rats

The addition of cofactors of phase II enzymes such as UGA, a co-factor of UGT, significantly reduced the production of revertant colonies in a concentration-dependent fashion. At the highest concentration, 10 mM, the production of the revertants was reduced to 63%, 64%, 55%, and 42% in rats, deer, cattle and horses, respectively, as shown in Figure 4A. The addition of GSH, a cofactor for GST, also significantly reduced the production of the revertants in a concentration-dependent manner. At 30 mM GSH, the production of the colonies was reduced to 65%, 58%, 76%, and 49% in rats, deer, cattle and horse S9 fractions, respectively (Fig. 4B). The co-factors did not show any significant effect on cell viability in the absence of the liver microsomal or S9 fractions.

To determine the other phase II enzymes activities, specific substrates were used. In the case of UGT, ρ-nitrophenol was used as a specific substrate, but no significant difference was found in the UGT activity in the liver microsomes of cattle, deer, and horses compared to rats because their activities were 14.0±2.0, 11.3±1.1, 12.7±3.1, and 14.7±4.2 nmol/min/mg microsomal protein, respectively (Fig. 5A). CDNB was used as the specific substrate to measure the cytosolic GST activity in these animals; horse had

the highest activity at 1.2±0.1 after rat at 1.5±0.3 compared to cattle and deer, whose GST activities were 0.6±0.1and 0.6±0.1 μmol/min/mg cytosolic protein, respectively (Fig. 5B).

4. Discussion

Cytochrome P450, a superfamily of heme proteins, is involved in the metabolism of a vast array of carcinogens, drugs and endogenous compounds. The liver is the major organ involved in P450-mediated xenobiotic metabolism (Conney, 2003). CYP1A1, a member of the P450 superfamily, contributes notably to the toxicity of many carcinogens, especially polycyclic aromatic hydrocarbons, because it is the principal enzyme that bioactivates inert hydrocarbons into DNA-binding reactive metabolites (Kommaddi et al., 2007). Polycyclic aromatic hydrocarbons are ubiquitous environmental carcinogens that have been shown to become carcinogenic only after metabolic activation by cytochrome P450, especially CYP1A1 (Conney, 1982; Shimada, 2006).

Cattle, deer and horses are important sources of animal-derived food products; nonetheless, our understanding about the expression of drug-metabolizing enzymes, either phase I or II enzymes, in food-producing animals still remains superficial, despite the obvious toxicological consequences (Giantin et al., 2008). Although several separate studies have investigated the drug metabolizing enzymes in deer, cattle, or horses as shown by several researchers (Machala et al., 2003; Nebbia et al., 2003; Sivapathasundaram et al., 2001; Sivapathasundaram et al., 2003a; Darwish et al., 2010), no study of the ability of the liver microsomes of these animals to mutagenically activate promutagens has been reported. Tometsko et al. (1981) found that chickens had the highest B[a]P activation level compared to rats, followed by cattle, pigs and sheep. Smith et al. (2007) quantitatively compared the different testicular and hepatic microsomal metabolisms of B[a]P in rats, mice, hamsters, rams, boars, bulls, and

monkeys. Also, Harris et al. (2009) reported the concentrations of B[a]P different metabolites generated by the microsomes of liver and ovaries of rats, mice, goats, sheep, pigs, and cows. Higher CYP1A-dependent activity and protein expression were observed in these animals than in rats (Machala et al., 2003; Nebbia et al., 2003; Sivapathasundaram et al., 2001; Sivapathasundaram et al., 2003a; Darwish et al., 2010), and higher epoxide hydrolase activity, a very important enzyme in the B[a]P metabolic pathway, was observed in ungulates compared to rats (Sivapathasundaram et al., 2003b). These activities may indicate a high risk of mutagenesis and carcinogenesis in these ungulates if they are exposed to promutagens. Thus, we performed this study not only to investigate the ability of cattle, deer and horses to activate B[a]P, an ideal promutagen, but also to investigate the contribution of phase II enzymes against this mutagenic activity and the mechanism of protection against the adverse effects produced by phase I metabolism. In addition, other phase II enzymes activities in this group of animals were studied in comparison to rats.

In this study, we found that the liver microsomes of these animals mutagenically activated B[a]P in the Ames mutagenicity assay. Interestingly, horse liver microsomes had the highest ability to produce revertant colonies compared to the other animals examined. Consistent with this finding, Nebbia et al. (2003) and Darwish et al. (2010) found that horse liver microsomes had the highest EROD, CYP1A-dependent activity compared to several agricultural and laboratory animals. This correspondence may indicate that the high mutagenic activation of B[a]P in horses is attributable to the high CYP1A activity in this animal species. To confirm that this mutagenic activity was because of the CYP1A subfamily, we studied the inhibition of this activity by chemical means, using a specific inhibitor of the CYP1 family, α-naphthoflavone, or by

immunological means, using the anti-rat CYP1A1 antibody. These inhibitors inhibited this mutagenic activity in a concentration-dependent manner. In order to evaluate the contribution of other cytochrome P450 subfamilies to this activity, we used anti-rat CYP3A2 and anti-rat CYP2E1; however, these antibodies had no significant effect on the mutagenic activity. This result highly suggests that as in the rat, the mutagenic activity of B[*a*]P in the meat-producing animals examined is mainly because of the CYP1A subfamily. In correspondence with our finding, Shimada and Fuji-Kuriyama (2004) declared that CYP3A4, 2A6, 2C9, and 2C19 had no significant contribution on the mutagenic activation of B[a]P in mice using umu gene expression test. While CYP1A1 metabolism generates reactive metabolites, the importance of CYP1B1, which is mostly extrahepatic, cannot be ruled out. As, Kim et al. (1998) indicated that CYP 1B1 carries out metabolism of B[*a*]P along the pathway to the postulated ultimate carcinogen, the diol epoxide 2, at rates much higher than P450 1A2 but less than P450 1A1 in rodent tumor models and in humans. The role of CYP 1B1 in B[*a*]P metabolism in ungulates is still to be investigated in our future study.

In rats and humans, phase II enzymes like UGT and GST play an important role in protection from the adverse effects produced by the metabolites of phase I, as reported previously by Fujita et al. (1988) and Shimada, (2006). To affirm the effects of the phase II enzymes against this mutagenic activity in the meat-producing animals, we added GSH and UGA, co-factors for GST and UGT, to the reaction mixture in the Ames assay. Phase II enzymes reduced the production of the revertant colonies in a concentration-dependent fashion in all examined animals, and this result agrees with that obtained by Fujita et al. (1988) in rat experiments. Surprisingly, the highest significant reduction percentage was recorded in horses, suggesting that horses had also

the highest UGT- and GST- dependent activities of all the examined animals. This finding may explain in part how these animals, and in particular horses, protect themselves from the adverse effects of environmental pollutants. Moreover, Shimada et al. (2007) reported that the parent polycyclic aromatic hydrocarbons may act as inhibitors of further metabolism of their own metabolites to prevent the carcinogenic effects either through direct inhibition or in a competitive mechanism.

Because of the lack of information about the activities of phase II enzymes in these meat-producing animals, and to confirm the high UGT- and GST- dependent activities in horses compared to cattle, deer and rats, we investigated the UGT and GST activities using their specific substrates. In the case of UGT, we used ρ-nitrophenol as a specific substrate, but we did not find any significant difference between horses and the other animals examined compared to the results of the Ames assay. Thus, further studies are needed to investigate the activities of the phase II enzymes in horses. CDNB was used as a specific substrate for GST activity. Horses also had the highest GST activity compared to other meat-producing animals.

In conclusion, although environmental pollution endangers meat-producing animals in many locations around the world, and although this may lead to mutagenesis and/or carcinogenesis, the bodies of these animals adapt to these changes by increasing the activities of phase II enzymes. Phase II enzymes, particularly in horses, play a vital role in producing a state of balance between the bioactivation and bioinactivation of environmental chemicals in living organisms.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Figure legends

Figure 1. Mutagenic activation activity of CYP1A in three meat producing-animals and rats

The number of revertant colonies reflects the mutagenic activation activity of CYP1A in the microsomes of different meat-producing animals compared to rats when benzo[*a*]pyrene is used as the substrate with (A) 1µM or (B) 10µM. The data represent the mean±SD for five animals from each species. Identical letters are not significantly different from each other. $P<0.05$.

Figure 2. Inhibition of B[*a*]P mutagenic activation in three meat-producing animals and rats by CYP1A specific inhibitors

A) Effect of α-naphthoflavone on B[*a*]P (10µM) mutagenic activation in three meat-producing animals compared to rats, under different concentrations of the inhibitor, α-naphthoflavone, (2.5, 5, 10, and 20 µM). The data represent the mean±SD for five animals from each species. B) Effect of anti-rat CYP1A1 antibody on B[*a*]P (10µM) mutagenic activation in cattle, deer and horses hepatic microsomes compared to rats, under different concentrations of the inhibitor anti-rat CYP1A1 antibody (2.5, 5, and 10 µg) after incubation of both microsomes and the antibody for 30 min at room temperature. Data at 0 concentration represent the B[*a*]P (10µM) mutagenic activation without addition of antibody. Rabbit IgG was used as a negative control with the following concentrations (2.5, 5, and 10 µg) instead of anti-rat CYP1A1 antibody in all animals (data are not shown in this figure). The data represent means±SD of three experiments performed at different times using liver microsomes of five animals of each

species. The data at concentrations of 2.5, 5, and 10 μg of the antibody are significantly different from the data at concentration of 0 μg antibody in all animals. $P<0.05$.

Figure 3. Immunoinhibition of B[a]P mutagenic activation in three meat-producing animals and rats by inhibitors of other CYPs

A) Effect of anti-rat CYP3A2 antibody on B[a]P (10μM) mutagenic activation in cattle, deer and horse hepatic microsomes compared to rats under different concentrations (2.5, 5, and 10 μg) after incubation of both microsomes and the antibody for 30 min at room temperature. Data at 0 concentration represent the B[a]P (10μM) mutagenic activation without addition of antibody. Rabbit IgG was used as a negative control with the following concentrations (2.5, 5, and 10 μg) instead of anti-rat CYP3A2 antibody in all animals (data are not shown in this figure). The data represent means±SD of three experiments performed at different times using liver microsomes of five animals. B) Effect of anti-rat CYP2E1 antibody on B[a]P (10μM) mutagenic activation in cattle, deer and horse hepatic microsomes compared to rats under different concentrations (2.5, 5, and 10 μg) after incubation of both microsomes and the antibody for 30 min at room temperature. Data at 0 concentration represent the B[a]P (10μM) mutagenic activation without addition of antibody. Rabbit IgG was used as a negative control with the following concentrations (2.5, 5, and 10 μg) instead of anti-rat CYP2E1 antibody in all animals (data are not shown in this figure). Data represent means±SD of three experiments performed at different times using liver microsomes of five animals. $P<0.05$.

Figure 4. Effect of UGT and GST on B[a]P mutagenic activation in three meat-producing animals and rats

A) Effect of UGT on B[*a*]P (10μM) mutagenic activation in cattle, deer and horse hepatic microsomes compared to rats under different concentrations of UGA, a co-factor of UGT (0.2, 1, and 10 mM). Data at 0 concentration represent the B[*a*]P (10μM) mutagenic activation without adding UGA. Data represent means ± SD of three experiments performed at different times using liver microsomes of five animals of each species. B) Effect of GST on B[*a*]P (10μM) mutagenic activation in cattle, deer and horse hepatic S9 compared to rats under different concentrations (10, 20, and 30 mM) of GSH, a co-factor of GST. Data at 0 concentration represent the B[*a*]P (10μM) mutagenic activation without addition of GSH. Data represent means±SD of three experiments performed at different times using liver S9 of five animals of each species. $P<0.05$.

Figure 5. UGT and GST activities in three meat-producing animals and rats

A) UGT activity towards ρ-nitrophenol (nmol/min/mg protein) of hepatic microsomes from cattle, deer and horses compared to rats, as determined by the assay described in the Methods section. Data represent means±SD of three experiments performed at different times using liver microsomes of five animals of each species. Identical letters are not significantly different from each other. $P<0.05$. B) GST activity towards 1-chloro-2,4-dinitrobenzene (μmol/min/mg protein) of hepatic cytosols from cattle, deer and horses compared to rats, as determined by the assay described in the Methods section. Data represent means±SD of three experiments performed at different times using cytosol of five animals of each species. Identical letters are not significantly different from each other. $P<0.05$.

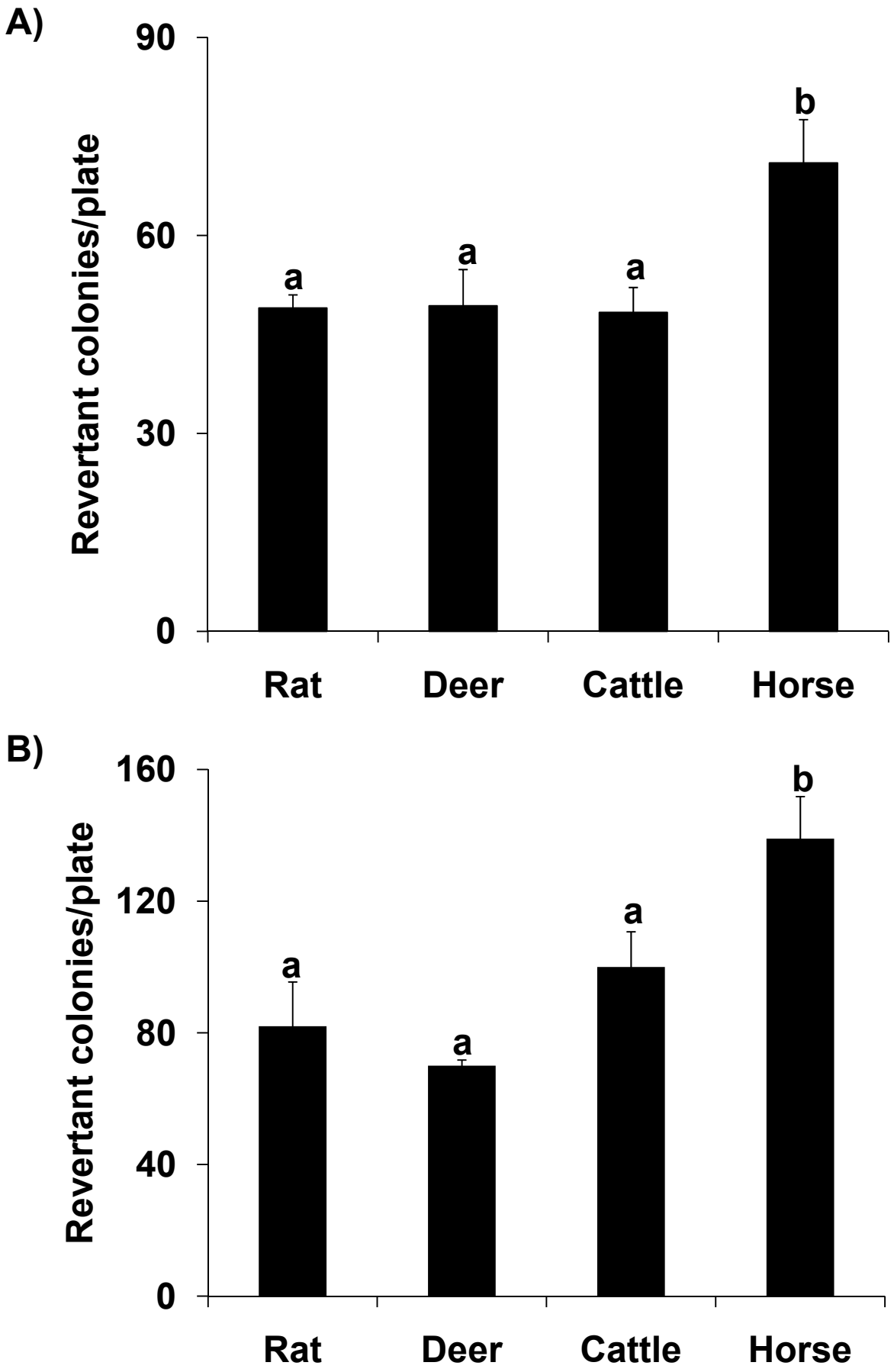
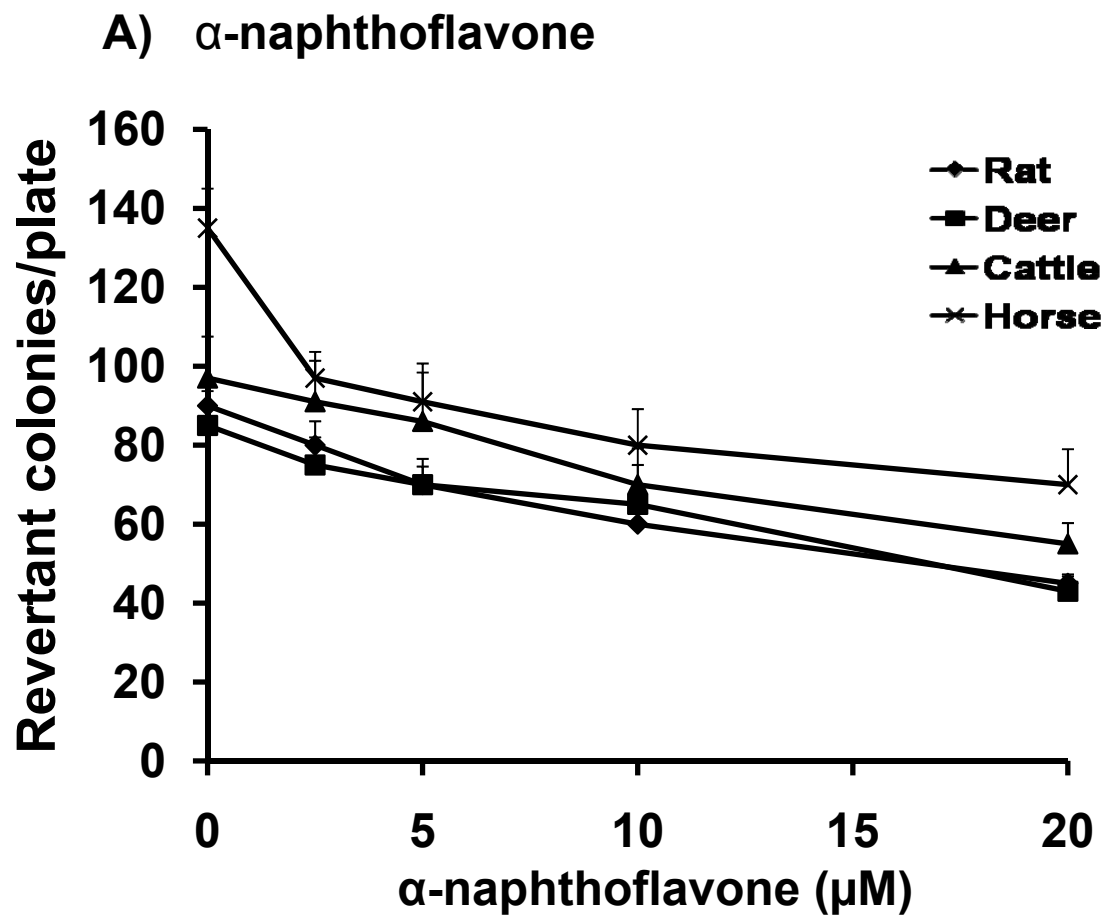

A)
Revertant colonies/plate
90
60
30
0
a
a
a
b
Rat
Deer
Cattle
Horse
B)
Revertant colonies/plate
160
120
80
40
0
a
a
a
b
Rat
Deer
Cattle
Horse

Figure 1


A) α-naphthoflavone
Revertant colonies/plate
160
140
120
100
80
60
40
20
0
Rat
Deer
Cattle
Horse
0
5
10
15
20
α-naphthoflavone (μM)

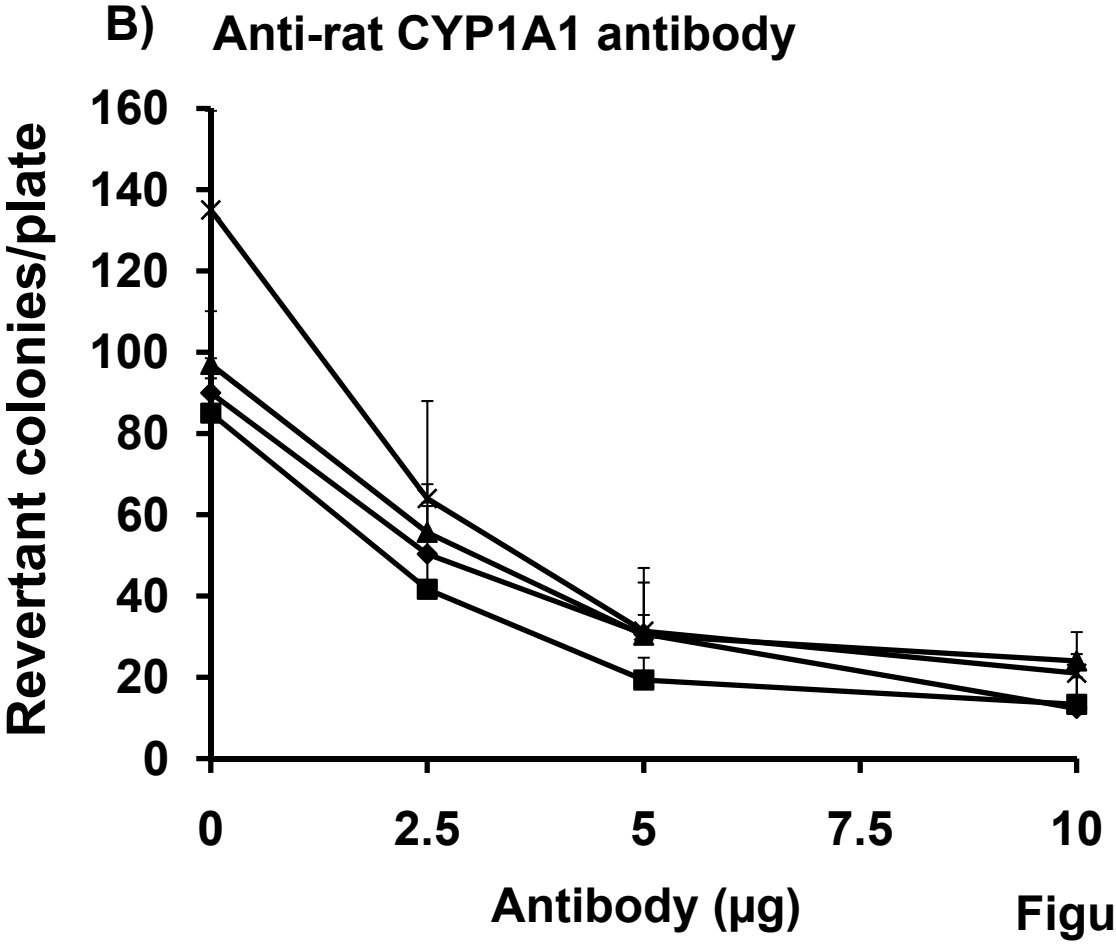

B) Anti-rat CYP1A1 antibody
Revertant colonies/plate
160
140
120
100
80
60
40
20
0
0
2.5
5
7.5
10
Antibody (μg)

Figure 2

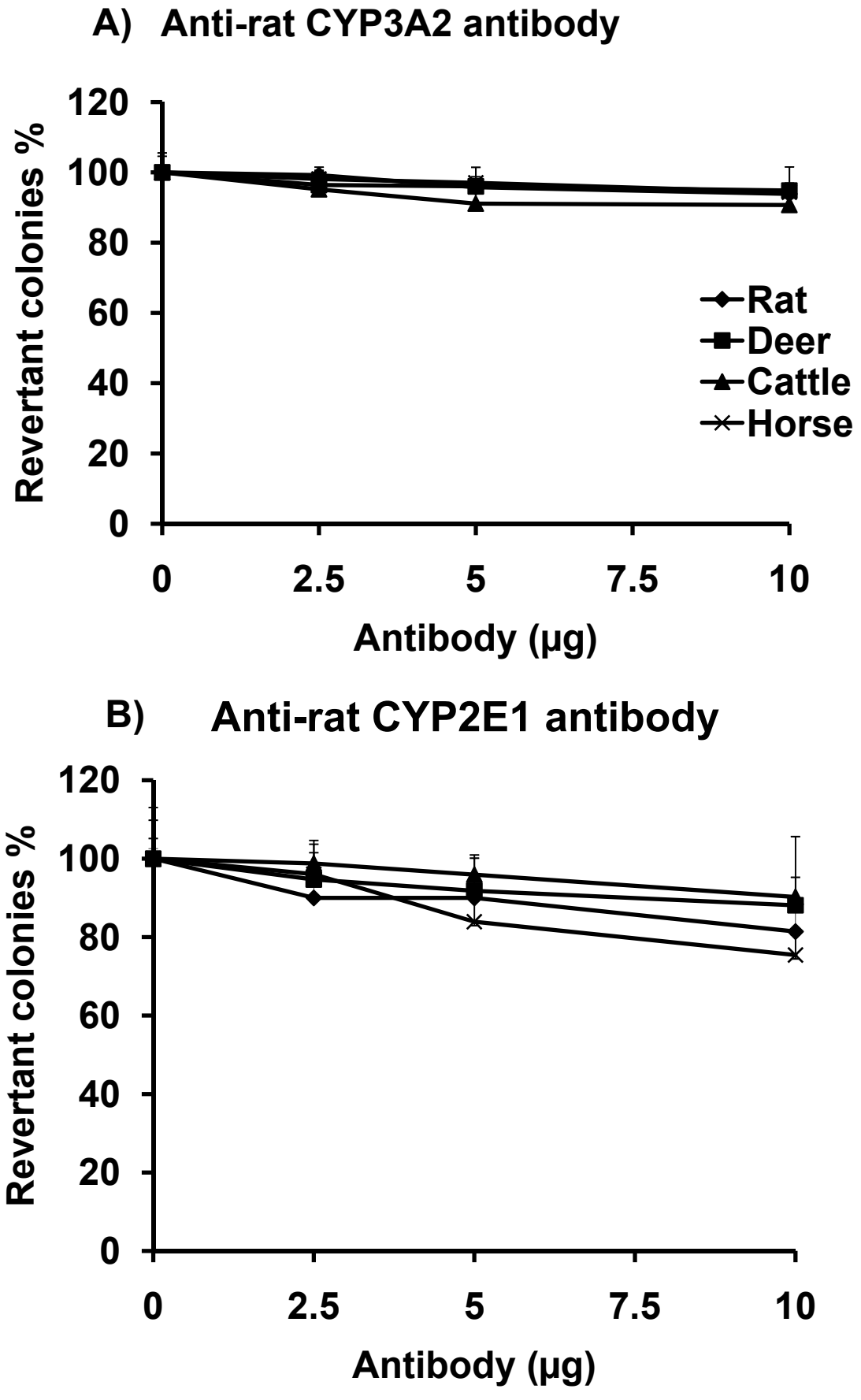

A) Anti-rat CYP3A2 antibody
Revertant colonies %
120
100
80
60
40
20
0
0
2.5
5
7.5
10
Antibody (µg)
Rat
Deer
Cattle
Horse
B) Anti-rat CYP2E1 antibody
Revertant colonies %
120
100
80
60
40
20
0
0
2.5
5
7.5
10
Antibody (µg)

Figure 3

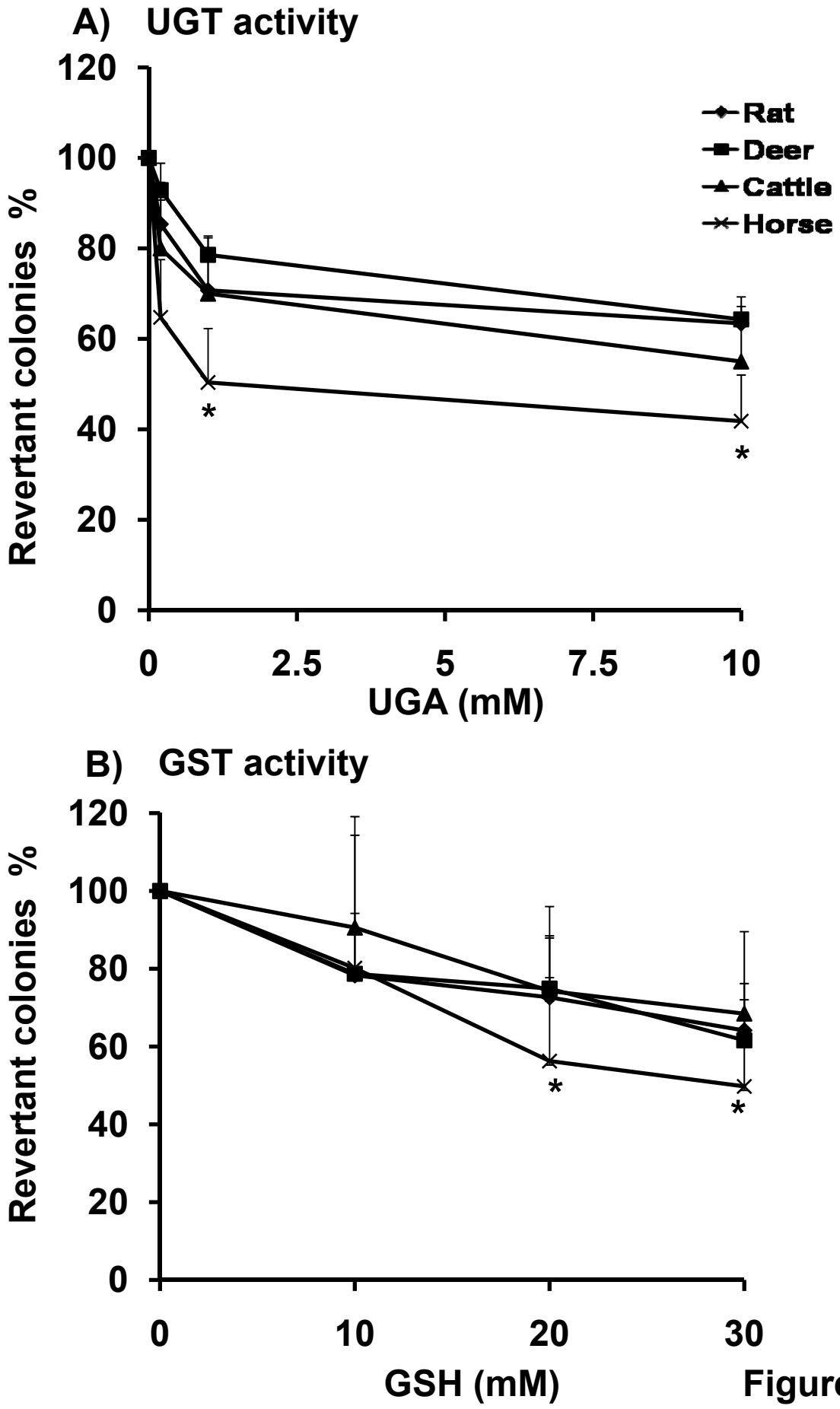

A) UGT activity
Revertant colonies %
120
100
80
60
40
20
0
Rat
Deer
Cattle
Horse
*
*
0
2.5
5
7.5
10
UGA (mM)
B) GST activity
Revertant colonies %
120
100
80
60
40
20
0
*
*
0
10
20
30
GSH (mM)

Figure 4

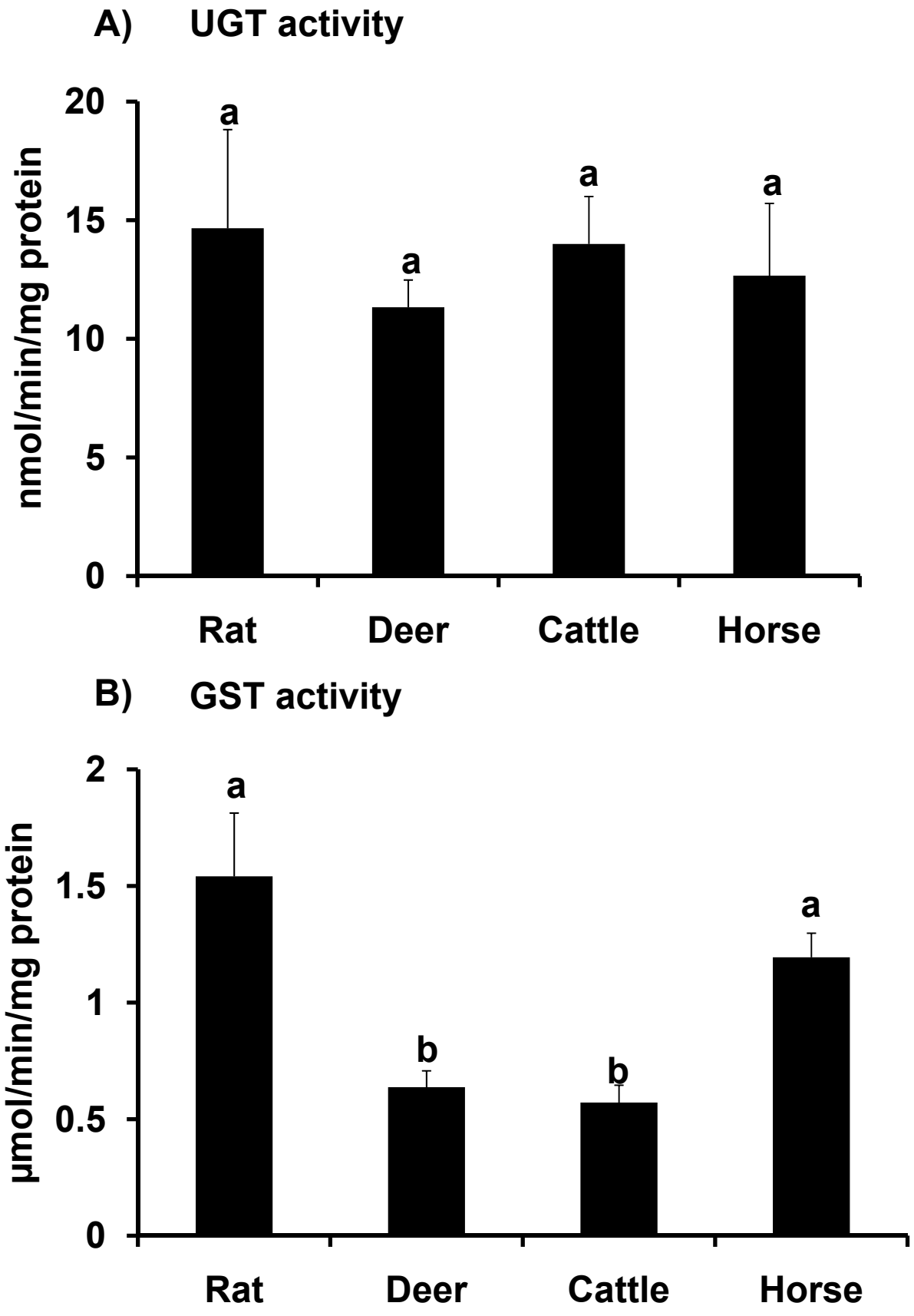

A) UGT activity
nmol/min/mg protein
20
15
10
5
0
a
a
a
a
Rat
Deer
Cattle
Horse
B) GST activity
µmol/min/mg protein
2
1.5
1
0.5
0
a
b
b
a
Rat
Deer
Cattle
Horse

Figure 5