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# Disposition kinetics of albendazole and metabolites in laying hens

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An increasing prevalence of roundworm parasites in poultry, particularly in litter-based housing systems, has been reported. However, few anthelmintic drugs are commercially available for use in avian production systems. The anthelmintic efficacy of albendazole (ABZ) in poultry has been demonstrated well. The goal of this work was to characterize the ABZ and metabolites plasma disposition kinetics after treatment with different administration routes in laying hens. Twenty-four laying hens *Plymouth Rock Barrada* were distributed into three groups and treated with ABZ as follows: intravenously at 10 mg/kg (*ABZ i.v.*); orally at the same dose (*ABZ oral*); and in medicated feed at 10 mg/kg·day for 7 days (*ABZ feed*). Blood samples were taken up to 48 h posttreatment (*ABZ i.v.* and *ABZ oral*) and up to 10 days poststart feed medication (*ABZ feed*). The collected plasma samples were analyzed using high-performance liquid chromatography. ABZ and its albendazole sulphoxide (ABZSO) and $ABZSO_2$ metabolites were recovered in plasma after *ABZ i.v.* administration. ABZ parent compound showed an initial concentration of 16.4 ± 2.0 μg/mL, being rapidly metabolized into the ABZSO and $ABZSO_2$ metabolites. The ABZSO maximum concentration ($C_{max}$) (3.10 ± 0.78 μg/mL) was higher than that of $ABZSO_2$ $C_{max}$ (0.34 ± 0.05 μg/mL). The area under the concentration vs time curve (AUC) for ABZSO (21.9 ± 3.6 μg·h/mL) was higher than that observed for $ABZSO_2$ and ABZ (7.80 ± 1.02 and 12.0 ± 1.6 μg·h/mL, respectively). The ABZ body clearance (Cl) was 0.88 ± 0.11 L·h/kg with an elimination half-life ($T_{1/2el}$) of 3.47 ± 0.73 h. The $T_{1/2el}$ for ABZSO and $ABZSO_2$ were 6.36 ± 1.50 and 5.40 ± 1.90 h, respectively. After *ABZ oral* administration, low ABZ plasma concentrations were measured between 0.5 and 3 h posttreatment. ABZ was rapidly metabolized to ABZSO ($C_{max}$, 1.71 ± 0.62 μg/mL) and $ABZSO_2$ ($C_{max}$, 0.43 ± 0.04 μg/mL). The metabolite systemic exposure (AUC) values were 18.6 ± 2.0 and 10.6 ± 0.9 μg·h/mL for ABZSO and $ABZSO_2$, respectively. The half-life values after *ABZ oral* were similar (5.91 ± 0.60 and 5.57 ± 1.19 h for ABZSO and $ABZSO_2$, respectively) to those obtained after *ABZ i.v.* administration. ABZ was not recovered from the bloodstream after *ABZ feed* administration. AUC values of ABZSO and $ABZSO_2$ were 61.9 and 92.4 μg·h/mL, respectively. The work reported here provides useful information on the pharmacokinetic behavior of ABZ after both i.v. and oral administrations in hens, which is a useful first step to evaluate its potential as an anthelmintic tool for use in poultry.

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## INTRODUCTION

Coccidia are the most important group of parasites causing infections in poultry, in terms of distribution, frequency, and economic losses (Mc Dougald & Reid, 1997). However, different field trials had shown that helminth infections are widely spread in poultry industry, mainly because of their extremely high fecundity combined with the longevity of their eggs, being the prevalence of these parasitic infections often underestimated (Mungube *et al.*, 2008). Different studies have demonstrated that

helminth infections have a negative effect on the behavior and overall health of the animals, which indirectly impact on the productive performance. Parasitic infections account for production losses owing to decreased appetite, reduced body weight and/or egg production, and/or clinically relevant disease. There are several species of helminth parasites that can infect hens. *Ascaridia galli* and *Capillaria* spp. are the most important parasites in terms of economic losses. In several cases of *Heterakis gallinarum* infection, there is an increased chance of developing a *Histomonas meleagridis* infection (Blackhead) (Ruff, 1999). Additionally, tapeworms as *Railletina* spp. also complicate the sanitary situation of the avian farms, especially when breeders are involved.

Chemotherapy is the most widely used method to control parasitic infections. Benzimidazoles (BZD) are anthelmintic drugs widely used in veterinary and human medicine (Campbell, 1990; McKellar & Scott, 1990). The BZD methylcarbamate compounds, such as albendazole (ABZ), fenbendazole (FBZ), and flubendazole (FLBZ), are broad-spectrum anthelmintics with an excellent nematodicidal and cestodicidal activity. ABZ exhibits high efficacy against BZD-susceptible adult and larval stages of nematodes, cestodes, and trematodes (Campbell, 1990; McKellar & Scott, 1990).

Among the different BZD methylcarbamate compounds, FLBZ is the only BZD registered for poultry in several countries (CODEX, 1995; EMEA, 2006). However, there are evidences of the extralabel use of ABZ in avian production systems. In fact, the anthelmintic efficacy of ABZ has been demonstrated in poultry (Tucker *et al.*, 2007; Oryan *et al.*, 2009; Lalchhandama, 2010).

Although ABZ pharmacokinetics has been extensively investigated in several animal species (Marriner & Bogan, 1980; Prichard *et al.*, 1985; Alvarez *et al.*, 1996; Sánchez *et al.*, 1997), very scarce information is available on the disposition kinetics and metabolic pattern of ABZ in poultry. A detailed characterization of the kinetic behavior, metabolic fate, and residual profile of ABZ in poultry may contribute to its optimized use in this species. The assessment of the ABZ pharmacokinetic behavior after single intravenous (i.v.) and oral treatments as well as after administration of medicated feed in laying hens was carried out in the work reported here.

## MATERIALS AND METHODS

### *Reagents and chemicals*

Pure reference standards (97–99% purity) of ABZ and its metabolites, albendazole sulphoxide (ABZSO), albendazole sulfone ($ABZSO_2$), and the internal standard (IS) oxibendazole (OBZ) were provided by Toronto Research Chemicals Inc. (Toronto, ON, Canada). Acetonitrile solvent used during the extraction and drug analysis were HPLC grade and purchased from Sintorgan® S.A. (Buenos Aires, Argentina). Ammonium acetate (HPLC grade) was from Baker (Phillipsburg, NJ, USA). Water was double distilled and deionized using a water purification system (Simplicity®; Millipore, São Paulo, Brazil).

### *Animals*

Twenty-four healthy breeder laying hens (*Plymouth Rock Barrada*) of 6 month old, weighing 2.2 ± 0.3 kg b.w., were used in the current experiment. The hens were monitored daily during 2-week acclimatization period. The hens were housed with water and balanced commercial food *ad libitum*. Before the experiments, the hens were not medicated with any anthelmintic drug.

### *Drug formulation*

Drug formulations used in the current experiment were prepared in our laboratory as follows. Intravenous treatment: A 2% ABZ solution was prepared by dissolving 0.5 g of pure ABZ in 5 mL of dimethyl sulphoxide (DMSO) and 20 mL of propylene glycol. The formulation was shaken until total dissolution of the drug. An ABZ 2% suspension was prepared for oral dose. The formulation was prepared after weighing 0.5 g of pure ABZ standard and mixed with 25 mL of HPLC water and 0.125 g of carboxymethylcellulose. An ABZ-medicated feed at 0.150 mg pure ABZ/g food was used. An ABZ premix was prepared by mixing 3 g of pure ABZ standard with 297 g of starch. The premix was mixed mechanically with 19.7 kg of balanced hen feed. Calculations were carried out considering that the daily dose to be administered was 10 mg/kg·day; the hen mean daily food intake was 150 g, and the average hens weight was 2.2 kg.

### *Experimental design*

Animals were randomly distributed into three groups of eight animals, which were treated as follows: *ABZ i.v.*, a single ABZ dose (solution 2%) of 10 mg/kg was administered by i.v. route using a catheter (MCM 24 G, China) previously placed into the right wing vein; *ABZ oral*, a single ABZ dose (suspension 2%) was administered by oral route through a 25-cm length plastic cannula; and *ABZ feed*, received ABZ-medicated feed at 10 mg/kg·day for 7 days simulating a therapeutic treatment.

After treatments, blood samples (1 mL) were taken by an intravenous catheter previously placed into the left wing vein as follows: *ABZ i.v.*, samples were collected from the contralateral administration wing vein at time 0 (blank) and 0.08, 0.25, 0.5, 1, 3, 6, 9, 12, 15, 24, 30, 36, and 48 h posttreatment; *ABZ oral*, at 0, 0.5, 1, 3, 6, 9, 12, 15, 24, 30, 36, and 48 h posttreatment; and *ABZ feed*: At 0, 3, 6, 12 h and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 10 days poststart feed medication. The volume of blood taken in each sample was replaced by i.v. infusion (1 mL) of sterile physiological saline solution. Blood samples previously placed in heparinized tubes were centrifuged at 2000 g for 10 min, and the plasma collected were frozen at −20 °C to be later analyzed.

### *HPLC system*

A high-performance liquid chromatographic (HPLC) method (Bistoletti *et al.*, 2011) was used for the analysis of ABZ and its ABZSO and $ABZSO_2$ metabolites in hen plasma. The analytical

method was developed using a Shimadzu Chromatography system (Shimadzu Corporation, Kyoto, Japan). The equipment was composed for two LC-10AS solvent pumps, an automatic sample injector (SIL-10A), an ultraviolet visible spectophotometric detector (UV) (SPD-10A), a column oven (Eppendorf TC-45; Eppendorf, Madison, WI, USA) set at 35 °C, and a CBM-10A data integrator. Data and chromatograms were collected and analyzed using the Class LC10 software (SPD-10A; Shimadzu Corporation). A $C_{18}$ reversed-phase column (Kromasil®; Eka Chemicals AB, NY, USA) of 250 × 4.6 mm with 5-$\mu$m particle size was used for separation. Elution from the stationary phase was carried out at a flow rate of 1.2 mL/min using acetonitrile and ammonium acetate buffer (0.025 M, pH 5) as the mobile phase. The gradient changed linearly from 23:77 (acetonitrile: ammonium acetate buffer) to 43:57 in 7 min and then was maintained for 1 min and modified to 50:50 in 3 min, which was maintained over 4 min to be modified in 2 min to 23:77, conditions maintained until the run finished at 25 min. Drugs/metabolites were detected at a wavelength of 292 nm.

### *Plasma sample extraction*

Following the methodology previously described (Bistoletti *et al.*, 2011), plasma sample (0.5 mL) was spiked with 20 $\mu$L of the IS (20 $\mu$g/mL). Drug molecules were extracted from plasma by the addition of 1 mL acetonitrile (twice) under a high-speed vortexing shaker (Multi-tube Vortexer; VWR Scientific Products, West Chester, PA, USA) over 5 min. After centrifugation (BR 4i Centrifuge, Jouan®; Saint Herblain, France) at 2000 *g* for 10 min at 10 °C to allow phase separation, the clear supernatant was transferred to a 5-mL plastic tube. The total extraction solvent (2 mL) was evaporated (40 °C) to dryness in a vacuum concentrator (Speed-Vac®; Savant, Los Angeles, CA, USA) and then reconstituted with 200 $\mu$L of mobile phase. A 50-$\mu$L aliquot was injected into the HPLC system.

### *Method validation*

The HPLC method to quantify ABZ and its metabolites in hen plasma was previously validated (Bistoletti *et al.*, 2011). The calibration curves for each analyte was constructed by least squares linear regression analysis, showing good linearity with correlation coefficients >0.9964. The absolute recoveries for ABZ, ABZSO, and $ABZSO_2$ ranged between 81.8 and 98.2. Precision (intra- and inter-assay) (CV) was lower than 7.79%. The limits of quantification (LOQ) for ABZ and its metabolites ranged between 0.05 and 0.125 $\mu$g/$\mu$L.

### *Pharmacokinetic and statistical analysis*

The pharmacokinetic analysis of the plasma concentration vs. time curves for ABZ and its metabolites for each animal was carried out using the PK Solution 2.0 software (Summit Research Services, Ashland, OH, USA). Pharmacokinetic analysis of the experimental data was performed using noncompartmental (area) and compartmental (exponential terms) methods without presuming any specific compartmental model. The concentration–time profile for ABZ parent drug in plasma after its i.v. administration was best fitted to a bioexponential equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and B are primary and secondary disposition intercepts; $\alpha$ and ß were the primary and secondary disposition rate constants ($h^{-1}$); and $C_p$ was the plasma concentration of ABZ at time $t$. The distribution and elimination half-lives were calculated as ln 2 divided by the rate constants. The estimated plasma concentration of ABZ parent drug at zero time ($C_{p0}$) after its i.v. administration was the sum of the extrapolated zero-time concentrations of the coefficients A and B. Estimation of the volume of the central compartment (Dose/$C_{p0}$) and microconstants were also obtained. The total body clearance (Cl) was calculated by the following formula:

$$Cl = Dose/AUC$$

The volume of distribution ($Vd_{area}$) was estimated by the following equation:

$$Vd_{area} = Dose/(AUC)(\beta)$$

The overall extraction ratio ($E$) was calculated by the following formula:

$$E = Cl/Q, \text{were } Q = \text{Cardic output} = 180\ BW^{-0.19};$$
$$\text{with BW} = \text{kg and } Q = \text{mL} \cdot \text{kg/min}$$

The following equation was used to describe the biexponential plasma concentration–time curves for ABZSO and $ABZSO_2$ after the oral or i.v. administration (Notari, 1987):

$$C_p = Be^{-\beta t} - Be^{-Kt}$$

where $C_p$ = concentration at time $t$ after administration ($\mu$g/mL); $B$ = concentration at time zero extrapolated from the elimination phase ($\mu$g/mL); e = base of the natural logarithm; $\beta$ = terminal slope ($h^{-1}$) and $K$ is the rapid slope obtained by feathering which represents the first-order metabolite formation rate constant ($K_{for}$) ($h^{-1}$). The elimination half-life ($T_{1/2el}$) was calculated as ln $2/\beta$. The peak concentration ($C_{max}$) and time to peak concentration ($T_{max}$) were read from the plotted concentration–time curve for each analyte. The area under the concentration–time curve (AUC) for ABZ/metabolites in plasma was calculated by the trapezoidal rule (Gibaldi & Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope ($\beta$).

Statistical moment theory was applied to calculate the mean residence time (MRT) for ABZ and metabolites in plasma as follows (Gibaldi & Perrier, 1982).

$$MRT = AUMC/AUC$$

where AUC is defined previously and AUMC is the area under the curve of the product of time and the plasma drug concentration vs. time from 0–∞ (Gibaldi & Perrier, 1982).

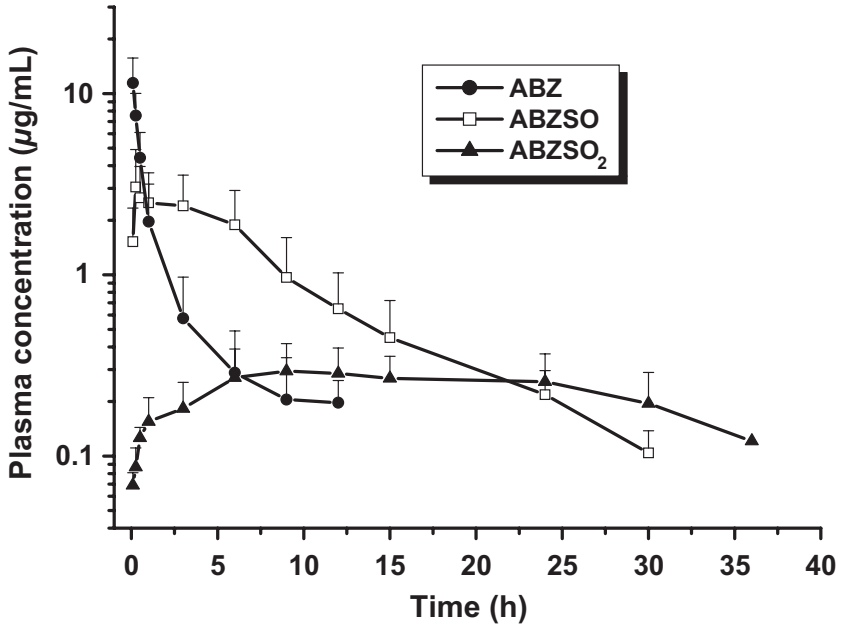


**Fig. 1.** Mean (±SEM) albendazole (ABZ), albendazole sulphoxide (ABZSO), and albendazole sulphone ($ABZSO_2$) plasma concentration profiles vs. time obtained after ABZ single intravenous administration (10 mg/kg) to laying hens.

Bioavailability ($F$) of ABZ was calculated as follows:

$$F = [(AUC_{0-\infty\,oral} \times D_{i.v.}) / (AUC_{0-\infty\,i.v.} \times D_{oral})] \times 100$$

The pharmacokinetic parameters and concentration data are reported as mean ± SEM. The time-based parameters (MRT, $T_{1/2\ for}$, $T_{1/2el}$) are expressed as harmonic means.

## RESULTS

Albendazole and its ABZSO and $ABZSO_2$ metabolites were recovered in plasma after the i.v. administration of ABZ formulated as injectable solution in hens. The mean plasma drug concentration profiles after i.v. administration are shown in Fig. 1. ABZ showed an initial concentration (5 min) of 16.4 ± 2.0 μg/mL, which rapidly decreased as a typical i.v. administration until the lowest concentration (0.1 μg/mL) attained at 12 h postadministration. ABZ was rapidly metabolized to the ABZSO and $ABZSO_2$ metabolites. ABZSO was detected at 5 min posttreatment and rapidly increased to reach its $C_{max}$ (3.10 ± 0.78 μg/mL) at 1.23 ± 0.56 h ($T_{max}$) postdosing. ABZSO concentration was higher than that of ABZ from $T_{max}$ up to 30 h posttreatment. Lower $ABZSO_2$ plasma concentrations were measured, being detected at 5 min posttreatment and increased to reach its $C_{max}$ (0.34 ± 0.05 μg/mL) at 13.7 ± 2.9 h ($T_{max}$) postdosing. Table 1 shows the complete plasma disposition kinetics data for ABZ and its ABZSO and $ABZSO_2$ metabolites after ABZ i.v. administration.

The AUC for ABZSO (21.9 ± 3.6 μg·h/mL) was higher than that observed for $ABZSO_2$ and ABZ. The ABZ body Cl was 0.88 ± 0.11 L·h/kg with a $T_{1/2el}$ of 3.47 ± 0.84 h. The $T_{1/2el}$ for ABZSO and $ABZSO_2$ were 6.36 ± 1.50 and 5.40 ± 1.90 h, respectively. The different permanence in plasma was evidenced by MRT with values of 4.73 ± 0.73, 8.51 ± 2.21, and 19.4 ± 1.9 h for ABZ, ABZSO, and $ABZSO_2$ h, respectively.

**Table 1.** Mean plasma pharmacokinetic parameters (±SEM) ($n$ = 8) for albendazole (ABZ), albendazole sulphoxide (ABZSO), and albendazole sulphone ($ABZSO_2$) obtained after single ABZ intravenous administration (10 mg/kg) to laying hens

| PK parameter | ABZ | ABZSO | $ABZSO_2$ |
|---|---|---|---|
| $C_0$ (μg/mL) | 16.4 ± 2.0 | – | – |
| $T_{½\ for}$ (h) | – | 0.19 ± 0.63 | 2.56 ± 0.42 |
| $C_{max}$ (μg/mL) | – | 3.10 ± 0.78 | 0.34 ± 0.05 |
| $T_{max}$ (h) | – | 1.23 ± 0.56 | 13.7 ± 2.9 |
| $AUC_{0\text{-}LOQ}$ (μg·h/mL) | 12.0 ± 1.6 | 21.9 ± 3.6 | 7.80 ± 1.02 |
| $AUC_{0-\infty}$ (μg·h/mL) | 12.4 ± 1.5 | 22.9 ± 3.6 | 8.70 ± 1.07 |
| $Vd_{area/kg}$ (L/kg) | 4.03 ± 0.52 | – | – |
| $CL_{area/kg}$ (L·h/kg) | 0.88 ± 0.11 | – | – |
| $T_{½\ el}$ (h) | 3.47 ± 0.84 | 6.36 ± 1.50 | 5.40 ± 1.90 |
| MRT (h) | 4.73 ± 0.73 | 8.51 ± 2.21 | 19.4 ± 1.9 |

$C_0$: initial concentration extrapolated to time '0'; $T_{½\ for}$: formation half-life; $C_{max}$: peak plasma concentration; $T_{max}$: time to peak concentration; $AUC_{0\text{-}LOQ}$: area under the plasma concentration vs. time curve from 0 up to the quantification time; $AUC_{0-\infty}$: area under the concentration vs. time curve extrapolated to infinity; $Vd_{area}$: apparent volume of distribution; $CL_{area}$: clearance; $T_{½\ el}$: elimination half-life; MRT: mean residence time.

The mean plasma concentration profiles for ABZ and its metabolites after oral administration at 10 mg/kg to hens are shown in Fig. 2. Low ABZ plasma concentrations were measured between 0.5 up to 3 h posttreatment. The higher mean ABZ plasma (0.250 μg/mL) was quantified in the first time (30 min) and then found declining until 3 h. The low concentrations detected during a short time period precluded the development of a complete pharmacokinetic analysis. ABZ was also rapidly metabolized to ABZSO and $ABZSO_2$ after oral administration. ABZSO was detected in plasma from 30 min to 36 h posttreatment, achieving the $C_{max}$ (1.71 ± 0.62 μg/mL) at 4.60 ± 0.92 h ($T_{max}$). The concentrations of $ABZSO_2$ were much lower than ABZSO concentrations and were detected in plasma from 3 to 36 h posttreatment with a $C_{max}$ of

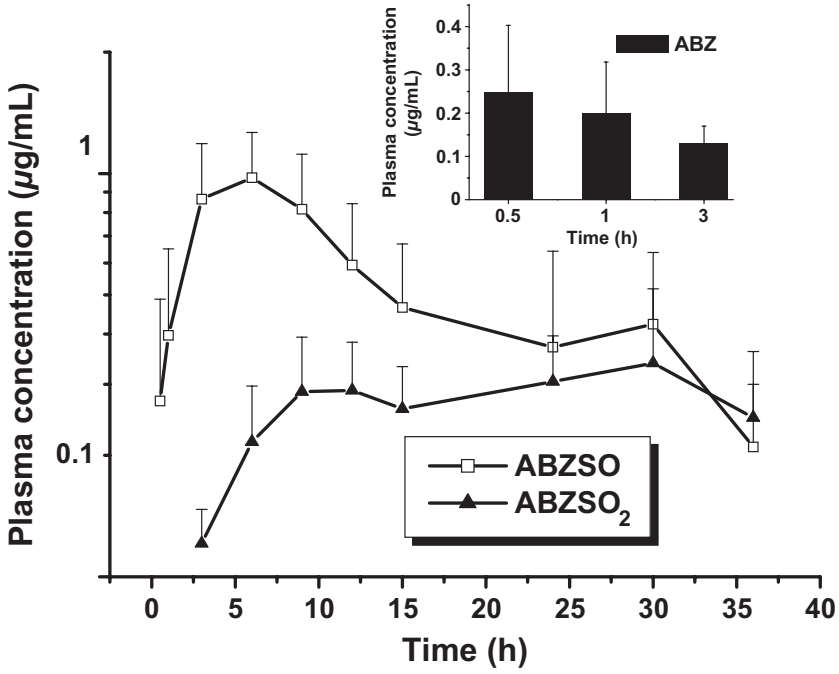


**Fig. 2.** Mean (±SEM) albendazole (ABZ), albendazole sulphoxide (ABZSO), and albendazole sulphone ($ABZSO_2$) plasma concentration profiles vs. time obtained after oral administration of ABZ (10 mg/kg) to laying hens.

**Table 2.** Mean plasma pharmacokinetic parameters (±SEM) ($n$ = 8) for albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) metabolites obtained after single albendazole oral administration (10 mg/kg) to laying hens

| PK parameter | ABZSO | $ABZSO_2$ |
|---|---|---|
| $T_{½\ for}$ (h) | 1.34 ± 0.43 | 1.89 ± 0.33 |
| $C_{max}$ (μg/mL) | 1.71 ± 0.62 | 0.43 ± 0.04 |
| $T_{max}$ (h) | 4.60 ± 0.92 | 19.1 ± 3.6 |
| $AUC_{0\text{-}LOQ}$ (μg·h/mL) | 18.6 ± 2.2 | 10.6 ± 0.9 |
| $AUC_{0-\infty}$ (μg·h/mL) | 18.9 ± 2.3 | 11.1 ± 0.9 |
| $T_{½\ el}$ (h) | 5.91 ± 0.60 | 5.57 ± 1.19 |
| MRT (h) | 12.2 ± 1.8 | 21.2 ± 1.7 |
| $F$ (%) | 84.9 | 135.8 |

$T_{½\ for}$: formation half-life; $C_{max}$: peak plasma concentration; $T_{max}$: time to peak concentration; $AUC_{0\text{-}LOQ}$: area under the plasma concentration vs. time curve from 0 up to the quantification time; $AUC_{0-\infty}$: area under the concentration vs. time curve extrapolated to infinity; $T_{½\ el}$: elimination half-life; MRT: mean residence time; $F$ (%): relative bioavailability.

0.43 ± 0.04 μg/mL at 19.1 ± 3.4 h ($T_{max}$). The plasma disposition kinetics data for ABZ and its metabolites after oral administration of ABZ are summarized in the Table 2. The metabolite plasma disposition (AUC) values were 18.6 ± 2.2 and 10.6 ± 0.9 μg·h/mL for ABZSO and $ABZSO_2$, respectively. The half-life values after oral administration for both metabolites were similar (5.91 ± 0.60 and 5.57 ± 1.19 h) to the found after i.v. administration. The MRT values obtained after oral administration were 12.2 ± 1.8 and 21.2 ± 1.7 h for ABZSO and $ABZSO_2$, respectively.

The mean plasma concentration profiles for ABZ metabolites after ABZ-medicated feed administration at 10 mg/kg·day for 7 days to hens are shown in Fig. 3. Opposite to that found after single i.v. or oral administrations, ABZ was not measured after medicated feed administration. Metabolite concentrations were measured in plasma from 3 h to 7 days (ABZSO) and from 6 h to 9 days ($ABZSO_2$) after the start treatment. ABZSO and $ABZSO_2$ metabolites plasma drug exposure represented by AUC were 61.9 and 92.4 μg·h/mL, respectively.

The comparative AUC values obtained after the three ABZ administrations to laying hens are presented in Fig. 4.

## DISCUSSION

In recent years, an increasing prevalence of roundworms, especially in litter-based housing systems, associated with increased exposure to parasites with a fecal–oral route of transmission has been reported (Stafford *et al.*, 2011; Höglund & Jansson, 2011; Jansson *et al.*, 2010; Papini & Cacciuttolo, 2008). Strategies to deworming are being introduced in the avian production systems with the limitation that FLBZ is currently the only anthelmintic licensed to be used in poultry. However, there are evidences that ABZ is extralabeled used without the knowledge of its pharmacokinetic behavior in poultry. The work reported here contributes in that direction. After i.v. administration in hens, ABZ initial concentration ($C_0$) was high and rapidly depleted from the bloodstream as reported in other species (Alvarez *et al.*, 1999). ABZ plasma concentrations in hens decreased rapidly and were not detectable beyond 12 h posttreatment. The metabolites ABZSO and $ABZSO_2$ appeared rapidly in the bloodstream ($T_{1/2\ for}$: 0.19 ± 0.63 and 2.56 ± 0.42 h, respectively) after the i.v. administration of ABZ, which indicates a fast ABZ biotransformation process. ABZSO and $ABZSO_2$ were measured in plasma up to 30 and 36 h, respectively, after the i.v. treatment. The plasma concentration

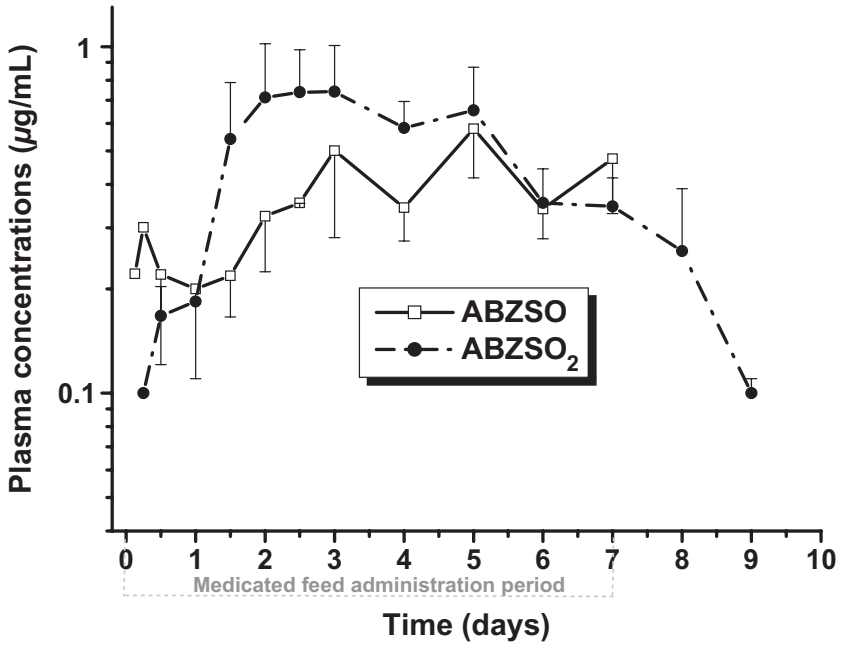


**Fig. 3.** Mean (±SEM) albendazole (ABZ), albendazole sulphoxide (ABZSO), and albendazole sulphone ($ABZSO_2$) plasma concentration profiles vs. time obtained after ABZ medicated feed administration (10 mg/kg·day) for 7 days to laying hens.

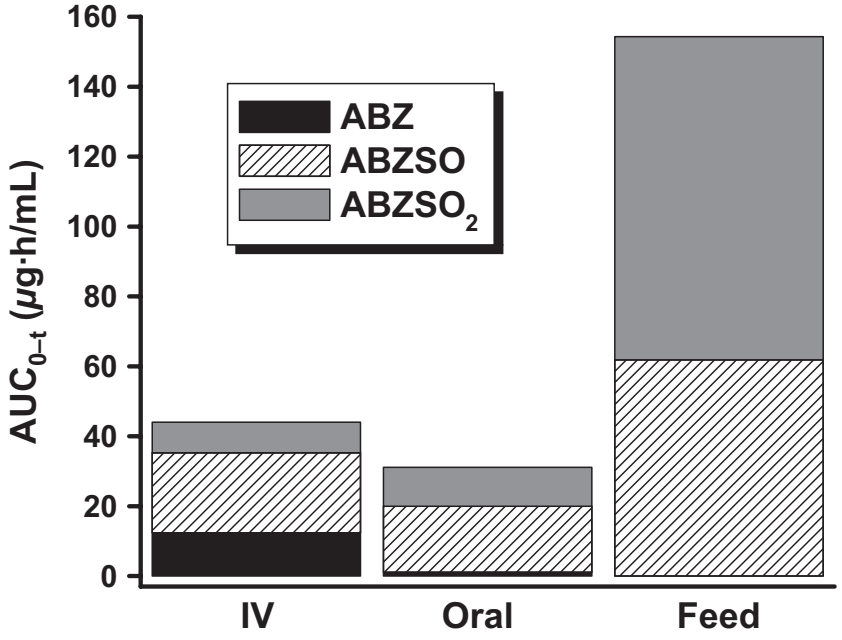


**Fig. 4.** Mean values of area under the plasma concentration–time curve (AUC) ($\mu$g·h/mL) for albendazole (ABZ), albendazole sulphoxide (ABZSO), and albendazole sulphone ($ABZSO_2$) obtained after ABZ administration by intravenous and oral routes (10 mg/kg single doses) or medicated feed (10 mg/kg·day for 7 days) to laying hens.

profiles obtained after the i.v. treatment of ABZ in hens was similar to that reported in sheep (Alvarez *et al.*, 1999). In both species, after ABZ i.v. administration, the highest plasma concentration profile was for ABZSO, followed by ABZ and $ABZSO_2$. However, a higher ABZ volume of distribution, longer $T_{1/2el}$ and MRT and lower overall extraction ratio (0.09 against 0.19), were observed in hens compared to sheep, which indicates longer permanence in plasma probably due to a slower metabolic process in hens.

Consistently with the i.v. administration, ABZ and its ABZSO and $ABZSO_2$ metabolites were detected in plasma after ABZ oral administration. Low parent drug concentrations were quantified from 30 min to 3 h. In previous works, after oral administration to hens (Bistoletti *et al.*, 2011) and chicken (Csiko *et al.*, 1996), also ABZ plasma concentrations were measured in plasma. In contrast, ABZ was not measured in several mammalian species after oral administration (Marriner & Bogan, 1980; Hennessy *et al.*, 1989; Benchaoui *et al.*, 1993; Alvarez *et al.*, 1996; Sánchez *et al.*, 1997, 2000). This difference has been associated with a fast absorption process or a slow metabolism in hens compared with ruminants (Csiko *et al.*, 1996). The gastrointestinal absorption of ABZ is limited by its poor water solubility, which is markedly

improved by low pH values. This low solubility reduces the dissolution of suspended particles of the drug. As we had previously reported (Bistoletti *et al.*, 2011), the low physiological pH of the poultry muscular stomach (gizzard), considered a powerful triturating machine, may contribute to a better ABZ dissolution in poultry compared to other animal species.

Both the ABZ absorption and metabolism processes were fast, as the metabolite $t_{1/2\,for}$ values were 1.34 ± 0.43 and 1.89 ± 0.33 h for ABZSO and $ABZSO_2$, respectively. ABZSO concentrations were measured from 30 min to 36 h posttreatment achieving a $C_{max}$ (1.71 ± 0.62 μg/mL) at 4.60 ± 0.92 h ($T_{max}$). Meanwhile, the sulfone metabolite ($ABZSO_2$) was quantified the first time at 3 h, achieving a lower peak plasma concentration (0.43 ± 0.04 μg/mL) much later at 19.1 ± 3.6 h ($T_{max}$), being measured also until 36 h postadministration. The plasma disposition of the active metabolite ABZSO was higher than that of the inactive metabolite ($ABZSO_2$). Results found after previous studies carried out in chicken (Csiko *et al.*, 1996) and hens (Bistoletti *et al.*, 2011) agree with those reported in the current work. In most of the mammalian species, after the same or lower ABZ dose administered by oral route (except in calves (Sánchez *et al.*, 1997) where the metabolic pattern is reverse), the AUC values of the ABZSO active metabolite are higher than that found in hens in the present work. Also, the $T_{max}$ was longer in sheep, goat (Hennessy *et al.*, 1993, Benchaoui *et al.*, 1993), calf (Sánchez *et al.*, 1997), pig (Alvarez *et al.*, 1996), and dogs (Sánchez *et al.*, 2000) than that found in the present and previous works in poultry (Csiko *et al.*, 1996; Bistoletti *et al.*, 2011). These differences indicate that ABZ absorption and metabolism patterns differ between avian and mammalians species.

Although ABZ concentrations were measured after oral administration to hens, the bioavailability was very low (8.9%) as a consequence of the first pass effect after intestinal absorption. However, when we compare the plasma metabolite exposure between routes ($AUC_{0-\infty\ oral}/AUC_{0-\infty\ i.v.}$), values of 84.9% and 135.8% were found for ABZSO and $ABZSO_2$, respectively.

Different from that found after single i.v. or oral administration, ABZ was not recovered in plasma after its administration in feed at 10 mg/kg·day for 7 days. ABZSO was quantified from 3 h to 7 days after the medicated treatment, reaching a $C_{max}$ 0.58 ± 0.16 μg/mL at the 5 day and suffering a rapid elimination process. Interestingly, opposite to the single oral administration, the $ABZSO_2$ mean plasma concentrations were higher than that measured for ABZSO. The permanence of the $ABZSO_2$ metabolite was longer, being measured by the first time at 6 h, all days during treatment (7 days) and 2 days after finishing treatment. The mean $ABZSO_2$ peak plasma concentration was 0.74 ± 0.26 μg/mL at third treatment day. According to the mean concentration profiles, after ABZ-medicated feed administration, the $ABZSO_2$ plasma disposition (92.4 μg·h/mL) was higher than that found for ABZSO (61.84 μg·h/mL). The variability in plasma drug concentrations after ABZ administration in food was high, probably associate with individual variations in the drug kinetic and in the physiological food intake between hens. In addition, the palatability of the medicated feed could be lower, because it was observed that the intake of hens fed with ABZ-medicated feed was lower than that of not treated hens. On the other hand, the fact that the ABZ parent drug was not measured at any time after feed medication could be associated with a slower intake of the ABZ daily dose, slower drug absorption process, and as a consequence, all the absorbed ABZ was metabolized in the liver without reach quantifiable plasma concentrations.

In accordance with the European legislation on the welfare of laying hens, a progressively increasing number of farmers can adopt breeding programs on soil; as a consequence, a high prevalence of helminth infections has been reported (Kaufmann *et al.*, 2011). Although quite a large number of organic layer farmers used homeopathic, phytotherapeutic, or other alternative medicines, the use of chemotherapeutics is currently inevitable to prevent animal suffering or distress in organic husbandry (Van Der Meulen *et al.*, 2007). The work reported here provides useful information on the pharmacokinetic behavior of ABZ after i.v. or oral administrations in hens, which is a useful first step to evaluate its potential as an anthelmintic tool for use in poultry. Follow-up studies to characterize the pattern of ABZ metabolites tissue and egg residue profiles in hens are required to establish suitable withdrawal times to ensure consumer health.

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