Metabolism of albendazole and albendazole sulphoxide by ruminal and intestinal fluids of sheep and cattle

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1. The metabolism of albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) by ruminal, abomasal and ileal fluids of sheep and cattle was investigated under anaerobic conditions *in vitro*.

2. None of the compounds was metabolically changed by incubation with abomasal fluids of sheep and cattle.

3. ABZ and ABZSO were extensively metabolized by sheep and cattle ruminal and ileal fluids. $ABZSO_2$ was unaffected by incubation with these gastrointestinal fluids.

4. The rate of ABZ oxidation into ABZSO was greater for cattle ruminal and ileal fluids than for sheep fluids.

5. ABZSO was reduced back to ABZ by ruminal and ileal fluids of both species. This reducing activity was significantly higher for both ruminal and ileal fluids of sheep compared with those of cattle.

Introduction

Benzimidazole and pro-benzimidazole anthelmintics are widely used in human and veterinary medicine. These compounds are thought to exert their antiparasite effects by binding to parasite tubulin, which produces subsequent disruption of the tubulin–microtubule dynamic equilibrium (Lacey 1990). Benzimidazole and pro-benzimidazole compounds are extensively metabolized in the host. Their metabolic pattern and resultant pharmacokinetic behaviour are relevant in the attainment of high and sustained concentrations of pharmacologically active drug/metabolites at the parasite target.

Following the administration of albendazole (ABZ), a propylthio-benzimidazole compound, in sheep (Marriner and Bogan 1980, Delatour *et al.* 1990) and cattle (Prichard *et al.* 1985; Delatour *et al.* 1990), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) were the major metabolites found in plasma. Figure 1 shows the proposed main biotransformation pathway for ABZ. However, other side and minor metabolic changes resulting in the formation of amino and hydroxy derivatives have been described (Gyurick *et al.* 1981). Netobimin is an anthelmintically inactive pro-drug (pro-benzimidazole) which exerts its anthelmintic activity by reduction and cyclization into ABZ in the gastrointestinal tract of the animal host (Lanusse and Prichard 1990). Following this conversion, the metabolic sequence for netobimin is as described in figure 1. ABZSO and $ABZSO_2$ are also the

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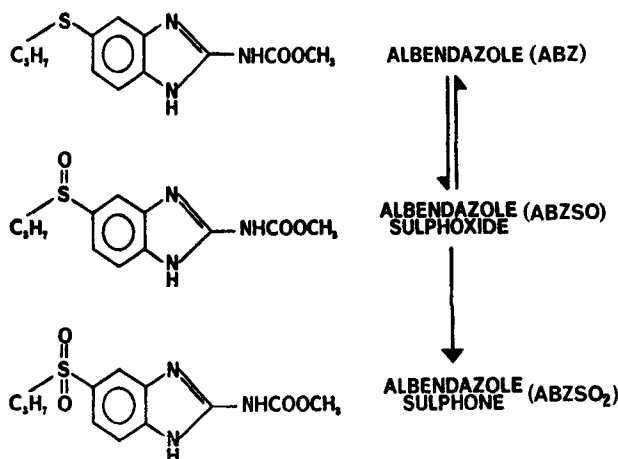‡ To whom correspondence should be addressed.

Figure 1.   The main albendazole biotransformation pathway.

principal metabolites recovered in the plasma of sheep (Lanusse and Prichard 1990) and cattle (Lanusse *et al.* 1991 a,b) treated with netobimin. Although the same metabolites are found in sheep and cattle plasma, the disposition kinetics of these metabolites, after either ABZ or netobimin treatment, differ markedly between species. These differences have been attributed to differential metabolic patterns between sheep and cattle (Prichard *et al.* 1985, Lanusse *et al.* 1991 a).

The liver microsomal fraction is thought to be the main site for the sequential biotransformation of benzimidazole thioether compounds (such as ABZ, fenbendazole, etc.). This process leads to the production of more polar and less anthelmintically effective metabolites (Lubega and Prichard 1991). However, the importance of the biotransformation of these compounds in the gastrointestinal tract should not be underestimated. Recently, we have characterized the gastrointestinal distribution of ABZ metabolites in cattle following oral administration of netobimin (Lanusse *et al.* 1991 b). These metabolites are reversibly exchanged between plasma and different gastrointestinal compartments in a pH gradient-mediated distribution process. While ABZ was not found in plasma, ABZSO was found, being detected in the plasma for only 30–36 h post-treatment. These two anthelmintically active metabolites were present in different gastrointestinal compartments for at least 72 h post-treatment. It is therefore likely that not only the plasma–gastrointestinal tract exchange, but also some metabolic interconversion in the digestive tract, accounts for the presence of these two anthelmintically active metabolites for an extended period of time in the gastrointestinal tract. This is of major relevance to the efficacy of the drug against gastrointestinal parasites.

Taking into consideration the extent of the exchange surface between the plasma and the digestive tract in ruminants, and the large volume of the forestomach, any potential bioconversion of these compounds taking place in the gastrointestinal tract could have a significant impact on both the pharmacokinetic behaviour and the availability of anthelmintically active metabolites at the sites where gastrointestinal and tissue-dwelling parasites are located.

Since parasite uptake and binding to tubulin may differ greatly amongst different metabolites, a complete understanding of the metabolic pattern of these compounds and their complex pharmacokinetic behaviours becomes crucial to optimize their

broad-spectrum efficacy. The *in vitro* experiments reported in this article were designed to investigate on a comparative basis the ability of ruminal, abomasal and ileal fluids of sheep and cattle to metabolize ABZ and ABZ-related metabolites.

Materials and methods

Chemicals

ABZ and oxibendazole were supplied by Smith Kline Beecham Corp., West Chester, PA, USA. ABZSO and $ABZSO_2$ were generous gifts from Schering Plough, Kenilworth, NJ, USA. All these compounds were between 96% and 99·5% pure, and stock solutions were prepared in methanol. Fresh working solutions were prepared immediately before each incubation assay. H.p.l.c. grade solvents were obtained from Fisher Scientific, Ontario, Canada. $(NH_4)H_2PO_4$, $NaHCO_3$ and ammonium acetate were purchased from Aldrich Chemical Co., Milwaukee, WI, USA.

Animals and collection of gastrointestinal fluids

Healthy Holstein Friesian calves (200–220 kg) and Finn Dorset crossbred sheep (40–45 kg) were used as a source of ruminal, abomasal and ileal fluids. The animals were exclusively fed on high-quality hay in the 3 months prior to slaughter, and water was permitted *ad libitum*. The animals were stunned by captive bolt and exsanguinated immediately. The abdomen was opened and the digestive organs were properly identified and removed. Ruminal fluid was obtained by making an incision in the dorsal sac of the rumen. The abomasum and distal region of the ileum were tied off, separated from the rest of the digestive tract and their contents drained into labelled containers. Aliquots of the collected fluids were kept at 37–38°C, transported from the abattoir to the laboratory and processed for incubation within 2 h of their collection. Samples were filtered through a hydrophilic gauze to remove solid material and the filtrate kept saturated with pure N_2 at 38°C until the incubation assays were carried out. The pH of the different fluids was determined for each individual animal used in the experiment and the range of values for both species was as follows: ruminal fluid 6·4–6·7; abomasal fluid 1·5–2·8; ileal fluid 7·4–7·6. Blank samples were prepared by boiling each fluid for 60 min (Beretta *et al.* 1987).

Incubation assays

One hundred microlitres of one of the compounds under investigation were added to 1·9 ml of the filtrate of ruminal, abomasal or ileal fluid to a final drug concentration of 2 μg/ml of incubation mixture. Each incubation mixture was gently gassed with pure N_2 for 5 min at 38°C. Incubations were carried out in a thermostatic shaking water bath at 38°C under anaerobic conditions for 30, 60 and 360 min. Metabolic conversions were found to be linear at least during 60 min when incubations were performed with both ruminal and ileal fluids. The long incubation time (360 min) was included based on data reported for the metabolism of other benzimidazole thioethers by ruminal microflora (Beretta *et al.* (1987) and also to allow sufficient time for the slow $ABZSO_2$ formation. Blank samples of each fluid (boiled) were prepared and incubated under the same conditions. Immediately after the incubation period the samples were frozen at −20°C. Extractions and analyses were undertaken within 2–3 days post-incubation. Incubation assays were performed in duplicate and repeated several times with fluids obtained from different animals of both species. Incubations were always done using fresh digesta fluids and within 2 h of slaughter.

Analytical procedures

Sample extraction. Oxibendazole (1 μg in 10 μl) as an internal standard, was added to an aliquot (1 ml) of each ruminal, abomasal and ileal fluid incubation mixture. Albendazole and its metabolites were extracted by solvent (ethyl acetate) extraction and subsequently by solid phase extraction using C_{18} Sep Pak Cartridges (Waters Associates, Milford, MA, USA). The solvent extraction procedure was a modification of that described by Hennessy (1985), whereby the samples were made alkaline (1 ml satd.aq.$NaHCO_3$), mixed with 15 ml of ethyl acetate and shaken on a mechanical shaker for 15 min. This procedure was repeated three times and the combined ethyl acetate extracts were evaporated under a stream of nitrogen. The residue was redissolved in 4 ml of 1 M HCl mixed with 5 ml of 0·017 M $(NH_4)H_2PO_4$ (pH 5·5), the final pH adjusted to 5·0 with NaOH and finally injected into a Sep Pak cartridge. Each cartridge had previously been conditioned with 5·0 ml of methanol followed by 5·0 ml of 0·017 M $(NH_4)H_2PO_4$ (pH 5·5). All samples were applied into the cartridge and subsequently eluted, concentrated and prepared for h.p.l.c. analysis as previously described (Lanusse and Prichard 1990).

Drug/metabolites analysis. Incubation samples were analysed for ABZ, ABZSO and $ABZSO_2$ by h.p.l.c. Fifty microlitres of each extracted sample were injected by an autosampler into an LKB Bromma HPLC system (LKB, Bromma, Sweden) fitted with a Bondex 10 μm C_{18} reverse-phase column (Phenomenex, CA, USA). The rest of the h.p.l.c. equipment and analysis conditions were as previously reported (Lanusse and Prichard 1990).

Known amounts of each analyte (0·01–5 μg/ml) were added to aliquots of sheep and cattle ruminal, abomasal and ileal fluids, extracted and analysed by h.p.l.c. (triplicate determinations) to establish the

percentage of recovery and calibration curves for each fluid. Recovery values for the different analytes in the different fluids of both species ranged from 81% to 92%. Concentrations were calculated by comparison of unknown compounds and internal standard peak area, using Nelson Analytical software, model 2600 (Nelson Analytical Inc., CA, USA) on an IBM-compatible AT computer. The sensitivity of the assay was $0\cdot010$–$0\cdot020\,\mu g/ml$ for ABZ and ABZSO in ruminal and abomasal fluid; $0\cdot020$–$0\cdot040\,\mu g/ml$ for ABZ and ABZSO in ileal fluids and for ABZSO$_2$ in each fluid.

Data and statistical analyses. The results presented are mean values of at least four determinations. The products (metabolites) formed after each incubation are expressed as percentage of the total recovered products. The ratio ABZ/ABZSO represents the mean value of the ratio between the amount of ABZ and ABZSO found in all the incubation assays carried out for a particular compound under particular conditions. Mean results were statistically compared using Student's *t*-test for either paired or unpaired data. A value of $P<0\cdot05$ was considered statistically significant.

Results

No chemical modification of ABZ, ABZSO or ABZSO$_2$ were observed upon incubation with abomasal fluids for 30, 60 or 360 min. While ABZ and ABZSO were extensively metabolized by both ruminal and ileal fluids of sheep and cattle, the ABZSO$_2$ metabolite was not affected by incubation with those fluids.

ABZ was oxidized to ABZSO by both sheep fluids, but the production of ABZSO after 60 min of incubation was higher for the ileal (44% of total recovered products) than for the ruminal fluid (23·6% of total products) which resulted in a significantly lower ($P<0\cdot05$) ABZ/ABZSO ratio for ileal than ruminal fluid of sheep (table 1). ABZSO was oxidized to ABZSO$_2$ at a very low rate by both digestive fluids. However, the most important metabolic change was the reduction of the sulphoxide metabolite (ABZSO) back to the thioether (ABZ) (figures 1 and 2). Ruminal fluid showed a greater efficiency to reduce ABZSO than that of ileal fluid of sheep following a 60 min incubation period under anaerobic conditions (table 1).

Comparisons of the ability of sheep and cattle ruminal and ileal fluids to biotransform both ABZ and ABZSO are presented in tables 2 and 3. The rate of ABZ oxidation was significantly higher for cattle than for sheep ileal fluid (table 2). Furthermore, ABZSO was more efficiently reduced to ABZ by sheep than by cattle ruminal and ileal fluids following both 60 min and 360 min (table 3) of incubation. The ratio ABZ/ABZSO was significantly lower for cattle than for sheep ruminal and ileal fluids both after 60 ($P<0\cdot05$) and 360 ($p<0\cdot01$) min of incubation (table 3).
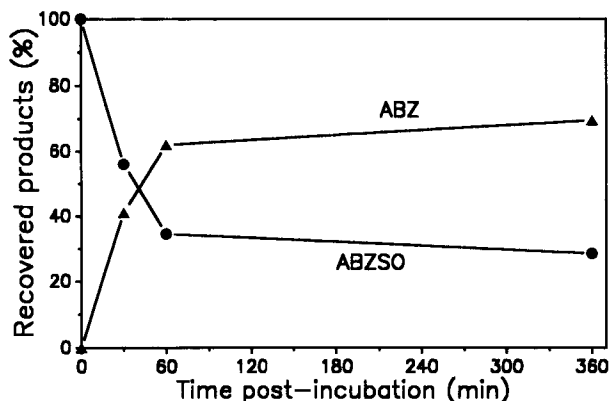

Figure 2.   Reduction of albendazole sulphoxide (ABZSO) to albendazole (ABZ) following anaerobic incubation of ABZSO with sheep ruminal fluid.

Values for the ABZSO$_2$ metabolite were $0' = 0\%$, $30' = 2\cdot8\%$, $60' = 4\cdot1\%$ and $360' = 3\cdot9\%$, and are not included in this figure. The concentration of ABZSO was $2\,\mu g/ml$.

Table 1. Bioconversion *in vitro* of albendazole (ABZ) and albendazole sulphoxide (ABZSO) by sheep ruminal and ileal fluids following anaerobic incubation.

| Drug added | Fluid | Products recovered | | | | |
| | | Total (μg/ml) | Percentage total recovery | | | Ratio ABZ/ABZSO |
			ABZ	ABZSO	ABZSO$_2$	
ABZ	Ruminal	1·7 ± 0·1	76·4 ± 2·9	23·6 ± 2·4	0·0 ± 0·0	4·0
	Ileal	1·8 ± 0·1	49·7 ± 7·1	44·0 ± 7·8	6·3 ± 0·6	1·5*
ABZSO	Ruminal	1·8 ± 0·3	64·0 ± 9·6	31·5 ± 4·4	4·5 ± 0·3	2·9
	Ileal	1·9 ± 0·1	29·3 ± 2·6	64·9 ± 2·0	5·9 ± 0·4	0·5**

Values are expressed as means of six determinations ± SEM. Incubations (60 min), sample extraction and h.p.l.c. determination were as described in Material and methods.

* Statistically different from the value obtained for the incubation of ABZ with ruminal fluid at $P < 0.05$.

** Statistically different from the value obtained for the incubation of ABZSO with ruminal fluid at $P < 0.01$.

Table 2. Oxidation *in vitro* of albendazole (ABZ) by sheep and cattle ruminal and ileal fluids following anaerobic incubation.

| Fluid | Species | Products recovered | | | | |
| | | Total (μg/ml) | Percentage total recovery | | | Ratio ABZ/ABZSO |
			ABZ	ABZSO	ABZSO$_2$	
Ruminal	Sheep	1·7 ± 0·1	77·6 ± 4·0	19·4 ± 3·3	3·0 ± 1·1	4·4
	Cattle	1·6 ± 0·1	68·3 ± 1·6	28·6 ± 1·8	3·1 ± 0·2	2·4
Ileal	Sheep	1·6 ± 0·1	81·2 ± 1·6	17·6 ± 1·8	1·2 ± 0·4	4·8
	Cattle	1·8 ± 0·2	64·9 ± 8·1	32·5 ± 8·0	2·6 ± 0·3	2·2*

Values are expressed as means of four to six determinations ± SEM. Incubations (6 h), sample extraction and h.p.l.c. determination were as described in Methods.

* Statistically different from the value obtained for sheep ileal fluid at $P < 0.05$.

Discussion

Albendazole oxidation

The importance of gastrointestinal microorganisms in the metabolism of ABZ and ABZSO was demonstrated by the complete absence of metabolic activity in boiled samples of ruminal and ileal fluids. Upon incubation with ruminal and ileal fluids of both species, ABZ was converted into its sulphoxide and this, in turn, further oxidized into ABZSO$_2$ (table 2). After a 60 min incubation period, sheep ileal microflora were more efficient at oxidizing ABZ than those in sheep ruminal fluid. These results are well illustrated by the significantly different ratio of ABZ/ABZSO obtained for these fluids (table 1); this ratio is a good indicator of the rate of sulphoxidation and of the relative concentrations of these two molecules. The previously proposed first-pass liver microsomal oxidation of ABZ is quantitatively the principal site of ABZSO formation and accounts for the absence, or detection of only trace amounts, of ABZ in jugular plasma following treatment with either netobimin pro-drug or ABZ itself in sheep and cattle (Delatour *et al.* 1990, Lanusse and Prichard 1990). The efficient sulphoxidation of ABZ by sheep liver microsomes (Galtier *et al.* 1986) may also explain the rapid disappearance of the parent ABZ following its intravenous administration in sheep (Galtier *et al.* 1991). However, our

Table 3. Bioconversion *in vitro* of albendazole sulphoxide (ABZSO) by sheep and cattle ruminal and ileal fluids.

| Fluid | Species | Time (min) | Total (μg/ml) | Percentage total recovery | | | Ratio ABZ/ABZSO |
				ABZ	ABZSO	ABZSO$_2$	
Ruminal	Sheep	60	1.8 ± 0.3	64.0 ± 9.6	31.5 ± 4.4	4.5 ± 0.3	2.9
		360	1.8 ± 0.1	67.7 ± 2.2	30.1 ± 2.6	2.2 ± 0.8	1.8
	Cattle	60	1.6 ± 0.1	41.1 ± 3.5	56.5 ± 3.3	2.4 ± 0.7	0.8*
		360	1.7 ± 0.2	15.1 ± 2.8	79.9 ± 2.9	5.0 ± 0.1	0.2**
Ileal	Sheep	60	1.8 ± 0.1	29.3 ± 2.6	64.9 ± 2.0	5.9 ± 0.4	0.5
		360	1.6 ± 0.1	62.4 ± 1.4	36.3 ± 1.4	1.4 ± 0.2	1.8
	Cattle	60	1.7 ± 0.2	11.9 ± 1.3	83.1 ± 2.5	5.0 ± 0.1	<0.1*
		360	1.7 ± 0.3	2.8 ± 0.1	95.7 ± 0.1	1.5 ± 0.1	<0.1**

Values are expressed as means of four to six determinations $\pm$ SEM. Incubations, sample extraction and h.p.l.c. analysis were as described in Methods.
 * Statistically different from the value obtained for sheep after 60 min of incubation at $P < 0.05$.
 ** Statistically different from the value obtained for sheep after 360 min of incubation at $P < 0.01$

results demonstrated that ABZ oxidation can also take place in the gastrointestinal tract, as earlier postulated by Delatour *et al.* (1986). This would explain the rapid detection of ABZSO and ABZSO$_2$ in the digestive tract and plasma at 15–20 min post-treatment with orally administered netobimin pro-drug in cattle (Lanusse *et al.* 1991 b). Consistently, Galtier *et al.* (1991) have postulated the occurrence of extrahepatic sulphoxidation of ABZ, which may explain the fact that the total ABZ body clearance was two to three times greater than the reported hepatic blood flow in sheep. The large volumes of distribution described for ABZ metabolites (Galtier *et al.* 1991) and the extent of the exchange surface between plasma and the digestive compartments in ruminants may facilitate the metabolism of ABZ in the gastrointestinal tract.

Albendazole sulphoxide reduction: clinical relevance

ABZSO was oxidized to a limited extent to ABZSO$_2$ by ruminal and intestinal fluids; the ABZSO$_2$ formed never reached more than 5–6% of the total recovered products. Perhaps the most relevant finding was the efficient reduction of ABZSO back to ABZ by ruminal and ileal fluids of both species. The reducing activity of sheep ruminal bacteria was greater than that of sheep ileal bacteria after 60 min of incubation (table 1). A similar pattern was observed in cattle (table 3). Comparison of ABZSO reduction activity between species was interesting. Both the ruminal and ileal microflora of sheep were significantly more efficient at converting ABZSO to ABZ than those of cattle. ABZ represented 67.7% of the products formed when ABZSO was incubated for 6 h with sheep ruminal fluid, and only 15% when ABZSO was incubated with cattle ruminal fluid.

Different studies have shown the gastrointestinal tract as the principal site for the reduction of different sulphoxide-containing drugs (Renwick *et al.* 1986). Although it has been previously suggested that the conversion of ABZ into ABZSO could be

reversible in the liver (Gyurick *et al.* 1981), we have recently demonstrated that neither sheep nor cattle liver microsomes are able to reduce ABZSO *in vitro* (unpublished observations). This metabolic reduction may be of prime importance for the antiparasite efficacy of benzimidazole thioethers. Although the liver cytosolic fraction might be involved, the gastrointestinal tract is the main site for this sulphoxide reduction. This *in vitro* evidence of ABZSO reduction correlates with the detection of ABZ in different gastrointestinal compartments for up to 72 h post-netobimin administration to cattle when the parent netobimin pro-drug has been completely removed from the digestive tract by 12–18 h post-administration (Lanusse *et al.* 1991 b). The reversible plasma–gastrointestinal tract exchange facilitates a pH gradient-mediated concentration of ABZSO in the digestive compartments, which could act as a source of ABZ. As ABZ has a greater affinity for parasite tubulin than ABZSO (Lubega and Prichard 1991), this bacteria-mediated reduction may have significant importance for efficacy against gastrointestinal parasites. As with ABZ, the thioether benzimidazole anthelmintic, fenbendazole, is rapidly and extensively converted *in vivo* into its sulphoxide, oxfendazole, which also has lower tubulin affinity that the parent thioether (Lubega and Prichard 1991). Reduction of oxfendazole to fenbendazole has been shown to occur in the ruminal fluids of sheep and cattle (Beretta *et al.* 1987). Thus, the high antiparasite efficacy of ABZSO and oxfendazole against gastrointestinal parasites may depend on this bacterial reduction of the sulphoxide to the more pharmacologically active thioethers. In fact, similar efficacies against sheep and cattle nematodes are obtained when animals are treated with either ABZSO, ABZ or even with netobimin pro-drug (McKellar and Scott 1990).

Pharmocokinetic differences between sheep and cattle

Pronounced differences in the plasma disposition and bioavailability of ABZSO between sheep and cattle have been described following the administration of either netobimin pro-drug (Lanusse *et al.* 1991 a) or ABZ itself (Delatour *et al.* 1990). The bioavailability of this active metabolite was significantly higher, and its elimination half-life and mean time of residence were markedly longer, in sheep compared to cattle. These differences were initially attributed to a differential pattern of liver biotransformation between species, in which a greater capacity for oxidation in cattle than in sheep could be expected. However, we have recently demonstrated the opposite. The rate of ABZ sulphoxidation by sheep liver microsomes in an NADPH-dependent reaction was significantly higher than that of cattle liver microsomes (Lanusse *et al.* unpublished observations) and there was no difference in the formation of $ABZSO_2$ between species. Surprisingly, the differential pattern of gastrointestinal metabolism of these benzimidazole compounds, reported in this article, may explain the sheep/cattle differences in pharmacokinetic behaviour and in the required dose rates for optimal clinical efficacy. This is also consistent with the prolonged elimination half-life and extended presence of ABZSO in sheep plasma for 100 h post-treatment (Lanusse and Prichard 1990), while in cattle ABZSO plasma removal is completed within 30–36 h post-treatment (Lanusse *et al.* 1991 a). The greater reductive capacity of sheep gastrointestinal fluids compared with those of cattle may also account for the higher $ABZSO_2/ABZSO$ plasma ratio observed in cattle (Lanusse *et al.* 1991 a). Incubation of $ABZSO_2$ with liver microsomes or digestive fluids did not appear to produce detectable chemical modification. Since $ABZSO_2$ is an anthelmintically-inactive metabolite (Lubega and Prichard 1991),

these results confirm that the sequential oxidation of benzimidazole thioethers represents a considerable decrease in antiparasite efficacy.

In conclusion, both ruminal and ileal fluids were able to biotransform ABZ and ABZSO. While oxidizing activity was greater in cattle, the reducing activity was more prevalent in the sheep ruminal and ileal fluids. These results contribute to the understanding of host metabolism of benzimidazole and pro-benzimidazole anthelmintics, and help explain the pharmacokinetic behaviour and clinical efficacy of these compounds in ruminants.

Acknowledgements

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