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Effect of Amphiphilic Surfactant Agents on the Gastrointestinal Absorption of Albendazole in Cattle

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ABSTRACT: Albendazole (ABZ) is a widely used broad-spectrum benzimidazole (BZD) anthelmintic. Low hydrosolubility and poor/erratic gastrointestinal (GI) absorption play against the systemic availability and resultant clinical efficacy of BZD compounds. Different strategies are currently investigated to improve their bioavailability and efficacy in different animal species and humans. Surfactant agents facilitate dissolution of lipophilic drugs and increase membrane permeability. The influence of amphiphilic surfactants on the pattern of absorption and systemic availability of ABZ and its metabolites in cattle was characterized in the current trial. Twenty (20) parasite-free Holstein calves (100–120 kg) were randomly allocated into four groups and treated intraruminally (10 mg/kg) using one of the following ABZ suspensions: *control* without surfactant (75/25 dimetyl sulphoxide/saline solution) (group A), *5 mM sodium taurocholate* (STC) in saline solution (group B), *8.27 mM sodium lauryl sulphate* (SLS) in saline solution (group C) and a *commercial formulation* (Valbazen®, Pfizer Inc. SA) (group D). Jugular blood samples were taken over 72 h post-treatment and plasma analysed by HPLC. Albendazole sulphoxide (ABZSO) and sulphone were the metabolites found in plasma. STC did not affect ABZ absorption while increased ABZSO peak plasma concentration (C_{max}) (158% higher, $P < 0.001$) was observed following co-administration of ABZ plus SLS, compared to the control group without surfactant. ABZSO plasma availability was significantly greater after the ABZ–SLS (164%) co-administration compared to that obtained in the control group without surfactant. A similar ABZSO plasma availability was obtained following the treatments with the ABZ-SLS and the commercially available formulation. SLS-mediated enhanced dissolution and absorption of ABZ accounted for the observed increased systemic availability of the active ABZSO metabolite in cattle. These results should be considered among strategies to improve the use of BZD anthelmintics.

Introduction

Albendazole (ABZ) is a broad-spectrum benzimidazole (BZD) anthelmintic widely used for the control of gastrointestinal (GI) round-worms, lungworms, tapeworms and liver flukes in livestock animals. This antiparasitic compound is also extensively recommended in human medicine to treat infections by different nematodes, hydatid cysts, giardiasis and microsporidiosis [1]. It is also potencially useful in the treatment of liver cancer [2].

The lack of water solubility reduces flexibility for formulation of ABZ (and other BZD compounds), allowing its formulation only as suspensions for oral/intraruminal administration in ruminant species [3]. Poor/erratic GI absorption is an important limitation in the bioavailability and resultant efficacy of enterally administered

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BZD suspensions in sheep and cattle. Development of more water-soluble pro-drugs (pro-BZD), chemical modification of the molecule of oxfendazole (OFZ) [4], preparation of ABZ loaded nanoparticles [5] or an aqueous solution of ABZ with cyclodextrins [6] are among other strategies used previously to overcome these problems. An injectable formulation (solution) of ricobendazole (RBZ) has been developed recently exploiting its greater hydrosolubility compared to other BZD molecules [7]. The subcutaneous treatment with this formulation resulted in greater plasma availability compared to the oral treatment with ABZ administered as an oral suspension at the same dose rate. On the other hand, several management practices have been investigated to improve the absorption and systemic bioavailability of BZD anthelmintics. In fact, starvation prior to treatment in cattle [8] and a reduction in feed intake level in sheep [9] enhanced the dissolution and GI absorption of BZD molecules by reducing the digesta transit time. Understanding factors affecting the GI absorption and subsequent bioavailability of BZD anthelmintics is relevant to optimize the anthelmintic therapy and to delay the development of parasite resistance.

Amphiphilic surfactants increase the dissolution and solubilization of lipophilic drugs [10]. They also seem to increase membrane permeability improving the GI absorption of both water-soluble and lipophilic molecules. It has been shown that surfactant agents enhance the intestinal absorption of ABZ in rats [11–13]. Considering the economical impact of parasite control in the veterinary pharmaceutical market, further research to optimize drug use in livestock species is required. The aim of this study was to asses the influence of amphiphilic surfactants on the pattern of GI absorption and systemic availability of ABZ and its metabolites in cattle.

Materials and Methods

Experimental animals and treatments

Twenty (20) parasite-free Holstein calves in optimal nutritional condition (100–120 kg) were randomly allocated into four groups and treated intraruminally (10 mg/kg) using one of the following ABZ suspensions (100 mg/ml): control without surfactant (75/25 dimethyl sulphoxide/saline solution) (group A), 5 mM sodium taurocholate (STC) in saline solution (group B), 8.27 mM sodium lauryl sulphate (SLS) in saline solution (group C) and a commercially available micronized formulation (Valbazen, Pfizer Inc. SA, Buenos Aires, Argentina) (group D). Animals were fed on a high-quality lucerne hay and had free access to water. The health of the animals was monitored prior to and throughout the experimental period.

Samples collection

Samples of jugular blood were collected prior to treatment and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 60 and 72 h post-administration. The collected blood samples were centrifuged at 3000 *g* for 15 min and the recovered plasma stored at −20°C until the time of analysis.

Analytical procedures

Sample clean-up/extraction. Plasma samples (1 ml) were spiked with an internal standard (oxibendazole, 99.2% pure, 1 μg/ml of methanol) and ABZ and its metabolites were extracted using disposable C_{18} columns for solid-phase extraction (Supelclean, Supelco Inc., Bellefonte, USA). The extraction of the analytes from plasma samples was done according to the technique described by Hennessy *et al*. [14] and modified by Lanusse and Prichard [15]. The solvents and reagents (Baker Inc., Phillipsburg, NJ, USA) used during the extraction and drug analysis were of HPLC grade.

Drug and metabolites analysis. Experimental and fortified plasma samples were assayed for ABZ and its metabolites using high-performance liquid chromatography (HPLC). Fifty microlitres (50 μl) were injected into a Shimadzu 10 A HPLC system (Shimadzu Corporation, Kyoto, Japan) fitted with a Selectosil C_{18} (5 μm, 250 mm × 4.60 mm) reverse-phase column (Phenomenex, CA, USA) and UV detector (Shimadzu, SPD-10A UV detector, Shimadzu Corporation, Kyoto, Japan) reading at 292 nm. Chromatographic conditions and retention times for the different

analytes were as previously reported [15]. The analytes were identified with the retention times of 97–99% pure reference standards. Drug and metabolites recoveries were established by comparison of the detector responses obtained for plasma and abomasal fluid fortified samples and those of direct standards prepared in methanol. Recovery percentages ranged from 93 to 98%. The analytes were identified with the retention times of pure reference standards. Regression lines for each analyte were constructed from the least-squares linear regression analysis of HPLC peak area ratios of unknown analytes/internal standard and nominal concentrations of spiked plasma (0.05–3 μg/ml) samples. The correlation coefficients ranged between 0.998 and 0.999. The concentration of each compound was determined by comparison with the internal standard peak area, using Class LC 10 software (Shimadzu Corporation, Kyoto, Japan) on an IBM compatible computer. The quantification limits for ABZ and its metabolites, reading at 292 nm ranged between 0.01 and 0.03 μg/ml. These values were obtained by injection and HPLC analysis of blank plasma samples fortified with the internal standard and measurement of the baseline noise at the time of retention of each analyte. The mean baseline noise plus 10 standard deviations was defined as the theoretical quantification limit. Experimental concentration values below the quantification limits were not used for the pharmacokinetic analysis of the data. There was no interference of endogenous compounds in the chromatographic determinations.

Kinetic and statistical analysis of data

The plasma concentration vs time curves of ABZ metabolites for each individual animal were fitted with the PK Solutions 2.0 computer program (SUMMIT Research Services, Ashland, USA). Pharmacokinetic parameters were determined using a noncompartmental method. The following equation was used to describe the biexponential concentration–time curves for the different analytes detected in plasma [16]

$$C_p = C_1 e^{-\lambda_2 t} - C_2 e^{-k a^t}$$

where C_p is the plasma concentration at time t after administration (μg/ml); C_1 is the concentration at time zero extrapolated from the elimination phase (μg/ml); e is the base of the natural logarithm λ_2 is the terminal slope (h^{-1}) and k_a is the rapid slope obtained by feathering which represents either the first-order absorption rate constant (k_{ab}) or first-order appearance rate constant (k_{app}) (h^{-1}).

The elimination ($T_{1/2el}$) and appearance ($T_{1/2app}$) half-lives were calculated as $\ln 2/\lambda_2$ and $\ln 2/k_{app}$, respectively. The peak concentration (C_{max}) and time to peak concentration (T_{max}) were read from the plotted concentration-time curve of each analyte. The area under the concentration-time curves (AUC) was calculated by trapezoidal rule [17] and further extrapolated to infinity by dividing the last experimental plasma concentration by the terminal slope (λ_2). The Wagner–Nelson method [17] was used to estimate comparative absorption rates after the intraruminal administration of the different formulations under evaluation.

Statistical moment theory was applied to calculate the mean residence time (MRT) for either drug or metabolite as follows:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

where AUC is as defined previously and AUMC is the area under the curve of the product of time and the plasma drug concentration vs time from zero to infinity [17].

Pharmacokinetic parameters are presented as mean ± SD. The mean results obtained after each ABZ treatment were statistically compared using one-way analysis of variance (Instat 3.00, Graph Pad Software, Inc.), where significant overall differences ($P < 0.05$) were obtained, further analysis among individual groups was performed using the Tukey–Kramer Multiple Comparisons test.

RESULTS

ABZSO and albendazole sulphone ($ABZSO_2$) were the metabolites detected in plasma after the intraruminal administration of each ABZ formulation. The mean plasma concentration profiles for ABZSO obtained following the intraruminal administration of ABZ without surfactant

(control) and plus STC (5 mM) and SLS (8.27 mM) are shown in Figures 1 and 2, respectively. Table 1 shows the mean pharmacokinetic parameters obtained after administration of the different ABZ suspensions. STC did not affect ABZ absorption while increased ABZSO peak plasma concentration (C_{max}) (158% higher, $P < 0.001$) was observed following co-administration of ABZ plus SLS, compared to the control group without surfactant. Thus, the increased GI absorption of ABZ in the presence of the SLS tensioactive was consistent with the earlier detection, the higher C_{max} ($P < 0.001$) and AUC values obtained for ABZSO after administration of the ABZ-SLS formulation compared to the ABZ control suspension without surfactant agent. No statistically significant differences were observed between the pharmacokinetic parameters of ABZSO obtained after administration of ABZ plus SLS and those observed after treatment with the commercial formulation of ABZ. However, ABZSO C_{max} and AUC values were higher ($P < 0.05$) following the administration of the commercially available formulation compared with ABZ treatment without surfactant (control group). The comparison of the ABZSO plasma AUC values obtained after each ABZ intraruminal treatment is shown in Figure 3 . The greater extent of ABZ absorption in the presence of SLS was confirmed by the cummulative amount of ABZSO reaching the bloodstream after the ABZ–SLS treatment compared with ABZ suspension without surfactant (Figure 4). As shown for the AUC values, there were no differences between the cummulative amounts of ABZSO in plasma observed after the ABZ–SLS administration and the commercially available formulation treatment.

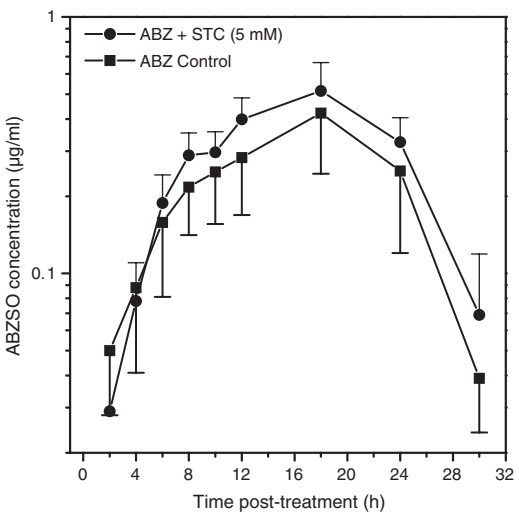

Figure 1. Comparative mean (± SD) plasma concentrations of albendazole sulphoxide (ABZSO) obtained after the intraruminal administration (10 mg/kg) of albendazole (ABZ) *without surfactant* (control) and ABZ plus 5 mM *sodium taurocholate* (STC) to cattle

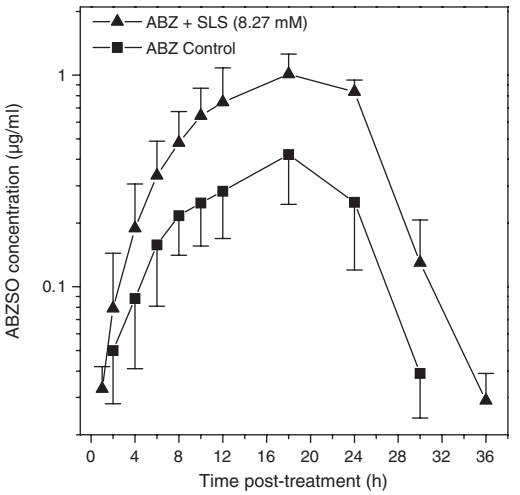

Figure 2. Comparative mean (± SD) plasma concentrations of ABZSO obtained after the intraruminal administration of albendazole (ABZ) *without surfactant* (control) and ABZ plus 8.27 mM *sodium lauryl sulphate* (SLS) to cattle

$ABZSO_2$ was detected in plasma between 2 and 36 h after the administration of ABZ (control without surfactant) and ABZ plus STC suspensions and from 1 to 36 h following ABZ-SLS and the commercial formulation treatments. Plasma pharmacokinetic parameters obtained for this metabolite after each ABZ treatment are shown in Table 2. The changes on ABZ absorption induced by the surfactant agents were also reflected in the plasma availability of the inactive sulphone metabolite.

DISCUSSION

A close relationship between pharmacokinetic behaviour and clinical efficacy for different anthelmintic drugs has been demonstrated [18,

Table 1. Comparative pharmacokinetic parameters for albendazole sulphoxide (ABZSO) obtained after the intraruminal administration (10 mg/kg) of different albendazole (ABZ) suspensions (prepared with and without surfactants) to cattle

Pharmacokinetic parameters	ABZ formulations			
	Control (without surfactant)	ABZ+STC (5 mM)	ABZ+SLS (8.27 mM)	Commercial[a] formulation
$T_{1/2app}$(h)	3.03 ± 0.78	3.79 ± 1.27	3.01 ± 0.51	2.45 ± 0.22
C_{max} (μg/ml)	0.43 ± 0.15	0.54 ± 0.11	1.11 ± 0.22[b]	0.85 ± 0.26[c]
T_{max} (h)	18.00 ± 3.79	15.60 ± 2.94	18.00 ± 3.79	18.00 ± 0.00
AUC $_{(0-\infty)}$ (μg/h ml^{-1})	7.02 ± 2.13	9.32 ± 0.88	18.52 ± 3.21[d]	13.50 ± 3.15[c]
MRT (h)	16.82 ± 0.89	18.24 ± 2.43	17.64 ± 1.01	15.56 ± 0.35
DP (h)	2–30	2–30	1–36	1–36

Note: Data are expressed as mean ± SD (n=5). STC, sodium taurocholate; SLS, sodium lauryl sulphate; ($T_{1/2app}$), appearance half-life; C_{max}, peak plasma concentration; T_{max}, time to peak concentration; $AUC_{(0-\infty)}$, area under the concentration vs time curve extrapolated to infinity; MRT, mean residence time; DP, detection period in plasma.

[a] Comercially available suspension for use in sheep and cattle (Valbazen, Pfizer Inc. SA, Argentina).

[b] Statistically different from control ($P<0.001$) and ABZ plus STC ($P<0.01$) values.

[c] Statistically different from control at $P<0.05$.

[d] Statistically different from control and ABZ plus STC at $P<0.001$.

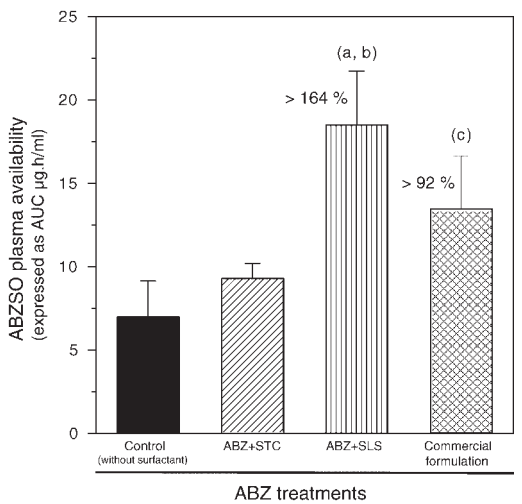

Figure 3. Comparative albendazole sulphoxide (ABZSO) plasma availability obtained after intraruminal administration of different albendazole suspension formulations to cattle. AUC values observed following ABZ+SLS and the commercial formulation treatments were higher (164 and 92%, respectively) compared to those obtained after administration of ABZ without surfactant agents (control). Values are expressed as mean (± SD). (a) Statistically different from ABZ control at $P<0.001$. (b) Statistically different from ABZ+STC (5 mM) at $P<0.001$. (c) Statistically different from ABZ control at $P<0.01$

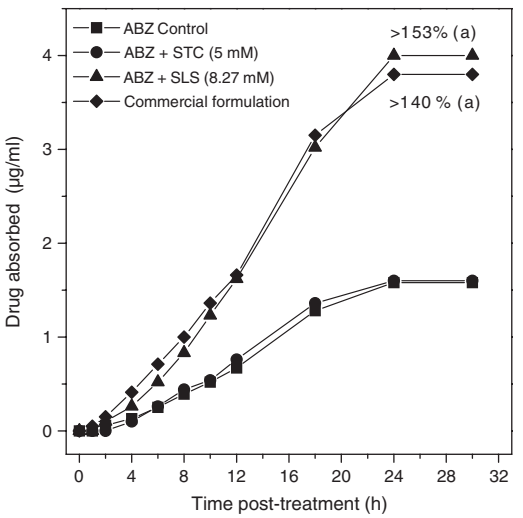

Figure 4. Estimation of albendazole (ABZ) absorption after its intraruminal administration as different formulations to cattle. The amount of drug absorbed (*Y*-axis) was indirectly estimated as the cumulative amount of albendazole sulphoxide reaching the bloodstream using the Wagner–Nelson method (Gibaldi and Perrier, 1982). Albendazole absorption following ABZ+SLS and the commercial formulation treatments was higher (153 and 140%, respectively) compared to that obtained after administration of ABZ without surfactant agent (control). Statistically different from ABZ Control and ABZ+STC (5 mM) at $P<0.01$

19]. The rate of absorption and the recycling between enteral and parenteral tissues are relevant for the exposure of worms residing in the lining of the gut to the active drug or its metabolites [20]. Moreover, the absorbed drug is more important for the efficacy against GI nematodes than unabsorbed drug passing down the GI tract [21]. Thus, the plasma concentration

Table 2. Comparative kinetic parameters for albendazole sulphone ($ABZSO_2$) obtained after the intraruminal administration of different albendazole (ABZ) suspensions (prepared with and without surfactants) to cattle

Pharmacokinetic parameters	ABZ formulations			
	Control (without surfactant)	ABZ+STC (5 mM)	ABZ+SLS (8.27 mM)	Commercial[a] formulation
($T_{1/2app}$) (h)	2.01 ± 0.12	2.49 ± 0.31	2.36 ± 0.35	2.27 ± 0.36
C_{max} (μg/ml)	0.96 ± 0.26	1.03 ± 017	1.85 ± 0.40[b]	1.64 ± 0.21[c]
T_{max} (h)	22.8 ± 2.40	21.60 ± 2.94	22.80 ± 2.40	21.60 ± 2.94
AUC $_{(0-\infty)}$ (μg/h ml^{-1})	17.30 ± 3.16	19.04 ± 2.22	30.75 ± 5.70[d]	26.26 ± 3.77[e]
MRT (h)	20.64 ± 0.61	20.36 ± 1.08	21.24 ± 0.76	18.56 ± 0.25
DP (h)	2–36	2–36	1–36	1–36

Note: Data are expressed as mean ± SD (n=5). STC, sodium taurocholate; SLS, sodium lauryl sulphate; $T_{\frac{1}{2}app}$, appearance half-life; C_{max}, peak plasma concentration; T_{max}, time to peak concentration; $AUC_{(0-\infty)}$, area under the concentration vs time curve extrapolated to infinity; MRT, mean residence time; DP, detection period in plasma.[a] Comercially available suspension for use in sheep and cattle (Valbazen, Pfizer Inc. SA, Buenos Aires, Argentina).

[b] Statistically different from control ($P<0.001$) and ABZ plus STC ($P<0.01$) values.

[c] Statistically different from control ($P<0.01$) and ABZ plus STC ($P<0.05$) values.

[d] Statistically different from control ($P<0.001$) and ABZ plus STC ($P<0.01$) values.

[e] Statistically different from control at $P<0.05$.

profiles of anthelmintically active BZD moieties reflect the pattern of exposure of worms in the GI tract (mucosas or lumen) as well in other tissues of parasite location such as liver or lung [20, 22, 23]. It has been recently shown that plasma profiles of ABZ metabolites correlate with those obtained in different tissues/fluids and in target parasites (*Moniezia spp.*, *Haemonchus spp.* and *Fasciola hepatica*) of ABZ-treated sheep [22, 24]. Moreover, a reduction in the level of feed intake enhanced OFZ plasma availability and efficacy against *Haemonchus contortus* in sheep [9, 25]. Altoghether these previous findings indicate that the characterization of the plasma disposition kinetics and systemic availability of anthelmintically active BZD molecules can be used to predict anthelmintic efficacy.

The absence of ABZ parent drug in plasma indicates the occurrence of first-pass microsomal oxidation in the liver [26–28] and intestine [29, 30]. This is in agreement with the rapid disappearance of ABZ from the bloodstream and the earlier detection of ABZSO and $ABZSO_2$ in plasma after the iv administration of the thioether in sheep [22, 31] and cattle [8]. Therfore, the extent of absorption of the enterally administered parent drug may be accurately assessed by the plasma availabilities of ABZ metabolites, as it has been previously shown in different animal species and man.

Poor drug absorption in the GI tract and the lack of water solubility are important limitations for the formulation, bioavailability and efficacy of BZD anthelmintics. The oral/intraruminal administrations of BZD anthelmintics formulated as aqueous suspensions have been the most commonly used in ruminants worldwide. Drug absorption in the digestive tract depends on lipid solubility and degree of ionization at GI pH levels. However, dissolution of drug particles is a crucial step that precedes the GI absorption of a drug formulated as a suspension [32]. The particles must dissolve in the enteric fluids to facilitate absorption of the BZD molecule through the GI mucosa. The dissolution rate of an enterally delivered compound influences the rate and extent of its absorption (systemic bioavailability). ABZ aqueous solubility is markedly higher at low pH values [3]. Dissolution of BZD particles given as drug suspensions is greater at the acid abomasal pH; the dissolution of drug particles in abomasum is a limiting factor for the absorption of enterally administered BZD. Thus, the time of residence of the administered BZD suspensions at the acid pH of the abomasum may notably affect the dissolution rate and the subsequent absorption of the drug in the gut. It has been also shown that different factors slowing the digesta transit time throughout the abomasum, such as the type and quantity of food

consumed in sheep [9, 25, 33] and fasting prior to post-treatment in cattle [8, 34], may enhance the BZD systemic availability as a consequence of a greater dissolution of drug particles in the acid pH of the abomasum.

Poor membrane permeation is also an important cause of low bioavailability of many drugs used both in domestic animals and humans. The low bioavailability may be consequence of poor partitioning into the lipid membrane or low membrane diffusivity [10]. The major absorption barrier for hydrophilic drugs is attributed to the cellular membranes of the intestinal epithelial cells, while the aqueous boundary layer adjacent to the intestinal wall is considered as the highest permeability barrier for lipophilic compounds [35]. Several formulation approaches could be employed to overcome low drug bioavailability. The effects of natural and synthetic surfactants on the passive GI absorption of different xenobiotics have been well documented [35–37]. Amphiphilic surfactants could affect the GI absorption by several mechanisms. They induce the formation of stable dispersions of lipophilic xenobiotics and increase the permeability of biological membranes. Surfactants can enhance the wetting and aqueous dissolution rate of water-insoluble drugs by micellar solubilization [10, 38]. Surfactant agents could also increase the permeability of enterocyte membranes to lipophilic compounds. The stagnant aqueous diffusion layer, adjacent to the brush border of epithelial cells, is disrupted by the synthetic tensioactives increasing the absorption rate of lipophilic drugs such as gryseofulvin [39, 40], amiodarone [37], cyclosporin [41] and sulfisoxazole [42].

Surfactant agents were used at critical micellar concentrations, since the absorption rate of lipophilic compounds could be reduced at supramicellar concentrations as a consequence of micellar solubilization of the drug, which reduces the free drug fraction available for absorption [13, 37, 38, 43]. The use of bile salts around the critic micellar concentration improves the dissolution, water solubility and absorption of lipophilic drugs across the intestine [10, 38]. It has been shown that both STC and SLS improve the intestinal absorption of ABZ in rats [11–13]. Therefore, both tensioactives were selected to test the influence of amphiphilic surfactants on the availability of ABZ metabolites in ruminants in the current experiment. The results observed after administration of ABZ plus STC in the trial reported here were unexpected, since solubilization of ABZ plus STC improved the enteral absorption of the thioether in rats, compared with ABZ formulated with either SLS or without a tensioactive agent [12, 13]. On the other hand, a higher amount of drug absorbed, measured as the cummulative concentration of ABZSO in plasma (Figure 4), was observed after ABZ plus SLS administration in the current work. The total plasma availabilitiy of this metabolite was also higher compared to control and ABZ plus STC groups. Thus, SLS-mediated enhanced dissolution and absorption of ABZ accounted for the observed increased systemic availability of the active ABZSO metabolite in cattle. Moreover, ABZSO plasma availability after administration of ABZ formulated with SLS resulted equivalent compared to that observed after the commercial formulation treatment. These results show that the simple addition of SLS is enough for producing a bioequivalent ABZ suspension compared to a commercial formulation. Thus, the use of surfactants should be considered, along with other strategies, to improve the availability of BZD anthelmintics in livestock animals. Since enhanced plasma availability has been shown to highly correlate to anthelmintic efficacy of BZD compounds in sheep and cattle, further pharmacotechnically oriented work should be encouraged. The findings reported here contribute in that direction.

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