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ALBENDAZOLE FAILURE TO CONTROL RESISTANT NEMATODES IN LAMBS: LACK OF EFFECT OF FASTING-INDUCED IMPROVEMENT ON DRUG ABSORPTION

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ABSTRACT: Enhanced plasma availability of albendazole sulphoxide (ABZSO), the active metabolite of albendazole (ABZ), has been described in feed-restricted sheep. The aim of the present work was to determine if the absorption-related pharmacokinetic changes derived from fasting animals prior to drug treatment would modify the clinical efficacy of ABZ against resistant gastrointestinal nematodes in lambs. Forty Corriedale lambs, naturally infected with resistant gastrointestinal nematodes, were divided into 4 groups. Controls were fed ad libitum and did not receive any drug treatment. Treated animals were fed ad libitum up to 30 min prior to treatment with ABZ (3.8 mg/kg) by the intraruminal route. The control (fasted) animals were not fed during the 24-hr period prior to the start of the experiment and did not receive any drug treatment. A second treated group of animals were fasted 24 hr prior to the treatment with ABZ, as previously described for the fed-treated group. Blood samples were collected over a period of 72 hr post-treatment from 6 animals in each treated group. Plasma samples were analyzed by high performance liquid chromatography. The pharmacokinetic parameters were statistically compared using parametric statistical tests. The estimation of the efficacy of the different treatments was performed by the fecal egg count reduction test (FECRT). Additionally, 4 animals randomly chosen from the control-fed and treated groups were killed 13 days post-treatment to evaluate the efficacy against different adult nematode parasites. The results were statistically compared by parametric and non-parametric tests. Significantly ($P < 0.05$) higher Cmax and AUC values were observed for both the ABZSO and ABZ-sulphone ($ABZSO_2$) metabolites in the fasted compared to the fed animals. These kinetic results may be due to a fasting-induced delay in the GI transit time which increases ABZ dissolution and GI absorption. However, a poor ABZ efficacy (measured as FECRT), compatible with a high degree of nematode resistance, was obtained in both fed (48%) and fasted (49%) animals. *Haemonchus contortus* and *Trichostrongylus colubriformis* appeared as the more reluctant species with respect to ABZ treatment. The efficacy against *H. contortus* ranged between 37 (fed) and 54% (fasted) and against *T. colubriformis* between 0% (fed) and 16% (fasted). Under these experimental conditions, the fasting-induced improvement on ABZ systemic availability (>60%) did not improve its activity against nematodes with a high degree of resistance. However, the data described here for a highly resistant nematode population should not discourage the use of fasting as a practical and well-proven management tool for parasite control in ruminants.

A significant part of the economic impact of parasitism in animal production is represented by the investment in control measures. Although alternative methods have been developed, chemically based treatments are the most important tool to control parasitism. There are many drugs available on the veterinary market to control parasitic diseases. Benzimidazoles (BZD), imidazothiazoles, and macrocyclic lactones are the most important chemical groups used to control gastrointestinal (GI) nematode infections in ruminants. The widespread use of BZD anthelmintics, particularly the methylcarbamate derivatives such as albendazole (ABZ), is based on their high efficacy, low toxicity, and a broad spectrum of activity against parasites including nematodes, cestodes, and trematodes (mature flukes) (McKellar and Scott, 1990). For many years, nematode infection control in livestock has been largely based on the overuse of broad-spectrum antiparasitic drugs. Thus, the high level of anthelmintic resistance in sheep and cattle nematodes is an increasing economic problem in several areas of the world (Kaplan, 2004; Wolstenholme et al., 2004).

Modified feeding management has been recommended to restore the anthelmintic action of those BZD compounds whose potency has been compromised by resistance (Hennessy et al., 1995). An enhanced plasma availability of oxfendazole (OFZ), induced by temporary feed restriction in sheep, accounted for increased efficacy of the drug against BZD-resistant nematode strains (Ali and Hennessy, 1995). Fasting the animals prior to intraruminal (i.r.) treatment resulted in pronounced modifications to the absorption and disposition kinetics of ABZ metabolites in cattle, in which the administered drug appeared to be absorbed to a greater extent than in fed animals (Sanchez et al., 2000). Starvation decreases digesta flow rates (Pearson et al., 1992). A delayed GI transit time that decreased the rate of passage of the anthelmintic drug down the GI tract may have accounted for the enhanced ABZ absorption observed in fasted compared to fed animals. The fasting-induced changes to the kinetic behaviour, and the quantitative tissue distribution of BZD methylcarbamates, may have particular relevance to design strategies to increase activity against susceptible parasites and to delay the development of resistant strains.

The anthelmintic efficacy of BZD compounds not only depends on their affinity by parasite tubulin but also on their ability to reach high and sustained concentrations at the site of parasite location (Lanusse and Prichard, 1993). The enhanced drug concentrations, and the prolonged persistence of active BZD molecules observed in fasted animals, may account for a greater parasite exposure to active drug and increased anthelmintic efficacy. This statement is supported by previously reported work, where a general upward trend in the efficacy against nematodes was observed after the enhancement of drug systemic availability (Ali and Hennessy, 1995; Hennessy et al., 1995; Moreno et al., 2004; Sanchez Bruni et al., 2005). The increased concentration profiles of active drug (both parent ABZ and its sulphoxide metabolite), measured in tissues or fluids where target parasites are located, i.e., GI mucosas and fluids, lung tissues, etc. (Sanchez et al., 2000), are a strong scientific argument to recommend the "fasting approach" to improve parasite control in cattle, an approach which is now recommended worldwide.

There is, however, a lack of information on how reduced feed or fasting of the animals prior to treatment could affect drug efficacy against highly resistant parasites. The aim of the work reported here was to determine if the pharmacokinetic changes

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derived from fasting animals prior to drug treatment are sufficient to modify the clinical efficacy of ABZ against resistant GI nematodes in lambs.

MATERIAL AND METHODS

Animals and selection procedures

Forty Corriedale lambs (7- to 8-mo-old, 27.3 ± 4.3 kg), naturally infected with resistant GI nematodes, were involved in this trial. The experimental lambs were selected from a farm where the failure of ABZ to control GI nematodes had been previously demonstrated by the fecal egg counts reduction test (FECRT) (Entrocasso et al., 2008). The selection of the animals was based on worm egg per gram (epg) counts. On day 1, all lambs were checked for epg counts, ear tagged, and the individual body weights were recorded. Experimental animals had an average of 5,675 ± 3,028 epg. Animals were allocated a paddock and fed on a lucerne–white and red clover pasture during the experiment and for 20 days before starting the study. All the animals had free access to water. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar) and according to internationally accepted animal welfare guidelines (American Veterinary Medical Association, 2001).

Chemicals

Standards of ABZ, ABZ-sulphoxide (ABZSO), ABZ-sulphone ($ABZSO_2$), and oxibendazole (OBZ), used as internal standards, were obtained from Sigma Chemical Company (St. Louis, Missouri). The commercial formulation of ABZ was provided by Pfizer Animal Health, Buenos Aires, Argentina (Valbazen®, 10% suspension).

Experimental design, treatments, and sampling

All parasitized lambs were randomly allocated into 4 experimental groups (n = 10) and subjected to the following feeding conditions and treatments. Control fed lambs were fed ad libitum and did not receive any drug treatment. Treated fed animals were fed ad libitum up to 30 min prior to treatment and treated with ABZ (3.8 mg/kg) by the i.r. route. Control fasted animals were fasted 24 hr prior to the start of the experiment and did not receive any drug treatment. Finally, treated fasted lambs were fasted 24 hr prior to the treatment with ABZ, as described for the fed treated group.

Six animals randomly selected from either the fed or fasted treated groups were used in the pharmacokinetic study. Blood samples were taken from the jugular vein before drug administration (time 0) and at 1, 3, 6, 9, 12, 18, 24, 30, and 48 hr post-treatment. Blood samples were collected using 10-ml heparinized Vacutainer® tubes (Becton Dickinson, Franklin Lakes, New Jersey). Plasma was separated by centrifugation at 2,000 *g* for 15 min, placed into plastic tubes, and frozen at −20 C until analysis by high performance liquid chromatography (HPLC).

Efficacy assessment

The anthelmintic efficacy of the treatments was evaluated by the FECRT, calculated according to

$$\text{FECRT}(\%) = 100\left(1 - \frac{\text{Xt}}{\text{Xc}}\right),$$

where Xt is the arithmetic mean epg counts in the treated group at 13 days post-treatment and Xc is the arithmetic mean epg counts in the untreated control group at 13 days post-treatment. The 95% confidence intervals were calculated as reported by Coles et al. (1992).

Direct adult nematode counts of animals from the experimental groups of fed control, fed treated, and fasted treated (n = 4 for each group) were determined 13 days after treatment following the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Wood et al., 1995). The genus and species of the nematodes recovered from parasitized lambs was determined by the identification of the third-stage larvae recovered from fecal pool cultures obtained from each experimental group (Ministry of Agriculture, Fisheries and Food, 1986). The efficacy of each anthelmintic treatment was determined by the comparison of worm burdens in treated versus untreated animals. The following equation expresses the percentage of efficacy (%E) of a drug treatment against a given parasite species (S) in a single treatment group (T) when compared with an untreated control (C):

$$\%E = \frac{(\text{Mean of S in C} - \text{Mean of S in T})}{\text{Mean of S in C}} \times 100.$$

The geometric mean was used, as it most accurately represents the distribution of nematode populations within each group (Wood et al., 1995).

Analytical procedures

ABZ, ABZSO, and $ABZSO_2$ were extracted using disposable C_{18} columns (RP-18, 100 mg, Strata®; Phenomenex, Torrance, California). Ten µl of OBZ (50 µg/ml) was added to 500 µl of plasma in a glass test tube. Spiked samples were placed into a C_{18} column (preconditioned with 0.5 ml of methanol followed by 0.5 ml water) in a vacuum system (Lichrolut®; Merck, Darmstadt, Germany). Samples were washed (2 ml of water) and then eluted with 2 ml of HPLC-grade methanol. After elution, all samples were concentrated to dryness in a vacuum concentrator (Speed-Vac®; Savant, Minneapolis, Minnesota) and then reconstituted with 300 µl of mobile phase.

The HPLC equipment and conditions used were as previously described (Alvarez et al., 2008). Experimental and spiked plasma samples (used for validation) were analyzed by HPLC (10 A-HPLC System; Shimadzu, Kyoto, Japan) with a UV detector set at 292 nm. Fifty µl of each previously extracted sample were injected and the analytes were eluted (flow 1.2 ml/min) from the analytical column (5 µm, 250 mm × 4.6 mm, C_{18} column, Selectosil®; Phenomenex) by a binary gradient previously described (Alvarez and Sanchez, 1999). The compounds were identified by the retention times of pure reference standards. Retention times for ABZSO, $ABZSO_2$, OBZ, and ABZ were 5.32, 7.24, 9.55, and 11.14 min, respectively. There was no interference

of endogenous compounds in the chromatographic determinations. Plasma calibration curves for each analyte were constructed by least squares linear regression analysis, giving a correlation coefficient (r) between 0.9987 and 0.9995. Mean absolute recovery percentages for concentrations ranging between 0.25 and 5 µg/ml (n = 6) were 88 (ABZSO), 90 ($ABZSO_2$), and 85% (ABZ) with coefficients of variation (CV) of 4.1%, 5.2%, and 8.6%, respectively. The precision of the method (intra- and inter-assay) was determined by analyzing plasma samples (n = 6) fortified with OBZ and metabolites at 3 different concentrations (0.25, 1.0, and 5 µg/ml). The CV for the intra- and inter-assay precision ranged from 4.37 to 7.44%. The limit of quantification (LOQ) was defined as the lowest measured concentration with a CV <20%, an accuracy of ±20%, and an absolute recovery ≥70%. The LOQ obtained for the 3 molecules assayed was 0.1 µg/ml. Values below LOQ were not included in the pharmacokinetic analysis.

Pharmacokinetic analysis of the data

The concentration versus time curves for TCBZ metabolites in plasma for individual animals were fitted with the PKSolutions™ computer program (Summit Research Service, Ashland, Ohio). Pharmacokinetic analysis of the experimental data was performed by non-compartmental analysis. The first order metabolite formation rate constants (k_f) (h^{-1}) were calculated by the residual method (Gibaldi and Perrier, 1982). The elimination (T½el) and metabolite formation (T½for) half lives were calculated as ln2/β and ln2/k, respectively, where β represents the terminal slope (h^{-1}). The observed peak concentration (Cmax) and time to peak concentration (Tmax) were read from the plotted concentration-time curve of each analysis. The area under the concentration time-curve (AUC) was calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). Statistical moment theory was applied to calculate the mean residence time (MRT) for metabolites in plasma, as follows:

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

where AUC is as previously defined, and AUMC is the area under the curve of the product of time and the plasma drug concentration versus time from zero to infinity (Perrier and Mayersohn, 1982).

Statistical analysis of the data

Pharmacokinetic parameters are presented as mean ± SD. A Student's t-test was used for the statistical comparison of the pharmacokinetic data obtained from both treatments groups. Egg counts in each experimental group were compared by an ANOVA plus a Tuckey test using log-transformed data. Nematode counts in the groups of fed control, fed, and fasted treated were compared by non-parametric analysis (Kruskal–Wallis test). In all cases, a value of $P < 0.05$ was considered statistically significant.

RESULTS

ABZ parent drug was not detected in plasma at any time after its i.r. administration to sheep; the analytes ABZSO and $ABZSO_2$ were detected in the bloodstream up to 48 hr post-treatment. The mean (±SD) ABZSO plasma concentrations in fed and fasted animals obtained after the i.r. administration of ABZ are shown in Figure 1. Table I summarizes the plasma pharmacokinetic parameters for ABZSO and $ABZSO_2$ obtained after the i.r. administration of ABZ in fed and fasted parasitized lambs. ABZSO was the main metabolite found in plasma in ABZ-treated lambs; the amount of $ABZSO_2$ recovered from the bloodstream (expressed as AUC) represents approximately 32% of that measured for ABZSO. Fasting the animals prior to ABZ administration resulted in marked changes in the kinetic behavior of its metabolites. Significantly ($P < 0.05$) higher peak plasma concentration and AUC values were observed for both ABZSO and $ABZSO_2$ in fasted compared to fed animals (Table I). While ABZSO reached a Cmax value of 1.20 ± 0.21 µg/ml (fasted lambs), this value was 0.74 ± 0.06 µg/ml in animals fed ad libitum. On the other hand, the increased ABZSO plasma concentrations measured in fasted animals contributed to an AUC increment of 68% compared to that estimated for fed animals.

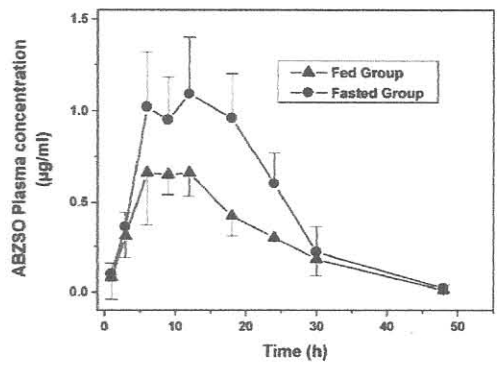

FIGURE 1. Comparative mean (±SD) plasma concentration profiles (n = 6) for albendazole sulphoxide (ABZSO) after the administration of albendazole (ABZ, 3.8 mg/kg) to lambs either fed ad libitum or subjected to a 24-hr pre-treatment fasting period.

The fecal egg counts (mean ± SD) obtained for all experimental groups, including the results of the FECRT and upper and low confidence limits (95%), are shown in Table II. The fecal egg counts observed at 13 days post-treatment in fed and fasted untreated controls did not result in significant differences, indicating that fasting the animals a single day did not modify the egg counts at 13 days post-treatment. The overall low efficacy levels observed indicates the presence of GI nematodes resistant to ABZ. A similar anthelmintic efficacy (obtained by the FECRT) was observed in fed (48%) and fasted (49%) lambs. The adult nematode counts and resultant clinical efficacy obtained in fed and fasted animals, as well as the nematode counts in the untreated controls, are shown in Table III. Since a rather low number of experimental animals (n = 4) were killed in each group, the direct nematode counts were only indicative of the efficacy of each treatment. Nematode resistance in the current experiment was related to *Haemonchus* spp., *Trichostrongylus colubriformis* and, in a much lower extension, *Trichuris ovis*. ABZ efficacy against *Haemonchus* spp. was 37 (fed lambs) and 54% (fasted lambs). The efficacy against *T. colubriformis* was 0% and 16% in fed and fasted animals, respectively. The observed efficacy of ABZ against *Teladorsagia circumcincta*, *Trichostrongylus axei*,

TABLE I. Plasma pharmacokinetic parameters (mean ± SD) for albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) obtained after the intraruminal administration of albendazole (ABZ, 3.8 mg/kg) to lambs either fed ad libitum or fasted for 24 hr prior to treatment.

	ABZSO		$ABZSO_2$	
Pharmacokinetic parameters*	Fed group	Fasted group	Fed group	Fasted group
Cmax (μg/ml)	0.74 ± 0.06	1.20 ± 0.21†	0.31 ± 0.12	0.47 ± 0.08†
Tmax (hr)	9.50 ± 2.26	11.5 ± 3.99	22.0 ± 3.10	24.0 ± 3.79
AUC_{0-t} (μg.hr/ml)	13.9 ± 2.32	23.4 ± 4.80†	6.45 ± 1.54	11.0 ± 1.08†
T½el (hr)	8.05 ± 2.88	5.55 ± 1.76	6.62 ± 1.88	5.55 ± 1.76
MRT (hr)	17.6 ± 2.80	16.2 ± 2.35	23.0 ± 1.05	24.3 ± 0.68

* Cmax, peak plasma concentration; Tmax, time to the Cmax; AUC_{0-t}, area under the plasma concentration vs. time curve from 0 to the detection time; T½el, elimination half-life; MRT, mean residence time (obtained by noncompartmental analysis of the data).
† Values statistically different from those obtained in the fed group ($P < 0.05$).

Cooperia punctata, *Nematodirus spatigher*, and *Oesophagostomun radiatum* in both treated groups ranged between 97–100% (Table III).

DISCUSSION

Consistent with kinetic data previously obtained in sheep (Marriner and Bogan, 1980; Hennessy et al., 1989; Lanusse et al., 1995), ABZSO and $ABZSO_2$ were the main metabolites recovered in plasma after the i.r. administration of ABZ in both fed and fasted animals. The efficient ABZ biotransformation is related to a first-pass oxidation occurring mainly in the liver. ABZSO was the main metabolite recovered in plasma for a 48-hr time period (Fig. 1), accounting for 68% of the total metabolites found in plasma in both treated groups (fed and fasted). The ABZSO plasma disposition kinetics observed in the current trial (fed-treated group) was equivalent to that previously described in sheep (Marriner and Bogan, 1980; Hennessy et al., 1989; Lifschitz et al., 1997).

Enterally administered BZD anthelmintics associate extensively with particulate digesta material in the rumen (Hennessy et al., 1994). The rumen acts as a drug reservoir, delaying BZD outflow throw the GI tract. This effect allows greater drug dissolution at the abomasal level, where the low pH value facilitates BZD water dissolution (McKellar and Scott, 1990). Dissolution of BZD particles in the enteric fluids from the administered suspension precedes its GI absorption. For BZD anthelmintics, dissolution is the rate-limiting step in the systemic availability of the active drug–metabolites (Lanusse and Prichard, 1993). Thus, the longer the time that administered BZD suspension resides at abomasal pH, the greater the dissolution and the amount of drug available to be absorbed. Worms residing in the lining of the GI tract are exposed to drug recycling from plasma to the GI fluids. As a consequence, the plasma concentration profiles of anthelmintically active BZD moieties reflect the pattern of exposure of worms in the GI tract, favoring its pharmacological effect.

The type and quantity of feed consumed affects the GI transit time in different animal species (Koritz, 1982). Previous studies (Lifschitz et al., 1997) indicate that fasting enhances ABZ dissolution and absorption by delaying its passage down the GI tract. The extended ABZ absorption process was correlated with a delayed peak plasma concentration of ABZ metabolites (Lifschitz et al., 1997). However, significantly higher plasma AUC and Cmax values for ABZSO were obtained in fasted animals compared to those obtained in adult sheep fed ad libitum (Lifschitz et al., 1997). Similar fasting-induced changes to ABZ metabolite kinetics were observed in the current trial. Marked enhancement on ABZSO peak plasma concentration (62%) and AUC (68%) were observed in fasted compared to fed lambs. However, while Lifschitz et al. (1997) reported differences in the time to peak plasma concentration (Tmax) for ABZSO, this kinetic parameter did not show changes in fasted compared to fed lambs. Some differences between treatments were also reflected in the disposition of the sulphone metabolite (Table I), the kinetics of which are dependent on the metabolic formation of ABZSO in the liver. Consistent with those results, a fasting-induced delay in the GI transit time substantially increased the plasma and abomasal concentrations of ABZ or its metabolites, or both, in cattle (Sanchez et al., 1997, 2000).

Pathological changes occurring in both abomasal and intestinal helminth infections may affect the plasma kinetics and GI disposition of the anthelmintic drug used for therapy. Some

TABLE II. Nematode egg counts (range) and reduction percentage of fecal egg counts (FECRT) after administration of albendazole (ABZ, 3.8 mg/kg) to lambs either fed ad libitum or fasted for 24 hr prior to treatment.*

	Mean fecal egg counts (range)				
Treatment group	Day 0	Day 13	FECRT (%)†	UCL	LCL
Control fed group	6,043 (1,440–9,960)	6773 (300–15,840)	–	–	–
Control fasted group	5,704 (1,500–11,800)	6613 (1,140–22,440)	–	–	–
Treated fed group	5,454 (1,380–11,280)	3534 (300–8,760)	48	75	0
Treated fasted group	5,574 (1,380–12,480)	3378 (120–14,640)	49	84	0

* Worm counts are arithmetic means.
† FECRT estimated according to Coles et al. (1992); UCL, upper confidence limit 95%; LCL, lower confidence limit 95%. Nematode egg counts at day 13 post-treatment in the different experimental groups are not statistically different ($P > 0.05$).

TABLE III. Adult nematode worm counts (range) and efficacy (%) of albendazole (ABZ, 3.8 mg/kg, n = 4) obtained at 13 days post-treatment after its intraruminal administration to parasitized lambs fed ad libitum (fed group) or fasted 24 hr prior to the treatment (fasted group). Nematode worm counts recorded in the untreated control group are also shown.*

		ABZ treated			
	Untreated control group	Fed treated group		Fasted treated group	
Parasites	Worm counts	Worm counts	Efficacy (%)	Worm counts	Efficacy (%)
Abomasum					
Haemonchus spp.	1,300 (900–1,710)[a]	1,050 (200–1,700)[a]	37	675 (300–1,200)[a]	54
Teladorsagia circumcincta	2,500 (800–3,650)[a]	0[b]	100	0[b]	100
Trichostrongylus axei	2,860 (500–3,740)[a]	0[b]	100	100 (0–400)[b]	97
Small intestine					
Trichostrongylus colubriformis	15,400 (1,650–35,000)[a]	12,450 (6,000–20,600)[a]	0	9,900 (2,200–14,000)[a]	16
Nematodirus spp.	2,025 (200–7,900)[a]	0[b]	100	0[b]	100
Large intestine					
Oesophagostomun spp.	230 (200–920)[a]	0[b]	100	0[b]	100
Trichuris ovis	40 (0–80)[a]	8[b]	89.1	13 (10–50)[b]	69

* Worm counts are arithmetic means (the percent of efficacy was calculated using geometric mean as suggested by Wood et al., 1995). Parasite means counts in groups with different superscripts are significantly different ($P < 0.05$).

studies on the influence of GI parasitism on the plasma kinetics of anthelmintic drugs have been reported (Marriner et al., 1985; Bogan et al., 1987; Debackere et al., 1993; Hennessy et al., 1993; McKellar et al., 1995; Alvarez et al., 1997). It is likely that, under the experimental conditions reported here, the effect of parasitism may have induced some pharmacokinetic changes on the ABZSO plasma kinetics. However, since lambs with similar parasite burdens were randomly allocated to the different experimental groups, any potential pharmacokinetic modification would have affected animals in both treated groups in a similar way. Thus, it is clear that the observed changes on ABZSO systemic availability between fed and fasted animals are related to the fasting-induced enhancement of ABZ absorption, as has been previously demonstrated in sheep and cattle.

Effective anthelmintic therapy requires exposure of the parasite to the active drug for a period of time necessary to obtain increased efficacy. Sangster et al. (1991) demonstrated enhanced anthelmintic efficacy against GI nematodes in sheep when oxfendazole plasma AUC was increased by administering 3 equivalent, 12-hourly spaced, single recommended doses. Furthermore, it has previously been demonstrated that even a temporary reduction on feed intake resulted in increased plasma oxfendazole AUC (Ali and Hennessy, 1995a, 1995b) and ABZ metabolites (Hennessy et al., 1995) in sheep. In both the experimental studies, the oxfendazole or ABZSO enhancement in the plasma AUC was associated with a higher clinical efficacy against resistant nematodes. However, the improved efficacy against resistant GI nematodes observed following the intravenous (i.v.) administration of ABZ, compared to that observed after the i.r. treatment (Entrocasso et al., 2008), was explained by the higher ABZ–metabolite plasma concentration observed after the parenteral i.v. administration (Alvarez et al., 2008). Furthermore, a high correlation between the concentration profiles of ABZ metabolites measured in the bloodstream and those recovered in tissues of parasite location, and within target parasites collected from treated sheep, has been demonstrated (Alvarez and Sanchez, 1999; Alvarez et al., 2000).

Although the time of parasite exposure to the active drug may be a limiting step on anthelmintic drug action, the level of drug concentrations appears to be an important factor on the final BZD anthelmintic effect of the drug (Moreno et al., 2004; Sanchez Bruni et al., 2005). However, the results reported in the present study fail to demonstrate this association. In fact, a similar efficacy (evaluated by the FECRT) against GI nematodes was obtained in fed (48%) and fasted (49%) lambs, which indicates that the enhancement in ABZSO plasma AUC or Cmax, or both, was not sufficient to improve the ABZ anthelmintic efficacy. Because the time of ABZSO presence in the bloodstream was not modified in fasted animals, it is likely that, for BZD anthelmintics, the time of parasite exposure to the drug would be more relevant than the achieved concentration level, at least if the fasting-induced increment in the plasma concentration of the active metabolite is less than 1-fold compared to that obtained in animals fed ad libitum.

The low efficacy levels observed in the current experiment confirm that the animals were naturally infected with at least some species of nematodes, such as *H. contortus* and *T. colubriformis*, that were resistant to ABZ. According to the criteria of Coles et al. (1992), which evaluate anthelmintic resistance by means of the FECRT, it is clear that resistance against ABZ was present. In fact, the percentage of reduction in egg fecal counts was less than 95% (with 95% confidence levels <90%) in both treated groups (Table II). Following the WAAVP guidelines (Wood et al., 1995), ABZ was shown to be highly effective (>98%) against *Te. circumcincta*, *Trichostrongylus axei* (fed treated group), *Nematodirus* spp., and *Oesophagostomun* spp. and insufficiently active (less than 80%) against *Haemonchus* spp. and *Trichostrongylus colubriformis*. ABZ treatment suggests that this drug is moderately effective (80–89%) against *Trichuris ovis* in the fed treated group and failed to control (69%) this parasite in the fasted treated group (Table III).

A "lack of fit" between the efficacy results obtained by the FECRT and direct nematode counts was observed in the fed treated group. While the FECRT showed an efficacy of 48%, the

treatment only reached an efficacy of 37% (*H. contortus*) and 0% (*Trichostrongylus. colubriformis*). These results may reflect a resilience stage of the female worms on egg laying, which could explain the higher efficacy level obtained by the FECRT compared to the adult nematode counts. This potential ABZ effect in the reduction of egg laying was not apparent in the fasted group.

Under our current experimental conditions, it is, therefore, clear that fasting the animals prior to ABZ treatment increases the Cmax and AUC of the active ABZSO metabolite. However, the enhancement of the relative availability of ABZSO did not improve the efficacy of ABZ against parasites with a high level of resistance.

In conclusion, diet management, or fasting, or both are still useful tools to enhance drug systemic availability and to achieve optimized parasite control. Following the research support from work done over many years, this simple animal management practice is recommended worldwide. However, the findings reported here seem to demonstrate that fasting-induced improvement on ABZ–metabolites absorption–availability may not be useful when a high level of drug resistance is already established in the treated nematode population.

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