DOI:10.1021/jf9033317

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effects of Illicit Dexamethasone upon Hepatic Drug Metabolizing Enzymes and Related Transcription Factors mRNAs and Their Potential Use As Biomarkers in Cattle

Mery Giantin,[‡] Rosa M. Lopparelli,[‡] Vanessa Zancanella,[‡] Pascal G. Martin,[†] Arnaud Polizzi,[†] Guglielmo Gallina,[‡] Flaviana Gottardo,[§] Clara Montesissa,[‡] Licia Ravarotto,[∥] Thierry Pineau,[†] and Mauro Dacasto*,[‡]

[‡]Dipartimento di Sanità pubblica, Patologia comparata e Igiene veterinaria, Università di Padova, viale dell'Università 16, I-35020 Agripolis Legnaro, Padova, Italy, [†]Laboratoire de Pharmacologie et Toxicologie, Institut National de la Recherche Agronomique (INRA)- UR66, 180 Chemin de Tournefeuille, F-31931 Toulouse, France, [§]Dipartimento di Scienze animali, Università di Padova, viale dell'Università 16, I-35020 Agripolis Legnaro, Padova, Italy, and [∥]Istituto Zooprofilattico Sperimentale delle Venezie, viale dell'Università 10, I-35020 Legnaro, Padova, Italy

In cattle fattening, the illicit use of growth promoters (GPs) represents a major problem. The synthetic corticosteroid dexamethasone (DEX) is the GP mostly used, alone or in combination with other steroids or β-agonists. Recently, GPs were shown to disrupt some cattle cytochromes P450 (CYPs) at the post-transcriptional level; therefore, the effects of two illicit protocols containing DEX (alone or together with 17β-estradiol, 17βE) upon main cattle liver drug metabolizing enzymes (DMEs) mRNAs and related transcription factors were investigated by quantitative real time RT-PCR. Eleven genes, out of the 18 considered, were significantly modulated by GPs. Corticosteroid-responsive genes did not respond univocally, whereas retinoic X receptor alpha (RXRα) and estrogen receptor alpha (ERα) were upregulated depending on the illicit protocol used. Nowadays, an increasing interest has been noticed toward the detection of biomarkers of response (BMRs) to be used in the screening of GPs misuse in cattle farming. In the present study, CYP2B6-*like*, CYP2E1, glutathione *S*-transferase A1- and sulfotransferase A1-*like* (GSTA1- and SULT1A1-*like*) mRNAs were significantly modulated regardless of the GP, the illicit protocol, and the animal breed, representing promising BMRs. The usefulness of these BMRs needs to be characterized more in depth.

KEYWORDS: cattle; drug metabolizing enzymes; growth promoters; dexamethasone; gene expression; biomarker

INTRODUCTION

In the meat cattle industry, a relevant concern is the use and abuse of growth promoters (GPs) to increase animal performances, and the fluorinated hydrocortisone derivative dexamethasone (DEX) is the GP more commonly used. It is usually administered orally at low dosages, either alone or within protocols containing other GPs (i.e., steroid hormones and β-agonists). The illicit use of GPs has been banned at the European Community level, and Member States official control programs consist of the GP analytical identification in urine samples; unfortunately, these assays are not useful whenever compounds of unknown chemical structure, present at levels below the limit of detection, or administered in cocktails, are used (*1*−*3*). Consequently, an increasing interest toward the discovery and, hopefully, the validation of molecular biomarkers of response (BMRs), to be used side by side with official analytical ones in the screening of GPs abuse, has been recently noticed (*4*−*8*).

*Corresponding author: E-mail: mauro.dacasto@unipd.it. Phone: +39.049.827.2935. Fax: +39.049.827.2973.

Drug metabolizing enzymes (DMEs) play an outstanding role in metabolism, detoxification (or bioactivation), and elimination of xenobiotics as well as of certain endogenous compounds (i.e., steroids, bile acids). These enzymes usually catalyze phase I (oxidation, reduction, and hydrolysis) or phase II (conjugation) reactions. Mostly abundant in the liver, they are also constitutively expressed in other tissues and organs, that is, gastrointestinal tract, lung, and kidney (*9*). The basic knowledge about cattle DMEs is still superficial; in past years, post-translational data were essentially published (*10*, *11*), and only recently, following progress in genetics and genomics culminating for many species in the completion of the entire genome sequencing, the molecular biology of cattle DMEs has become the subject of investigation, extending knowledge also at the pretranscriptional level (*12*−*14*). Such evidence, common to most veterinary species, is usually brought back to the overall lesser importance attributed in these species to drug metabolism studies. This is rather peculiar: cattle, and other farm animals, are exposed to xenobiotics (i.e., drugs, pesticides, pollutants) that might represent a risk for the animal itself but potentially also for humans, whenever the consumption

 Published on Web 12/30/2009

of edible tissues containing residues occurs (*10*). Consequently, most veterinary pharmacologists and toxicologists affirm that more comparative studies about the effects of xenobiotics upon DMEs expression and regulation phenomena are needed.

In humans, DEX undergoes hepatic oxidative and conjugative biotransformations (*15*); moreover, the involvement of certain transcription factors (TFs) in the regulation of its effects upon DMEs has been clearly demonstrated (*16*). In ruminants, DEX is thought to be mainly hydroxylated at the 6-position and reduced at the 3-carbonyl group; then, both the parent drug and the metabolite undergo glucuronidation or sulphation (*3*, *15*). Recently, DEX, used for growth promoting purposes alone or in combination with other GPs, was shown to affect post-transcriptionally and to a various extent cattle cytochromes P450 (CYPs) (*17*, *18*).

Therefore, in the present study the effects of two illicit protocols containing DEX were investigated on a set of candidate genes, consisting of most relevant oxidative and conjugative DMEs and their related TFs. The corticosteroid was administered alone or in combination with 17β-oestradiol (17βE); furthermore, chosen protocols (in terms of routes of administration and dosages) were similar to those illegally used in the field and gathered from previously published studies on GPs (*7*, *17*−*19*). As a whole, this study aimed at first to highlight a possible effect of DEX, when used at growth promoting purposes, upon cattle liver DMEs and TFs gene expression; on a second instance, to detect some (if any) potential molecular BMRs, whose employment in the screening of GP abuse in cattle might be envisaged, if confirmed and validated.

MATERIALS AND METHODS

Chemicals. Dexamethasone sodium phosphate (Desashock) was obtained from Fort Dodge Animal Health (Bologna, Italy); estradiol benzoate (Estradiolo AMSA) from AMSA srl (Roma, Italy). Chloroform, isopropyl, and ethyl alcohol are from Thermo Electron Corporation (Waltham, MA), whereas TRIzol reagent and agarose are from Invitrogen (Carlsbad, CA). The RNAlater solution, High Capacity cDNA Archive Kit and Power SYBR Green PCR Master Mix are from Applied Biosystems (Foster City, CA). Oligonucleotide primers were synthesized by Invitrogen.

Animals and Treatments. Two experiments were run in succession in an authorized facility located in the nearby Padua Faculty of Veterinary Medicine and according to the European Community Directive 86/609, recognized and adopted by the Italian Government (DLgs 116/92). The experimental plan was approved by the Italian Ministry of Health.

In the first one (experiment 1), 24 clinically healthy male Marchigiana beef cattle (about 490 kgs bw and 14−16 months old) were used. After an acclimatization period, they were allotted on a weight-basis into pens of three animals each and assigned to three different experimental groups: C_1 (n = 9, mean body weight 495 kg), D_1 (n = 9, mean bw 491 kg), and DIM (n = 6, mean bw 485 kg). The first one was the control group. Individuals from D_1 were orally administered with DEX, by using a balling gun, at a dose rate of 0.75 mg/per animal/per day and for 50 days; cattle from DIM group were intramuscularly injected with DEX (1.32 g/per animal), twice every 21 days, with the first injection scheduled on day 15 (see **Figure 1A**).

In the second experiment (experiment 2), 18 clinically healthy male French crossbred beef cattle (about 506 kg bw and 15−18 months old) were used. Animals were divided on a weight basis into three groups of six animals each: C_2 (control, 504 kg), D_2 (513 kg), and DE (502 kg). The D_2 group was orally administered with DEX, top-layered on the unifeed (0.75 mg/per animal/per day and for 43 days); individuals of DE group, besides DEX, were also intramuscularly injected with 20 mg/per animal 17βE, every 15 days for three times, with the first injection scheduled on day 0 (see **Figure 1B**).

Bovines were slaughtered three (experiment 1) or two (experiment 2) days after the suspension of the oral DEX administration (corresponding to 16 and 14 days after the last injection of DEX and E_2 for DIM and DE, respectively). After the bleeding step, aliquots (about 100 mg each) of the liver caudate lobe for total RNA extraction were collected in sterility and immediately stored in RNAlater solution at −80 °C, until use.

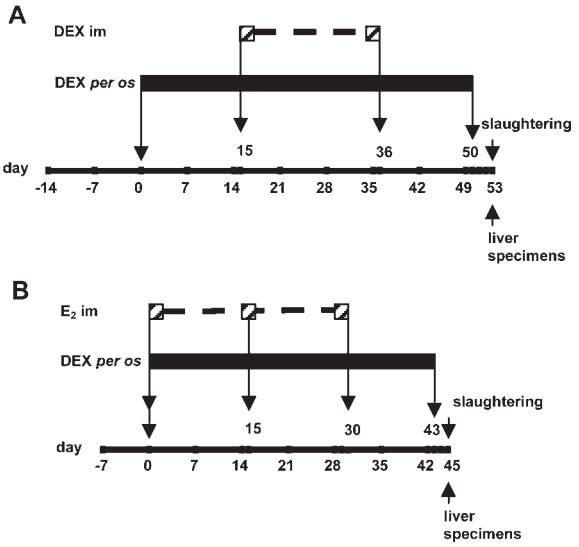

Figure 1. Treatments. In experiment 1 (**A**) cattle were administered DEX either orally (0.75 mg/per animal/day for 50 days) or intramuscularly (1.32 g/per animal, twice at 21-day intervals); in experiment 2 (**B**) cattle were orally treated with DEX alone (0.75 mg/per animal/day and for 43 days) or in association with 17βE (20 mg/per animal, intramuscularly injected at 15-day intervals).

Total RNA Isolation and Reverse-Transcription. Total RNA was isolated from bovine liver samples by using the TRIzol reagent, according to the manufacturer's instructions; briefly, 1 mL of TRIzol was added to about 80 mg of liver sample in a Lysing Matrice Tube (Qbiogene, MP Biomedicals, Illkirch, France) and immediately homogenized by means of the Fast-Prep FP120 (Qbiogene, MP Biomedicals, Illkirch, France), twice for 20 s. Samples were then purified with a standard phenol-chloroform extraction. Total RNA concentration and quality were determined by using the Nanodrop ND-1000 spectrophotometer (Labtech France, Paris, France). The isolated RNA quality was confirmed by denaturing gel electrophoresis.

The reverse transcription of 2 μg of total RNA was performed, in a final volume of 20 μL, by using the High Capacity cDNA Archive kit and following the purchaser's procedure.

Quantitative Real-Time RT-PCR (Q RT-PCR). *Bos taurus* mRNA sequences of target and reference genes were obtained from GenBank and Ensembl Genome Browser Web sites (http://ncbi.nlm.nih.gov/ and http://www.ensembl.org/, respectively). Primers sequences for Q RT-PCR were designed using Primer Express Software (version 2.0, Applied Biosystems, Foster City, CA). Oligonucleotides were designed at the exon−exon junctions to avoid genomic DNA amplification and were subjected to primer test analysis (Primer Test Document application in Primer Express Software) to exclude dimers synthesis.

GenBank accession number of genes chosen for primer design, oligonucleotides sequences and length, and the amplicon size are reported in **Table 1**.

Primers sequence specificity for each selected gene was checked against the NCBI BLAST database as well as with agarose gel electrophoresis and melting curves analysis. Each primers set was optimized in the 300−900 nM range to identify the primers concentration that provided the highest sensitivity and specificity. Calibration curves were obtained after the amplification of decreasing amounts of a cDNA pool diluted at 10-fold intervals to evaluate RT-PCR performances, that is, PCR efficiency (E_x, determined using the equation $E_x = 10^{-1/\text{slope}}$) and test linearity correlation. Only PCR with E_x comprised between 1.9 and 2.1 was considered as acceptable.

Bovine β-actin was chosen as the housekeeping gene among a group of three candidates (β-actin itself, β_2-microglobulin, and TATA-box binding protein) for the absence of statistically significant differences between groups, the lower variability between control and treated animals and,

Table 1. Primers Sequences, GenBank Accession Numbers, Primer Length and Amplicon Size of Candidate Drug Metabolizing Enzymes (DMEs), Nuclear Receptors (NRs), and Housekeeping Genes Used for Q RT-PCR

gene name	gene acronym	GenBank accession no.	5′ → 3′ primer sequence	primer length (bp)	amplicon size (bp)
cytochrome P450 1A1	CYP1A1	XM_588298	F: GACCTGAATCAGAGGTTCTACGTCT	25	81
			R: CCGGATGTGACCCTTCTCAA	20	
cytochrome P450 1A2	CYP1A2	NM_001099364	F: ACCATGACCCGAAGCTGTG	19	78
			R: CAATGGTGGTGCCATCAGAC	20	
cytochrome P450 2B6-*like*	CYP2B6-*like*	NM_001075173	F: GCGGACCTCATCCCCATT	18	80
			R: GTGCCCTTGGGAAGGATGT	19	
cytochrome P450 2C87	CYP2C87	XM_612374	F: TCCCTGGACATGAACAACCC	20	71
			R: TTGTGCTTTTCCTGTTCCATCTT	23	
cytochrome P450 2E1	CYP2E1	NM_174530	F: ACCCGGAGGTTGAAGAGAAAC	21	51
			R: GCCCAATCACCCTGTCAATTT	21	
cytochrome P450 3A28	CYP3A28	NM_174531	F: GCCAGAGCCCGAGGAGTT	18	77
			R: GCAGGTAGACGTAAGGATTTATGCT	25	
glutathione-*S*-transferase A1-*like*	GSTA1-*like*	NM_001078149	F: TTCCCTCTGCTAAAGGCCCTA	21	84
			R: CTTCCTCTGGCTGCCAGG	18	
glutathione-*S*-transferase P1-*like*	GSTP1-*like*	NM_177516	F: CCTCATTTACACCAACTACGAGGC	24	72
			R: AAAGGCTTCAGGTGCTGGG	19	
sulfotransferase 1A1-*like*	SULT1A1-*like*	NM_177521	F: CACGGCTCCTCAAGACACACT	21	84
			R: GGGCGATGTAGATCACCTTG	20	
UDP-glucuronosyltransferase 1A1-*like*	UGT1A1-*like*	NM_001105636	F: ACCATCCTACGTGCCCAGG	19	71
			R: TGTTCTTCACCCGCTGCAG	19	
UDP-glucuronosyltransferase 2B17-*like*	UGT2B17-*like*	NM_001075724	F: GCAAAGCCCCTACCTAAGGAATTA	24	72
			R: AGTAAACACCACGACTCCATCTTTT	25	
constitutive androstan receptor	CAR	NM_001079768	F: GAAGGACATGATCCTATCGACAGA	24	63
			R: CGTCGCTGGGCCTGTCT	17	
hepatocyte-nuclear factor 4-alpha	HNF4α	NM_001015557	F: CGACAACGAGTACGCCTGC	19	58
			R: CCCCTTGGCATCTGGGTC	18	
pregnane X receptor	PXR	NM001103226	F: TGAAGGCCTACATCGAGTTCAAC	23	68
			R: GGCCATGATCTTCAGGAACAA	21	
retinoic X receptor alpha	RXRα	XM_881943	F: GCCTCAATGGTGTCCTCAAAG	21	120
			R: AGCTGTACACCCCGTAGTGCTT	22	
estrogen receptor alpha	ERα	ENSBTAT00000009422	F: CGGCTACGCAAGTGCTATGA	20	51
			R: TTTCCGTATTCCGCCTTTCA	20	
glucocorticoid receptor	GR	ENSBTAT00000025941	F: AGCAGTGGAAGGACAGCACAA	21	71
			R: TTCTTCGAATTTTATCAATGATACAATCAT	30	
tyrosine aminotransferase	TAT	NM_001034590	F: CTGAAGTTACCCAAGCAATGAAAG	24	90
			R: CCTCCCGACTGGATAAGTAGCC	22	
beta-actin	β-actin	NM_173979	F: GTCGACACCGCAACCAGTT	19	85
			R: AAGCCGGCCTTGCACAT	17	

finally, for its amplification efficiency approximately equal to that of target genes.

The quantitative real-time RT-PCR was performed on 25 ng of cDNA, in a final volume of 25 μL, by using Power SYBR Green PCR Master Mix and an ABI-Prism 7000 thermal cycler (Applied Biosystems, Foster City, CA) under standard PCR conditions.

The ΔΔCt method (*20*) was used to analyze results. Relative quantification data were expressed as -fold change compared with the respective control (C_1 and C_2 for experiment 1 and 2, respectively).

Statistical and Data Analysis. Each target gene data were expressed as the arithmetic mean ± standard error (SE) of ΔΔCt values expressed as -fold change. The Grubbs' test was used to reveal potential outliers. Statistical analysis was performed by means of one-way analysis of variance (ANOVA) followed, if appropriate, by the Tukey's post-test (Graph Pad Instat 2.01, San Diego, California, USA). A $p < 0.05$ value was considered statistically significant.

RESULTS

A total of 19 candidate genes were chosen. Selection criteria were based on their relevance in the general pathway of drug metabolism (phase I and II DMEs), their involvement in the regulation of DMEs (TFs), or their known responsiveness to glucocorticoids (tyrosine aminotransferase, TAT: see **Figure 2**).

For each of them, the specific mRNA was always detected in hepatic specimens obtained from control and DEX-treated animals.

Gene expression profile data, measured by using a Q RT-PCR approach, and expressed as -fold change of the respective control, to whom a mean value of 1 was arbitrarily assigned, are reported in **Tables 2**−**4**. As a whole, DEX significantly modulated 11 genes out of the 18 tested. A more detailed description of results, for each subset of target genes, is hereby reported.

Cytochromes P450. The cytochrome P450 1A1, CYP1A2, CYP2B6-*like*, CYP2C87, CYP2E1, and CYP3A28 were chosen as candidate genes.

Cytochromes P450 1A1, 1A2, and 2C87 mRNAs were never affected by GPs, although in experiment 2 a down-regulation of both members of CYP1A subfamily was noticed in group D_2 ($p < 0.05$ against DE). As far as CYP3A28 is concerned, a significant increase ($p < 0.01$) of its mRNA was pointed out only in cattle administered with DEX plus 17β-E (**Table 2**). Interestingly, an extensive (more than 10-fold) and significant decrease of CYP2B6-*like* ($p < 0.05$ vs D_1, DIM, and DE; $p < 0.001$ vs D_2: **Table 2**) and CYP2E1 ($p < 0.05$ vs DIM, D_2, DE, and $p < 0.001$ vs D_1: **Table 2**) mRNAs was noticed in all treated animals.

Phase II DMEs. Glutathione *S*-transferases A1-*like* and P1-*like* (GSTA1-*like* and GSTP1-*like*, respectively), UDP-glucuronosyltransferases 1A1- and 2B17-*like* (UGT1A1-*like* and UGT-2B17-*like*), and sulfotransferase 1A1-*like* (SULT1A1-*like*) were selected among conjugative DMEs. The UDP-glucuronosyltransferases

1A1- and GSTP1-*like* mRNAs genes were measured only in experiment 2, owing to their almost exclusive involvement in steroid conjugation.

Growth promoters did not alter UGT1A1-, UGT2B17-, and GSTP1-*like* gene expression (**Table 3**); by contrast, GSTA1- and SULT1A1-*like* genes were upregulated, in both experiments and in all treated groups. Significant increases were recorded only in DIM and DE groups ($p < 0.01$ and $p < 0.05$) as well as in D_1 and DE groups ($p < 0.05$), respectively (**Table 3**).

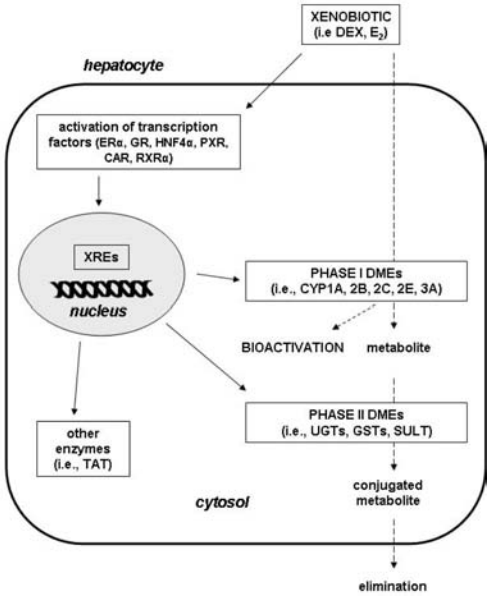

Figure 2. General scheme of liver drug metabolism. The xenobiotic, once entered the hepatocyte, usually undergoes reactions (mostly, oxidations) catalyzed by phase I DMEs (i.e., CYPs); the resulting metabolite is conjugated with an endogenous substrate by phase II DMEs (i.e., UGTs); finally, the conjugated metabolite leaves the hepatocyte and is eliminated. Dexamethasone and 17βE are ligands of GR and ERα, respectively; moreover, they interact with some members of the nuclear receptor superfamily of transcription factors (i.e., PXR, CAR, HNF4α, RXRα), which contribute to the regulation of phase I and II DMEs involved in their biotransformation as well as of other target enzymes (i.e., TAT).

Legend: 17βE, 17β-oestradiol; CAR, constitutive androstan receptor; CYP, cytochrome P450; DEX, dexamethasone; DMEs, drug metabolizing enzymes; ERα, estrogen receptor alpha; GR, glucocorticoid receptor; GSTs, glutathione *S*-transferase; HNF4α, hepatocyte nuclear factor 4-alpha; PXR, pregnane X receptor; RXRα, retinoic X receptor alpha; SULT, sulfotransferase; TAT, tyrosine aminotransferase; UGTs, UDP-glucuronosyltransferases; XREs, xenobiotic response elements.

Transcription Factors. The effects of illicit GPs were also investigated upon TFs involved in DMEs expression and regulation (pregnane X receptor, PXR; constitutive androstan receptor, CAR; retinoic X receptor alpha, RXRα; hepatocyte nuclear factor 4-alpha, HNF4α; glucocorticoid receptor, GR; estrogen receptor alpha, ERα).

No statistically significant differences were ever observed in PXR and HNF4α gene expression profiles, whereas a significant ($p < 0.05$) increase of CAR mRNA was recorded only in D_1 group (**Table 4**). When DEX was orally administered, an increase of RXRα mRNA, reaching the level of statistical significance in D_1 and DE groups ($p < 0.05$), was noticed.

Furthermore, significant differences ($p < 0.05$) were also pointed out among treated groups, particularly for CAR (both studies) and RXRα (only in experiment 2).

Interestingly, the glucocorticoid-responsive genes GR and TAT were never modulated by GPs; however, in the DE group the former gene was upregulated ($p < 0.05$ vs D_2).

Finally, an increase ($p < 0.05$) of ERα mRNA was pointed out only in the DE group; similar to GR, such an increase was significant vs D_2 ($p < 0.01$).

DISCUSSION

Effects of Illicit Protocols Containing DEX upon Cattle DMEs and Related TFs Gene Expression. In humans DEX induces CYP3A (*9*, *16*, *21*), a phenomenon that might interfere with the metabolism of drugs or endogenous steroids (*16*), but marked species differences in CYP3A responsiveness to DEX have been reported (*21*, *22*). In cattle hepatocytes, DEX did not induce CYP3A in a dose-dependent way (*10*); moreover, it has been recently hypothesized that neither therapeutic nor growth-promoting schedules upregulate target bovine DMEs (*3*). Usually, CYP3A upregulation follows DEX binding with GR or PXR (*16*), and TAT typically reflects changes in GR gene expression (*16*, *21*, *23*, *24*). In the present study, DEX did not affect CYP3A28 and TAT mRNA, similar to veal calves to whom similar amounts of the corticosteroid (about 10 times lower than therapeutic ones) were administered with a milk replacer (*17*, *18*). The glucocorticoid receptor and TAT gene did not respond in the same way, and GR data partially disagree with those obtained in the aforementioned experiment (*18*); nonetheless, contradictory results on GR-TAT parallel response have also been reported in humans (*25*, *26*).

Dexamethasone, besides CYP3A, also upregulates human CYP2B6 and rat CYP2B1/2 in vitro (*27*, *28*). By contrast, cattle CYP2B6-*like* was surprisingly down-regulated by illicit DEX. In human liver, CYP2B6 represents a minor subfamily, highly inducible by barbiturates and mostly playing a deactivating role,

Table 2. Liver Cytochromes P450 mRNA Relative Abundances (Arbitrary Units, a.u.) in Beef Cattle Treated with Two Illicit Protocols Containing DEX[a]

		-fold change (a.u.)					
		experiment 1			experiment 2		
gene name	gene acronym	C_1	D_1	DIM	C_2	D_2	DE
Cytochromes P450							
cytochrome P450 1A1	CYP1A1	1.00 ± 0.12	0.98 ± 0.15	0.90 ± 0.02	1.00 ± 0.10	0.87 ± 0.06^{f}	1.26 ± 0.12
cytochrome P450 1A2	CYP1A2	1.00 ± 0.07	1.02 ± 0.10	0.87 ± 0.13	1.00 ± 0.14	0.63 ± 0.03^{f}	1.07 ± 0.11
cytochrome P450 2B6-*like*	CYP2B6-*like*	$1.00 \pm 0.39^{a,b}$	0.22 ± 0.10	0.15 ± 0.06	$1.00 \pm 0.19^{ddd,e}$	0.03 ± 0.01	0.61 ± 0.33
cytochrome P450 2C87	CYP2C87	1.00 ± 0.09	1.16 ± 0.19	1.31 ± 0.67	1.00 ± 0.13	1.38 ± 0.08	1.20 ± 0.23
cytochrome P450 2E1	CYP2E1	$1.00 \pm 0.19^{aaa,b}$	0.03 ± 0.01	0.36 ± 0.16	$1.00 \pm 0.29^{d,e}$	0.10 ± 0.05	0.35 ± 0.17
cytochrome P450 3A28	CYP3A28	1.00 ± 0.14	0.82 ± 0.12	0.77 ± 0.10	1.00 ± 0.12^{ee}	1.49 ± 0.10	1.65 ± 0.20

[a] In Experiment 1, DEX was administered either per os (D_1) or injected im (DIM). In Experiment 2, DEX was administered per os either alone (D_2) or in combination with 17β-estradiol (DE). Groups C_1 and C_2 served as control. Data (arithmetic means ± SE) are expressed as -fold change (normalized to the $\triangle\triangle$Ct mean value of the respective control group, to whom an arbitrary value of 1 was assigned). Statistical analysis was made by ANOVA, followed by Tukey's post-test. [a, aaa] $p < 0.05$ and $p < 0.001$ between C_1 and D_1. [b] $p < 0.05$ between C_1 and DIM. [d, ddd] $p < 0.05$ and $p < 0.001$ between C_2 and D_2. [e, ee] $p < 0.05$ and $p < 0.01$ between C_2 and DE. [f] $p < 0.05$ between D_2 and DE.

Table 3. Liver Conjugative Drug Metabolizing Enzymes mRNA Relative Abundances (Arbitrary Units, a.u.) in Beef Cattle Treated with Two Illicit Protocols Containing DEX[a]

		-fold change (a.u.)					
		experiment 1			experiment 2		
gene name	gene acronym	C_1	D_1	DIM	C_2	D_2	DE
Conjugative enzymes							
glutathione *S*-transferase A1-*like*	GSTA1-*like*	1.00 ± 0.12^{bb}	1.51 ± 0.23	2.24 ± 062	1.00 ± 0.16^{e}	1.28 ± 0.11	1.72 ± 0.30
glutathione *S*-transferase P1-*like*	GSTP1-*like*				1.00 ± 0.16	0.97 ± 0.16	1.27 ± 0.22
sulfotransferase 1A1-*like*	SULT1A1-*like*	1.00 ± 0.13^{a}	1.55 ± 0.19	1.31 ± 0.32	1.00 ± 0.11^{e}	1.18 ± 0.06	1.33 ± 0.14
UDP-glucuronosyltransferase 1A1-*like*	UGT1A1-*like*				1.00 ± 0.11	1.08 ± 0.06	1.02 ± 0.15
UDP-glucuronosyltransferase 2B17-*like*	UGT2B17-*like*	1.00 ± 0.06	1.06 ± 0.13	1.08 ± 0.09	1.00 ± 0.08	1.01 ± 0.07	0.92 ± 0.10

[a] In experiment 1, DEX was administered either per os (D_1) or injected im (DIM). In experiment 2, DEX was administered per os either alone (D_2) or in combination with 17β-estradiol (DE). Groups C_1 and C_2 served as control. Data (arithmetic means $\pm$ SE) are expressed as -fold change (normalized to the $\triangle\triangle$Ct mean value of the respective control group, to whom an arbitrary value of 1 was assigned). Statistical analysis was made by ANOVA, followed by Tukey's post-test. [a] $p < 0.05$ and $p < 0.001$ between C_1 and D_1. [bb] $p < 0.01$ between C_1 and DIM. [e] $p < 0.05$ between C_2 and DE.

Table 4. Liver Nuclear Receptors, Tyrosine Aminotransferase, Glucocorticoid, and Estrogen Receptor Alpha mRNA Relative Abundances (Arbitrary Units, a.u.) in Beef Cattle Treated with Two Illicit Protocols Containing DEX[a]

		-fold change (a.u.)					
		experiment 1			experiment 2		
gene name	gene acronym	C_1	D_1	DIM	C_2	D_2	DE
Transcription factors							
constitutive androstan receptor	CAR	1.00 ± 0.09^{a}	1.44 ± 0.21^{c}	0.94 ± 0.14	1.00 ± 0.14	0.82 ± 0.07^{f}	1.26 ± 0.15
hepatocyte nuclear factor 4-alpha	HNF4α	1.00 ± 0.06	0.89 ± 0.12	0.91 ± 0.20	1.00 ± 0.13	1.15 ± 0.10	1.18 ± 0.15
pregnane X receptor	PXR	1.00 ± 0.16	1.02 ± 0.11	0.77 ± 0.08	1.00 ± 0.10	1.11 ± 0.10	1.26 ± 0.18
retinoic X receptor alpha	RXRα	1.00 ± 0.07^{a}	1.37 ± 0.14^{c}	0.88 ± 0.09	1.00 ± 0.15^{e}	1.41 ± 0.09	1.51 ± 0.10
glucocorticoid receptor	GR	1.00 ± 0.11	1.02 ± 0.06	0.87 ± 0.06	1.00 ± 0.06	0.84 ± 0.05^{f}	1.20 ± 0.12
estrogen receptor alpha	ERα				1.00 ± 0.10^{e}	0.87 ± 0.15^{ff}	1.66 ± 0.16
tyrosine aminotransferase	TAT	1.00 ± 0.14	0.73 ± 0.09	0.67 ± 0.13	1.00 ± 0.09	0.85 ± 0.08	1.08 ± 0.17

[a] In Experiment 1, DEX was administered either per os (D_1) or injected im (DIM). In Experiment 2, DEX was administered per os either alone (D_2) or in combination with 17β-estradiol (DE). Groups C_1 and C_2 served as control. Data (arithmetic means $\pm$ SE) are expressed as -fold change (normalized to the $\triangle\triangle$Ct mean value of the respective control group, to whom an arbitrary value of 1 was assigned). Statistical analysis was made by ANOVA, followed by Tukey's post-test. [a] $p < 0.05$ between C_1 and D_1. [c] $p < 0.05$ between D_1 and DIM. [e] $p < 0.05$ between C_2 and DE. [f, ff] Significant differences ($p < 0.05$ and $p < 0.01$, respectively) between D_2 and DE.

albeit it contributes to bioactivation of long-chain nitrosamines and aflatoxin B_1 (*10*). In cattle, CYP2B6-*like* is only constitutively expressed in the liver; phenobarbital oral administration increases its mRNA levels but CAR, the nuclear receptor mostly involved in human CYP2B6 gene regulation following the barbiturate oral administration, was not upregulated (Zancanella et al., personal data); finally, contrasting results were obtained at the post-transcriptional level (*10*). To the best of our knowledge, such an inhibition has never been reported in the literature, except in the case of inflammation, infectious or pathological diseases where, however, it was usually and essentially pointed out at the post-translational level (*29*).

Besides GR, some other TFs (namely, PXR, CAR, RXRα, and HNF4α) contribute to DEX upregulation of human CYP2B, 2C and 3A (*16*, *27*), and a dual dose-dependent mechanism of regulation (involving either GR or PXR) has been hypothesized to explain CYP3A induction (*16*, *21*, *25*). In cattle, neither PXR nor HNF4α mRNAs were ever modulated by DEX, whereas the common heterodimerizing partner RXRα (and, to a lower extent, CAR) was upregulated only when the corticosteroid was administered per os. Although comparable results (except for RXRα ones) were found in the veal calf (*18*), PXR behavior is difficult to explain on a knowledge basis: in fact, DEX (30 μg kg bw^{-1}, given twice a day and for five days) lowered veal calf PXR and CAR mRNAs (*13*). On the other hand, DEX amounts used in the above-mentioned study were definitely higher than those adopted at growth promoting purposes, and age and diet represent constitutional factors modulating cattle NRs mRNA abundance (*12*). Therefore, it should be inferred that DEX effects upon the proposed human GR-[PXR/CAR]-CYPs (TAT) signal transmission cascade might be different in cattle, and such an hypothesis would further confirm marked species differences in the CYP3A pattern of induction (*21*). Thus, further basic and applied clarifying molecular studies are needed.

Similar to CYP2B6-*like*, CYP2E1 mRNA was deeply downregulated. Such a finding was rather astonishing: basically, CYP2E1 expression is regulated at the post-transcriptional level via protein stabilization and, consequently, protection against rapid proteolysis (*30*); furthermore, CYP2E1 is neither considered a DEX molecular target nor involved in its metabolism. Nonetheless, DEX inhibits CYP2E1 expression in the rat, whereas in swine certain steroids decrease CYP2E1-dependent catalytic activities, CYP2E1 promoter activity, and, consequently, its gene transcription (*31*, *32*). Ruminants physiologically produce ketone bodies, which are metabolized by CYP2E1 (*33*); moreover, ketone bodies modulate CYP2E1 mRNA/protein, although contradictory results have also been reported (*34*). Consequently, CYP2E1 might play a major role in cattle drug metabolism (*35*, *36*). Dexamethasone, administered at high dosages, increase plasma insulin, glucose levels, and ketogenesis (*36*). In light of this evidence, it should be argued that DEX might effectively and indirectly modulate cattle liver CYP2E1 gene expression, similar to diabetes and ketone bodies.

Another interesting result may be gathered from CYP1A data. In humans, CYP1A1 and CYP1A2 genes, with CYP3A, code for key enzymes involved in 17βE biotransformation (*37*). Dexamethasone did not affect CYP1A constitutive expression in vitro (*38*); likewise, in cattle neither CYP1A1 nor CYP1A2 gene

expression profiles were modulated when DEX was administered alone. But in the DE group, ERα, CYP1A1, GR, and CYP3A28 genes were equally upregulated. Usually, 17β-E effects upon CYP1A result from a mechanism involving ERα, albeit species differences in response (induction or inhibition) have been reported (*39*, *40*); moreover, a functional cross-talk between GR and ERα has been recently hypothesized in women (*41*). Therefore, an involvement of ERα-GR in the molecular effect of 17β-E upon cattle CYP1A1/2-CYP3A28 cannot be excluded a priori.

Among phase II DMEs, GSTA1- and SULT1A1-*like* genes were in general upregulated. These data confirm previous rat studies, where high glucocorticoid amounts increased GSTα and SULT1A1 gene expression, respectively, through PXR activation and a molecular mechanism involving a glucocorticoid response element located in its 5′-flanking region (*42*, *43*).

On the whole, present results suggest that DEX illicit protocols modulate, to a various extent, DMEs and their related TFs gene expression in cattle liver. An intriguing question is whether these effects might be reflected at the post-translational level. Target CYP catalytic activities and apoprotein levels (immunoblotting) were measured, and contrasting results obtained: only CYP3A (in both experiments, but mostly when DEX was given im), CYP1A and CYP2E1 (in experiment 2) mirrored pretranscriptional data (albeit immunoblotting did not confirm always the enzyme activity). In veterinary species, a low correlation among catalytic activity, CYP protein amount, and relative gene expression profiles has been pointed out (*14*, *44*−*47*); such evidence has been justified with (a) post-translational (i.e., phosphorylation) or post-transcriptional phenomena (i.e., proteosomal degradation); (b) the substrate change or loss of selectivity toward the target DME; (c) the low specificity of antibodies used for immunoblotting or immunoinhibition studies; (d) the low number of substrates whose usefulness has been investigated by measuring K_m and V_{max} (*11*, *14*, *45*−*47*). By contrast, primers sequences for target and reference genes are usually species- and isoform-specific. Therefore, it is conceivable to be more confident about gene expression data rather than post-translational ones. Nonetheless, caution must be adopted before drawing final conclusions; in fact, a potential untoward effect of xenobiotics upon DMEs gene expression should be confirmed also post-transcriptionally before being considered a risk for the animal's health (i.e., drug−drug interactions) and, indirectly, for the bovine meat consumer.

Identification of Candidate BMRs. As a consequence of the increasing importance attributed to -omic methodologies, genomic and proteomic approaches have been applied in cattle to identify possible BMRs for GPs abuse; some potential candidates, often unrelated with the GP molecular mechanism of action or metabolism, have been identified (*4*−*8*, *48*−*50*). In the present study, cattle CYP2B6-*like*, CYP2E1, GSTA1-, and SULT1A1-*like* were significantly modulated by DEX (and 17β-E). The two CYPs showed a marked (more than 10-fold) down-regulation of their mRNAs; on the other hand, GSTA1- and SULT1A1-*like* gene expression was increased, although their fold-changes (up to 2.5-fold) were lower than those calculated for CYP2B6-*like* and CYP2E1. Noteworthy, the target gene up- or down-regulation was a constant finding in all treated cattle, thereby independent from the breed (Marchigiana or French cross-bred), the GP (DEX alone or in combination with 17β-E), the route of administration (oral or intramuscular), and dosage regimens. Of lesser importance, in this respect, was the RXRα increasing level of expression (up to 1.5-fold change), a constant finding in all groups where DEX was administered per os. In many pharmaco-toxicological -omic investigations, the usual cutoff value is represented by a 2-fold change; thus, the aforementioned genes could be considered, at least in perspective, as useful BMRs to be used in the screening of illicit GPs in cattle. An evident issue is how to use these BMRs in the screening of illicit GPs. Apart from a necessary confirmatory step in pilot monitoring campaigns, these same assays (using more sensitive probes such as TaqMan ones, if necessary) might be applied to liver specimens withdrawn ex vivo (at slaughterhouse). Interestingly, a minimally invasive liver biopsy technique has been developed (*51*), but such a procedure might be difficult to apply on animals still alive. Rather, blood has been considered as a surrogate tissue for invasive and not accessible organ biopsies (*52*), and mRNA levels of some candidate BMRs have been successfully measured in human blood (*53*, *54*) and in cattle treated with illicit GPs (*8*). Therefore, both approaches (mostly the second one) look promising, at least in perspective.

Nonetheless, a prototypical BMR should be quantitative, sensitive, noninvasive, specific, easily measurable, relate to the biochemical mechanism of action, and work at realistic doses (*55*). In light of this, it will be necessary to deepen those basic molecular mechanisms of regulation involved in cattle DMEs gene responses to GPs, to clarify whether present results arise from GPs administration or, rather, represent unspecific alterations (even more for those transcripts showing moderate variations of their mRNAs). Among these mechanisms, there are the dual dose-dependent effects of DEX upon DMEs expression and/or regulation, with possible and consequent induction or inhibition phenomena, the confirmation of these latter at the post-translational level, and the prospective cross-talks among genes (mostly TFs), which might harvest importance in the presence of a cocktail of GPs. All of this is envisaged in our laboratory.

ABBREVIATIONS USED

17β-E, 17β-estradiol; ANOVA, analysis of variance; BMRs, biomarkers of response; CAR, constitutive androstan receptor; CYP, cytochrome P450; DEX, dexamethasone; DMEs, drug metabolizing enzymes; ERα, estrogen receptor alpha; GPs, growth promoters; GR, glucocorticoid receptor; GSTA1-like, glutathione *S*-transferase A1-*like*; GSTP1-*like*, glutathione *S*-transferase P1-*like*; HNF4α, hepatocyte nuclear factor 4-alpha; PXR, pregnane X receptor; Q RT-PCR, quantitative real time RT-PCR; RXRα, retinoic X receptor alpha; SULT1A1-*like*, sulfotransferase A1-*like*; TAT, tyrosine aminotransferase; TFs, transcription factors; UGT1A1-*like*, UDP-glucuronosyltransferase 1A1-*like*; UGT2B17-*like*, UDP-glucuronosyltransferase 2B17-*like*.

ACKNOWLEDGMENT

The authors thank Alessia Pomaro, DVM, and Clara Sandron, DVM, for their helpful assistance during the animal phase. Moreover, Authors would like to dedicate this work in memory of Giovanni Vincenzi, DVM, from Servizi Veterinari, Regione del Veneto, that in the past decade strongly supported research on illicit GPs in cattle.

LITERATURE CITED

(1) Courtheyn, D.; Le Bizec, B.; Brambilla, G.; De Brabander, H. F.; Cobbaert, E.; Van de Wiele, M.; Vercammen, J.; De Wasch, K. Recent developments in the use and abuse of growth promoters. *Anal. Chim. Acta* **2002**, *473*, 71–82.

(2) Serratosa, J.; Blass, A.; Rigau, B.; Mongrell, B.; Rigau, T.; Tortadés, M.; Tolosa, E.; Aguilar, C.; Ribó, O.; Balagué, J. Residues from veterinary medicinal products, growth promoters and performance enhancers in food-producing animals: a European perspective. *Rev. Sci. Tech. Off. Int. Epiz.* **2006**, *25*, 637–653.

(3) Vincenti, M.; Girolami, F.; Capra, P.; Pazzi, M.; Carletti, M.; Gardini, G.; Nebbia, C. Study of dexamethasone urinary excretion

profile in cattle by LC-MS/MS: comparison between therapeutic and growth-promoting administration. *J. Agric. Food Chem.* **2009**, *57*, 1299–1306.

(4) Toffolatti, L.; Gastaldo, R. L.; Patarnello, T.; Romualdi, C.; Merlanti, R.; Montesissa, C.; Poppi, L.; Castagnaro, M.; Bargelloni, L. Expression analysis of androgen-responsive genes in the prostate of veal calves treated with anabolic hormones. *Domest. Anim. Endocrinol.* **2006**, *30*, 39–55.

(5) Reiter, M.; Walf, V. M.; Christians, A.; Pfaffl, M. W.; Meyer, H. H. Modifications of mRNA expression after treatment with anabolic agents and the usefulness for gene expression-biomarkers. *Anal. Chim. Acta* **2007**, *586*, 73–81.

(6) Carraro, L.; Ferraresso, S.; Cardazzo, B.; Romualdi, C.; Montesissa, C.; Gottardo, F.; Patarnello, T.; Castagnaro, M.; Bargelloni, L. Expression profiling of skeletal muscle in young bulls treated with steroidal growth promoters. *Physiol. Genomics* **2009**, *38*, 138–148.

(7) De Maria, R.; Divari, S.; Goria, M.; Bollo, E.; Cannizzo, F. T.; Olivero, M.; Barbarino, G.; Biolatti, B. 17β-Estradiol-induced gene expression in prostate cell culture and tissue from cattle: biomarkers to detect illegal use of growth promoters. *Vet. Rec.* **2009**, *164*, 459–464.

(8) Riedmaier, I.; Tichopad, A.; Reiter, M.; Pfaffl, M. W.; Meyer, H. H. D. Identification of potential gene expression biomarkers for the surveillance of anabolic agents in bovine blood cells. *Anal. Chim. Acta* **2009**, *638*, 106–113.

(9) Xu, C.; Li, C. Y.; Kong, A. N. Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch. Pharm. Res.* **2005**, *28*, 249–268.

(10) Ioannides, C. Cytochrome P450 expression in the liver of food-producing animals. *Curr. Drug Metab.* **2006**, *7*, 335–348.

(11) Fink-Gremmels, J. Implications of hepatic cytochrome P450-related biotransformation processes in veterinary sciences. *Eur. J. Pharmacol.* **2008**, *585*, 502–509.

(12) Greger, D. L.; Philipona, C.; Blum, J. W. Ontogeny of mRNA abundance of nuclear receptors and nuclear receptor target genes in young cattle. *Domest. Anim. Endocrinol.* **2006**, *31*, 76–87.

(13) Greger, D. L.; Blum, J. W. Effects of dexamethasone on mRNA abundance of nuclear receptors and hepatic nuclear receptor target genes in neonatal calves. *J. Anim. Physiol. Anim. Nutr.* (*Berl.*) **2007**, *91*, 62–67.

(14) Giantin, M.; Carletti, M.; Capolongo, F.; Pegolo, S.; Lopparelli, R. M.; Gusson, F.; Nebbia, C.; Cantiello, M.; Martin, P.; Pineau, T.; Dacasto, M. Effect of breed upon cytochromes P450 and phase II enzyme expression in cattle liver. *Drug Metab. Dispos.* **2008**, *36*, 885–893.

(15) Al Katheeri, N. A.; Wasfi, I. A.; Lambert, M.; Giuliano Albo, A.; Nebbia, C. In vitro and in vivo metabolism of dexamethasone in the camel. *Vet. J.* **2006**, *172*, 532–543.

(16) Pascussi, J. M.; Gerbal-Chaloin, S.; Drocourt, L.; Maurel, P.; Vilarem, M. J. The expression of CYP2B6, CYP2C9 and CYP3A4 genes: a tangle network of nuclear and steroid receptors. *Biochim. Biophys. Acta* **2003**, *1619*, 243–253.

(17) Cantiello, M.; Carletti, M.; Dacasto, M.; Martin, P. G. P.; Pineau, T.; Capolongo, F.; Gardini, G.; Nebbia, C. Cytochrome P450 inhibition profile in liver of veal calves administered a combination of 17β-oestradiol, clenbuterol, and dexamethasone for growth-promoting purposes. *Food Chem. Toxicol.* **2008**, *46*, 2849–2855.

(18) Cantiello, M.; Giantin, M.; Carletti, M.; Lopparelli, R. M.; Capolongo, F.; Lasserre, F.; Bollo, E.; Martin, P. G.; Nebbia, C.; Pineau, T.; Dacasto, M. Effects of dexamethasone, administered for growth promoting purposes, upon the hepatic cytochrome P450 3A expression in veal calf. *Biochem. Pharmacol.* **2009**, *77*, 451–463.

(19) Cannizzo, F. T.; Miniscalco, B.; Riondato, F.; Bollo, E.; Barbarino, G.; Giorgi, P.; Mazzini, C.; Biolatti, B. Effects of anabolic and therapeutic doses of dexamethasone on thymus morphology and apoptosis in veal calves. *Vet. Rec.* **2008**, *163*, 448–452.

(20) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2-$\triangle\triangle$Ct method. *Methods* **2001**, *25*, 402–408.

(21) Luo, G.; Guenthner, T.; Gan, L.-S.; Griffith Humphreys, W. CYP3A induction by xenobiotics: biochemistry, experimental methods and impact on drug discovery and development. *Curr. Drug Metab.* **2004**, *5*, 483–505.

(22) Tomlinson, E. S.; Maggs, J. L.; Park, B. K.; Back, D. J. Dexamethasone metabolism in vitro: species differences. *J. Steroid Biochem. Molec. Biol.* **1997**, *62*, 345–352.

(23) Pascussi, J.-M.; Drocourt, L.; Gerbal-Chaloin, S.; Fabre, J.-M.; Maurel, P.; Vilarem, M.-J. Dual effect of dexamethasone on CYP3A4 gene expression in human hepatocytes. Sequential role of glucocorticoid receptor and pregnane X receptor. *Eur. J. Biochem.* **2001**, *268*, 6346–6358.

(24) Ribarac-Stepić, N.; Vulović, M.; Koričanac, G.; Isenović, E. Basal and glucorticoid induced changes of hepatic glucorticoid receptor during aging: relation to activities of tyrosine aminotransferase and tryptophan oxygenase. *Biogerontology* **2005**, *6*, 113–131.

(25) Duret, C.; Daujat-Chavanieu, M.; Pascussi, J.-M.; Pichard-Garcia, L.; Balaguer, P.; Fabre, J.-M.; Vilarem, M. J.; Maurel, P.; Gerbal-Chaloin, S. Ketoconazole and miconazole are antagonists of the human glucocorticoid receptor: consequences on the expression and function of the constitutive androstane receptor and the pregnane X receptor. *Mol. Pharmacol.* **2006**, *70*, 329–339.

(26) Dvořák, Z.; Modrianský, M.; Pichard-Garcia, L.; Balaguer, P.; Vilarem, M.-J.; Ulrichova, J.; Maurel, P.; Pascussi, J.-M. Colchicine down-regulates cytochrome P450 2B6, 2C9, and CYP3A4 in human hepatocytes by affecting their glucorticoid receptor-mediated regulation. *Mol. Pharmacol.* **2003**, *64*, 160–9.

(27) Pascussi, J. M.; Gerbal-Chaloin, S.; Fabre, J. M.; Maurel, P.; Vilarem, M. J. Dexamethasone enhances androstane receptor expression in human hepatocytes: consequences on cytochrome P450 gene regulation. *Mol. Pharmacol.* **2000**, *58*, 1441–1450.

(28) Cui, X.; Thomas, A.; Han, Y.; Palamanda, J.; Montgomery, D.; White, R. E.; Morrison, R. A.; Cheng, K. C. Quantitative PCR assay for cytochrome P450 2B and 3A induction in rat precision-cut liver slices: correlation study with induction in vivo. *J. Pharmacol. Toxicol. Methods* **2005**, *52*, 234–243.

(29) Lee, C. M.; Kim, B. Y.; Li, L.; Morgan, E. T. Nitric oxide-dependent proteasomal degradation of cytochrome P450 2B proteins. *J. Biol. Chem.* **2008**, *283*, 889–898.

(30) Cederbaum, A. I. CYP2E1 − Biochemical and toxicological aspects and role in alcohol-induced liver injury. *Mt. Sinai J. Med.* **2006**, *73*, 657–672.

(31) Tambyrajah, W. S.; Doran, E.; Wood, J. D.; McGivan, J. D. The pig CYP2E1 promoter is activated by COUP-TF1 and HNF-1 and is inhibited by androsterone. *Arch. Biochem. Biophys.* **2004**, *431*, 252–260.

(32) Zamaratskaia, G.; Gilmore, W. J.; Lundström, K.; Squires, E. J. Effect of testicular steroids on catalytic activities of cytochrome P450 enzymes in porcine liver microsomes. *Food Chem. Toxicol.* **2007**, *45*, 676–681.

(33) Yao, H.-T.; Lin, P.; Chang, Y.-W.; Chen, C.-T.; Chiang, M.-T.; Chang, L.; Kuo, Y.-C.; Tsai, H.-T.; Yeh, T.-K. Effect of taurine supplementation on cytochrome P450 2E1 and oxidative stress in the liver and kidneys of rats with streptozotocin-induced diabetes. *Food Chem. Toxicol.* **2009**, *47*, 1703–1709.

(34) Abdelmegeed, M. A.; Carruthers, N. J.; Woodcroft, K. J.; Kim, S. K.; Novak, R. F. Acetoacetate induces CYP2E1 protein and suppresses CYP2E1 mRNA in primary cultured rat hepatocytes. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 203–213.

(35) Zweers-Zeilmaker, W. M.; Maas, R. F. M.; Horbach, G. J.; Van Miert, A. S. J. P. A. M.; Witkamp, R. F. Cytochrome P4502E in vivo and in vitro in the dwarf goat: effects of enzyme induction and the applicability of chlorzoxazone as marker substrate. *J. Vet. Pharmacol. Ther.* **1996**, *19*, 245–250.

(36) Maciel, S. M.; Chamberlain, C. S.; Wetterman, R. P.; Spicer, L. J. Dexamethasone influences endocrine and ovarian function in cattle. *J. Dairy Sci.* **2001**, *84*, 1998–2008.

(37) Badawi, A. F.; Cavalieri, E. L.; Rogan, E. G. Role of human cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16α-hydroxylation of 17β-estradiol. *Metabolism* **2001**, *9*, 1001–1003.

(38) Nishimura, M.; Koeda, A.; Suganuma, Y.; Suzuki, E.; Shimizu, T.; Nakayama, M.; Satoh, T.; Narimatsu, S.; Naito, S. Comparison of inducibility of CYP1A and CYP3A mRNAs by prototypical inducers in primary cultures of human, cynomolgus monkey, and rat hepatocytes. *Drug Metab. Pharmacokinet.* **2007**, *22*, 178–186.
(39) Navas, J. M.; Segner, H. Estrogen-mediated suppression of cytochrome P4501A1 (CYP1A) expression in rainbow trout hepatocytes: role of estrogen receptor. *Chem. Biol. Interact.* **2001**, *138*, 285–298.
(40) Son, D. S.; Roby, K. F.; Rozman, K. K.; Terranova, P. F. Estradiol enhances and estriol inhibits the expression of CYP1A1 induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a mouse ovarian cancer cell line. *Toxicology* **2002**, *176*, 229–243.
(41) Cuzzocrea, S.; Bruscoli, S.; Crisafulli, C.; Mazzon, E.; Agostani, M.; Muià, C.; Esposito, E.; Di Virgilio, R.; Meli, R.; Vegeto, E.; Maggi, A.; Riccardi, C. Estrogen receptor antagonist fulvestrant (ICI 182,780) inhibits the anti-inflammatory effects of glucocorticoids. *Mol. Pharmacol.* **2007**, *71*, 132–144.
(42) Duamnu, Z.; Kocarek, T. A.; Runge-Morris, M. Transcriptional regulation of rat hepatic aryl sulphotransferase (SULT1A1) gene expression by glucocorticoids. *Drug Metab. Dispos.* **2001**, *29*, 1130–1135.
(43) Schauss, S. J.; Henry, T.; Palmatier, R.; Halvorson, L.; Dannenbring, R.; Beckmann, J. D. Characterization of bovine tracheobronchial phenol sulphotransferase cDNA and detection of mRNA regulation by cortisol. *Biochem. J.* **1995**, *311*, 209–217.
(44) Nebbia, C.; Dacasto, M; Rossetto Giaccherino, A.; Giuliano Albo, A.; Carletti, M. Comparative expression of liver cytochrome P450-dependent monooxygenases in the horse and in other agricultural and laboratory species. *Vet. J.* **2003**, *165*, 53–64.
(45) Szotáková, B.; Baliharová, V.; Lamka, J.; Nožinová, E.; Wsól, V.; Velík, J.; Machala, M.; Neča, J.; Souček, P.; Šusová, S.; Skálová, L. Comparison of in vitro activities of biotransformation enzymes in pig, cattle, goat and sheep. *Res. Vet. Sci.* **2004**, *76*, 43–51.
(46) Skaanild, M. Porcine cytochrome P450 and metabolism. *Curr. Pharm. Res.* **2006**, *12*, 1421–1427.
(47) Tydén, E.; Olsén, L.; Tallkvist, J.; Tjälve, H.; Larsson, P. Cytochrome P450 3A, NADPH cytochrome P450 reductase and cytochrome b5 in the upper airways in horse. *Res. Vet. Sci.* **2008**, *85*, 80–85.
(48) Gardini, G.; Del Boccio, P.; Colombatto, S.; Testore, G.; Corpillo, D.; Di Ilio, C.; Urbani, A.; Nebbia, C. Proteomic investigation in the detection of illicit treatment of calves with growth-promoting agents. *Proteomics* **2006**, *6*, 2813–2822.
(49) Draisci, R.; Montesissa, C.; Santamaria, B.; D'Ambrosio, C.; Ferretti, G.; Merlanti, R.; Ferranti, C.; De Liguoro, M.; Cartoni, C.; Pistarino, E.; Ferrara, L.; Tiso, M.; Scaloni, A.; Cosulich, M. E. Integrated analytical approach in veal calves administered the anabolic androgenic steroids boldenone and boldione: urine and plasma kinetic profile and changes in plasma protein expression. *Proteomics* **2007**, *7*, 3184–3193.
(50) Nebbia, C.; Della Donna, L.; Carletti, M.; Balbo, A.; Barbarino, G.; Gardini, G. Use of hepatic protein biomarkers for tracing the exposure of veal calves to illegal growth promoters: investigations on experimental samples and preliminary application under field conditions. *J. Vet. Pharmacol. Ther.* **2008**, *31*, 272–275.
(51) Vels, L.; Røntved, C. M.; Bjerring, M.; Ingvartsen, K. L. Cytokine and acute phase protein gene expression in repeated liver biopsies of dairy cows with a lipopolysaccharide-induced mastitis. *J. Dairy Sci.* **2009**, *92*, 922–934.
(52) Mohr, S.; Liew, C.-C. The peripheral-blood transcriptome: new insights into disease and risk assessment. *Trends Mol. Med.* **2007**, *13*, 422–432.
(53) Liptrott, N. J.; Penny, M.; Bray, P. G.; Sathish, J.; Khoo, S. H.; Back, D. J.; Owen, A. The impact of cytokines on the expression of drug transporters, cytochrome P450 enzymes and chemokine receptors in human PBMC. *Br. J. Pharmacol.* **2009**, *156*, 497–508.
(54) Wang, A.-H.; Zhu, S.-M.; Qiu, Y.-L.; Zhu, R.; Qu, Y.-B.; Li, Y.-L.; Brandt-Rauf, P. W.; Xia, Z.-L. CYP2E1 mRNA expression, genetic polymorphism in peripheral blood lymphocytes and liver abnormalities in chines VCM-exposed workers. *Int. J. Occup. Med. Env.* **2008**, *21*, 141–146.
(55) Timbrell, J. A. Biomarkers in toxicology. *Toxicology* **1998**, *129*, 1–12.

Received for review May 20, 2009. Revised manuscript received November 25, 2009. Accepted December 14, 2009. This study was supported by grants from Ministero della Salute (IZS.VE.008/2001: studio sperimentale sulla somministrazione di desametasone nel bovino da carne. Impiego di alcuni parametri biologici come indicatori di benessere animale e come marker nella ricerca dei residui, to F.G. and L.R.; IZS.VE.009/03RC: identificazione di biomarcatori nell'allevamento del bovino da carne quali indicatori di trattamenti illeciti anche in relazione al benessere animale, to M.D., F.G. and L.R.); from Università degli Studi di Padova (CPDA054599: messa a punto di una tecnica omics per la valutazione dei profili di espressione genica di biomarcatori del metabolismo in bovini trattati con sostanze illecite) to M.D.; from Ministero Università e Ricerca Scientifica e Tecnologica and Università Italo-Francese (Programma Galileo 2006: valutazione degli effetti dei trattamenti illeciti sugli enzimi farmaco-metabolizzanti del bovino mediante tecniche di PCR quantitativa) to M. D. and T.P.; and, finally, a Ph.D. fellowship (to M.G.) from Università degli Studi di Padova. Preliminary results of this work were presented at the 10th European Regional ISSX Meeting, held in Vienna (Austria) on May 18−21, 2008.