Nutritional condition affects the disposition kinetics of albendazole in cattle

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1. The influence of nutritional status on the plasma and abomasal fluid disposition kinetics of albendazole (ABZ) and its metabolites, albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$), has been investigated in the calf.

2. Free fatty acid (FFA) and β-hydroxybutyrate (β-OHB) serum concentrations were significantly higher in the feed-restricted (poor nutritional status) compared with control calf (optimal nutritional status).

3. ABZ parent drug was not detected in plasma at any time post-treatment and ABZSO and $ABZSO_2$ were the metabolites detected in plasma. Both metabolites were rapidly depleted from the bloodstream. ABZ and its metabolites were recovered in abomasal fluid from 0·25 up to either 48 (ABZ) or 120 h (ABZSO and $ABZSO_2$) post-treatment.

4. The plasma disposition kinetics of both ABZ metabolites was significantly changed in the feed-restricted compared with control calf. ABZSO and $ABZSO_2$ plasma area under the curves (*AUC*s) were significantly higher in the restricted animal. These enhanced *AUC*s correlated with significantly longer plasma half-lives ($T_{1/2_{el}}$) and mean residence times (*MRT*s) for these metabolites.

5. The delayed elimination of ABZ metabolites from the bloodstream correlated with the higher concentration of these molecules recovered in the abomasal fluid of the calves subjected to a dietary restriction.

6. The changes observed on disposition kinetics may reflect an impairment on the hepatic metabolism and clearance of ABZ as a consequence of FFA mobilization from adipose tissue and overproduction of ketone bodies in the liver.

Introduction

Adequate parasite control programs are a major contributor to efficient animal production systems. The close relationship between pharmacokinetic behaviour and clinical efficacy for anthelmintic drugs in domestic animals has been demonstrated. Benzimidazole (BZD) and pro-benzimidazole (pro-BZD) are among the most widely used anthelmintic drugs in veterinary and human medicine. These compounds are thought to exert their antiparasite effects by selective binding to parasite tubulin, which produces subsequent disruption of the tubulin–microtubule dynamic equilibrium (Lacey 1990). The use of BZD and pro-BZD compounds quickly became widespread because they offered major advantages over previous available drugs in terms of spectrum, efficacy against immature stages and safety for the host animal (Campbell 1990).

The biotransformation processes in the body tend to transform drugs/xenobiotics into less lipid soluble and more polar metabolites for easier and faster elimination. BZD and pro-BZD are extensively metabolized in the host. Their metabolic pattern and resultant pharmacokinetic behaviour are relevant in the

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attainment of high and sustained concentrations of pharmacologically active drug/metabolites at the parasite target. Studies on metabolism and fate of BZD anthelmintics in different species have been useful in understanding differences in pharmacokinetic behaviour, clinical efficacy and level of drug residues amongst different BZD compounds. However, the biotransformation processes involved are complex and sites of metabolic conversion, pathways and metabolic products, particularly for the most recently introduced drugs, need to be elucidated. The current understanding on the clinical pharmacokinetics and metabolism of compounds in ruminants has been recently reviewed (Lanusse and Prichard 1993).

Several host-related factors may affect the pharmacokinetic behaviour and the resultant antiparasite activity of BZD compounds in production animals. Absorption and biotransformation are two of the most important processes affected by those factors. Feeding management (Hennessy 1993) and parasite disease-related factors (Marriner *et al.* 1984) could influence the amount of active BZD drug that reaches the parasites and, even more important, the length of time that parasites are exposed to active drug concentrations. Parasite-mediated liver damage with reduced enzymatic activity of different liver microsomal function oxidases has been reported in the *Fasciola hepatica*-infected rat (Teckwani *et al.* 1988) and sheep (Galtier *et al.* 1986), which could lead to altered patterns of drug/xenobiotic metabolism and clearance (Tufenkji *et al.* 1988). In fact, a significant decrease in the rate of albendazole metabolism and elimination has been demonstrated in sheep 8 weeks after experimental infestation with 150 metacercariae of *F. hepatica* (Galtier *et al.* 1991).

Because of its central position in the biotransformation and metabolic activities of the animal, the liver is vulnerable not only to damage by various foreign chemicals, microorganisms and parasites, but also to dietary induced metabolic disorders. Drug pharmacokinetic characterizations have traditionally been performed in healthy animals. However, different health disorders with altered liver function may induce important changes on the pharmacokinetic behaviour, side-effects and expected efficacy of the chosen anthelmintic for therapy. Thus, the understanding of these potential factors affecting the pharmacology of BZD anthelmintics in production animals reared under field conditions, may be relevant for achieving optimal parasite control and avoiding selection for drug resistance. The purpose of the current research was to evaluate the influence of the nutritional condition on the plasma and abomasal disposition kinetics of albendazole and its metabolites in the calf.

Materials and methods

Animals

The study was conducted on six Holstein calves (140–180 kg), in parasite-free conditions. Four animals were surgically fitted with a permanent cannula in the pyloric region of the abomasum. A 6-week post-surgery recovery period was then allowed, before starting the pharmacokinetic trial. Animals were housed in individual pens in an indoors cattle facility. They were handled and observed daily to ensure their good health prior to and throughout the course of the experiment.

Experimental design and treatments

The study was conducted in two different experimental phases. A 6-week wash-out period transpired before the same animals participated in the second phase of the study. Phase I: calves were in an 'optimal' nutritional status. They were fed *ad libitum* with a high-quality alfalfa hay plus a grain-based concentrate diet, and treated with albendazole (ABZ) (suspension, 100 mg/ml) by intraruminal administration at 10 mg/kg body weight (control group). Phase II: calves were subjected to a dietary restriction for 35 days (restricted group), and subsequently treated with ABZ as described above. This sustained feed restriction

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(covering only 40% of the animal's maintenance requirements) was intended to simulate a period of inadequate nutrition. The body weight of the animals was determined every 7 days during the restriction period. Water was provided *ad libitum* during both experimental phases.

Biochemical determinations

Serum concentrations of free fatty acid (FFA) and β-hydroxybutyrate (β-OHB) were measured in the experimental animals prior to the kinetics trial (control group), and every 7 days during the feed restriction period (restricted group). These biochemical 'markers' were determined to correlate potential changes on ABZ kinetics with metabolic disorders taking place in animals subjected to the different experimental conditions. FFA serum concentrations were determined according to a technique adapted from that of Falholt *et al.* (1973), using palmitic acid as standard to prepare calibration curves. β-OHB concentrations were measured following the procedure described by Gibbard and Watkins (1968) using test kits purchased from Sigma Chemical Co. (St Louis, MO, USA).

Collection of blood and abomasal fluid samples

In both experimental phases, samples of abomasal fluid (via cannula) and jugular blood were taken prior to and at 0·25, 0·5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 60, 72, 96 and 120 h post-treatment. Plasma was separated by centrifugation at 2600 *g* for 15 min. Plasma and abomasal fluid samples were placed into plastic vials and frozen at − 20°C until analysed.

Analytical methods

Immediately after thawing, plasma and abomasal fluid samples (1·0 ml) were spiked with an internal standard (oxibendazole, 1 μg/10 μl methanol) and ABZ, albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) extracted using disposable C_{18} SepPak cartridges (part # 51910, Waters Associates, MA, USA). For abomasal fluid samples, there was a solvent-mediated extraction before SepPak clean-up, which was performed following the method previously described (Lanusse *et al.* 1993a). Sample extraction procedures, hplc equipment and analysis conditions were as previously reported (Lanusse *et al.* 1991). The extraction efficiencies for the different analytes ranged from 87 to 96% (plasma) and from 84 to 93% (abomasal fluid).

Identification of ABZ, ABZSO and $ABZSO_2$ was undertaken by comparison with the retention time of pure reference standards (supplied by Schering Plough, NJ, USA). These standards were also used to prepare the calibration curve for each analyte. The linear regression lines for each analyte in the range 0·02–3·0 μg/ml (triplicate determinations) showed r between 0·977 and 0·991. Unknown concentrations were quantified by establishing the ratio between each analyte and the internal standard peak area, using Nelson Analytical Software, model 2600, version 3.0 (Nelson Analytical Inc., CA, USA) on an IBM-AT computer. The sensitivity of the assay (μg/ml) was as follows: 0·010 (ABZ and ABZSO), and 0·020 ($ABZSO_2$). There was no interference of any endogenous compounds in the chromatographic determination of either ABZ or its metabolites.

Pharmacokinetic analysis of data

The plasma and abomasal fluid concentration versus time curves for ABZ and/or its metabolites after each treatment were fitted with the PKCALC computer program (Shumaker 1986) coupled to an augmented copy of the stripping program ESTRIP (Brown and Manno 1978). Pharmacokinetic parameters were determined using model-independent methods. The following equation was used to describe biexponential concentration–time curves for both ABZ metabolites in plasma and for ABZ parent drug in abomasal fluid (Notari 1987):

$$C_p = B\exp^{(-\beta t)} - B\exp^{(-kt)},$$

where C_p is concentration at the time t after administration (μg/ml); B is concentration at time zero extrapolated from the elimination phase (μg/ml); e is the base of the natural logarithm; β is the terminal slope (h^{-1}); and k is the rapid slop obtained by feathering which represents either the first-order absorption rate constant (K_{ab}) or first-order metabolite formation rate constant (K_f) (h^{-1}). Appropriate lag times were used where necessary. The profiles of ABZSO and $ABZSO_2$ in abomasal fluid were best fitted using a triexponential equation.

The elimination half-life ($T_{1/2_{el}}$) and absorption ($T_{1/2_{ab}}$) or metabolite formation half-lives ($T_{1/2_{for}}$) were calculated as in $2/\beta$ and $\ln 2/k$ respectively. The peak concentrations (C_{max}) and time-to-peak concentrations (T_{max}) were read from the plotted concentration–time curve of each metabolite. The area under the concentration–time curves (AUC) was calculated by the trapezoidal rule (Gibaldi and Perrier 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (β).

Statistical moment theory was applied to calculate the mean residence time (MRT) for ABZ and its metabolites as follows (Perrier and Mayersohn 1982):

$$MRT = \frac{AUMC}{AUC} - \frac{1}{K},$$

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

Statistical analysis

Statistical comparison of mean pharmacokinetic parameters for ABZ and its metabolites obtained under the different experimental conditions was performed using Student's t-test for paired observations. $p < 0{\cdot}05$ was considered significant. The pharmacokinetic parameters are reported as mean ± SEM.

Results

Both FFA and β-OHB serum concentrations were significantly higher in feed restricted compared with control animals in an optimal nutritional condition. The comparative FFA and β-OHB serum concentrations obtained in the control and restricted calf, after 28 and 35 days of dietary restriction, are shown in figure 1. The experimental animals had an average weight loss of 22·1 ± 2·19 kg during the 35 days of reduced nutrient intake.

ABZ parent drug was not detected in plasma at any time post-treatment. ABZSO and $ABZSO_2$ were the main metabolites recovered in plasma after ABZ administration in both experimental phases. The plasma disposition kinetics of both ABZ metabolites was markedly changed in the feed-restricted compared with control animal. The comparative ABZSO and $ABZSO_2$ mean plasma concentrations obtained after ABZ administration in the control and restricted calf are shown in figures 2 and 3 respectively. The summarized kinetic analyses for ABZSO and $ABZSO_2$ are shown in table 1.

ABZ parent drug and its sulphoxide and sulphone metabolites were recovered in abomasal fluid in all experimental animals. They were detected from 0·25 up to either 48 (ABZ) or 120 h (ABZSO and $ABZSO_2$) post-treatment. Increased concentration profiles of the parent drug and its metabolites in abomasal fluid, were found in the feed-restricted compared with control calf. Figure 4 shows the concentration profile of ABZ parent drug in abomasal fluid of the control and feed-restricted animal. The comparative AUCs for ABZ, ABZSO and $ABZSO_2$ in abomasal fluid, obtained after intraruminal administration of ABZ in the control and restricted groups, are shown in figure 5. C_{max}, T_{max}, $T_{1/2_{el}}$ and ratio of AUC abomasum/plasma obtained for ABZ and its metabolites in abomasal fluid of calves in both experimental groups are compared in table 2. The correlations between β-OHB serum concentrations and different estimated pharmacokinetic parameters for ABZ (abomasal fluid) and ABZSO (plasma) in control and feed restricted animals are shown in figure 6.

Discussion

Free fatty acid (FFA) and β-hydroxybutyrate (β-OHB) determinations

The serum concentrations of FFA and β-OHB have been shown to be good indicators of nutritional status in ruminants (Bouchat *et al.* 1981, Barnouin *et al.* 1986), and plasma β-OHB represents over 80% of the total ketone bodies in cattle. Determination of β-OHB blood concentrations permits an evaluation of the nutritional condition and energy intake, being useful to diagnose subclinical ketosis and fatty liver disease. These biochemical 'markers' were determined to correlate potential changes on ABZ disposition with metabolic changes taking place in the experimental animals subjected to a period of dietary restriction. This mild and sustained feed restriction was intended to simulate a period of inadequate nutrition to which parasitized cattle reared under field conditions may be subjected. Decreased

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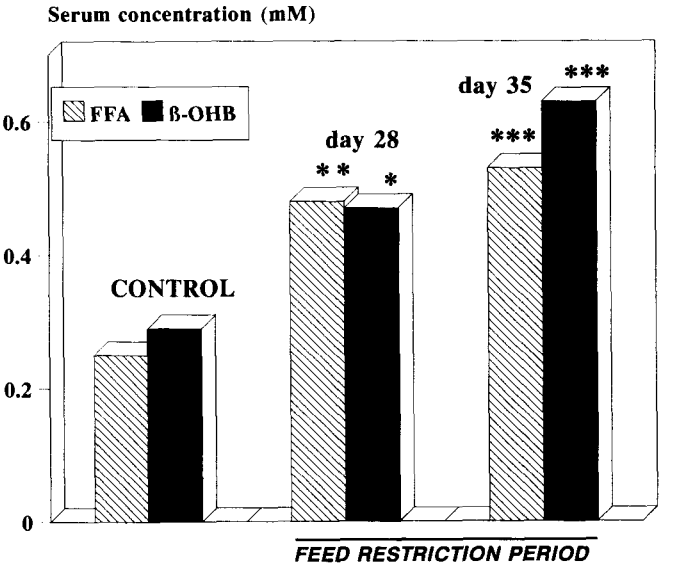

Figure 1. Mean serum concentrations ($n = 6$) of free fatty acid (FFA) and β-hydroxybutyrate (β-OHB) in the control calf (optimal nutritional status) and in the feed-restricted calf at 28 and 35 days of the restriction period. Values are statistically different from those of the control group at: $*p < 0.05$; $**p < 0.01$; and $***p < 0.001$. SEMs are as follows: control: FFA (0·017), β-OHB (0·032); day 28: FFA (0·022), β-OHB (0·022); and day 35: FFA (0·043), β-OHB (0·035) mM.

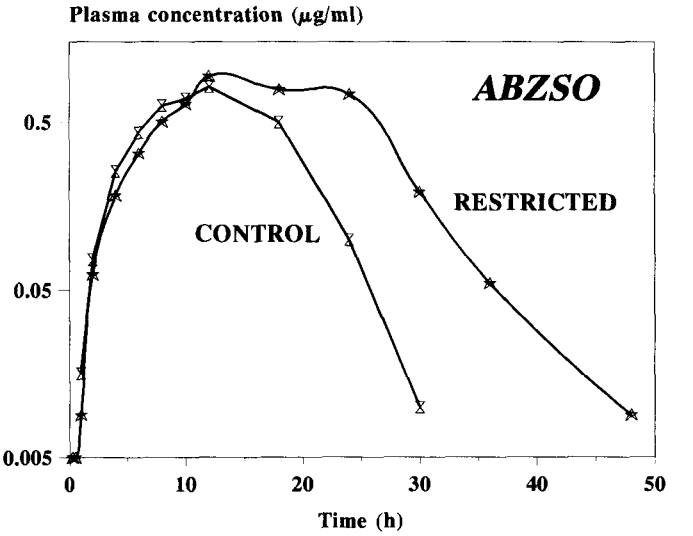

Figure 2. Mean plasma concentrations ($n = 6$) of albendazole sulphoxide (ABZSO) obtained after the intraruminal administration of albendazole (10 mg/kg) in the control and feed-restricted calf.

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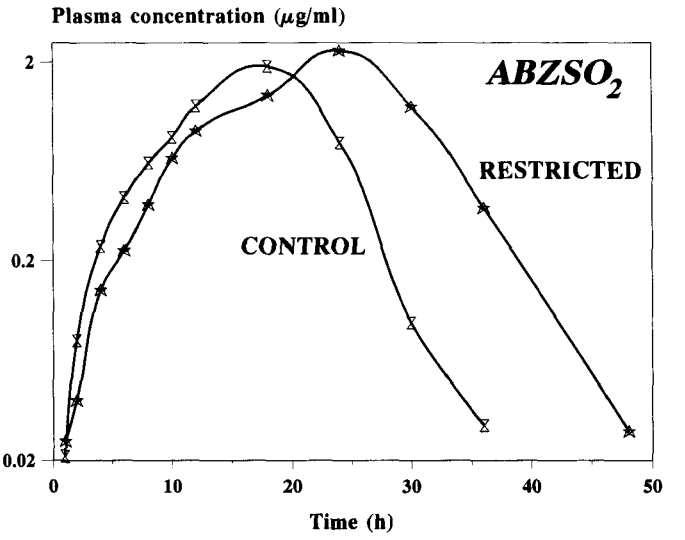

Figure 3. Mean plasma concentrations ($n = 6$) of albendazole sulphone ($ABZSO_2$) obtained after the intraruminal administration of albendazole (10 mg/kg) in the control and feed-restricted calf.

Table 1. Pharmacokinetic parameters for albendazole sulphoxide and albendazole sulphone in plasma obtained after the intraruminal administration of albendazole (10 mg/kg) to the control and feed-restricted calf.

	Albendazole sulphoxide		Albendazole sulphone	
Kinetic parameters	Control	Restricted	Control	Restricted
C_{max} (μg/ml)	0·82 ± 0·09	1·10 ± 0·09*	1·89 ± 0·19	2·53 ± 0·22*
T_{max} (h)	11·3 ± 0·52	18·0 ± 2·70*	18·0 ± 0·00	21·0 ± 3·00**
AUC (μg.h/ml)	10·2 ± 1·00	18·1 ± 1·75*	26·3 ± 2·92	38·4 ± 1·55*
$AUMC$ (μg.h^2/ml)[a]	140 ± 14·4	321 ± 35·5**	435 ± 55·0	835 ± 55·3**
$T_{1/2_{el}}$ (h)	2·57 ± 0·28	4·09 ± 0·49*	2·35 ± 0·28	3·65 ± 0·47*
MRT (h)[a]	9·63 ± 0·36	13·5 ± 0·50***	13·5 ± 0·49	16·8 ± 1·15**

C_{max}, peak plasma concentration; T_{max}, time of the peak plasma concentration; AUC, area under the concentration versus time curve; $AUMC$, area under the curve of the product of time and the plasma drug concentration versus time; $T_{1/2_{el}}$, elimination half-life; MRT, mean residence time.

Values are presented as mean ± SEM ($n = 6$). Values are statistically different from those of the control group at: *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$.

[a]Parameters obtained by non-compartmental analysis of the data based on the statistical moment theory.

appetite and poor growth rate are consistent features of subclinical parasitism in young cattle (Gibbs 1987). As a consequence of parasite activities and host responses to parasitism, various nutritional parameters can be influenced including nutrient intake, nutrient digestibility, nitrogen retention, and energy utilization. Where nutritional factors may limit production, any effect of the parasites on the utilization of nutrients will be of great importance. This situation may be more evident during the winter season where pasture availability is reduced.

The poor nutritional status of the dietary restricted animals in this trial was evidenced by an average loss of 22 kg body weight/animal during the 35 days of feed restriction. This poor nutritional condition was biochemically corroborated by the significantly higher serum concentrations of FFA and β-OHB (figure 1), obtained

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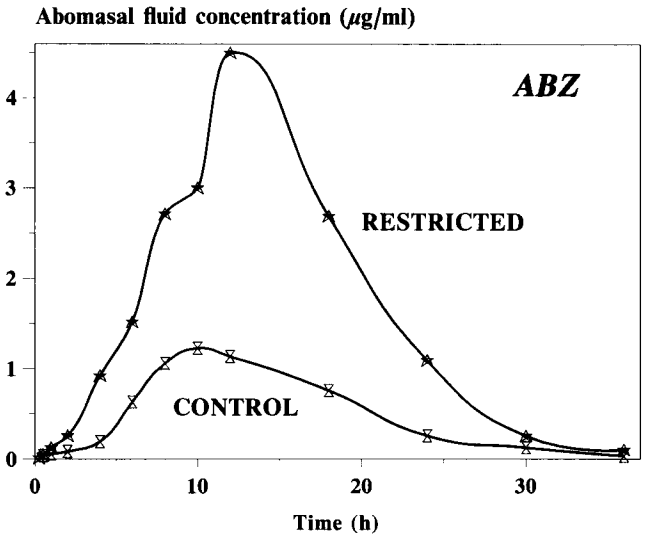

Figure 4. Mean albendazole (ABZ) parent drug concentrations ($n = 4$) in abomasal fluid, obtained after its intraruminal administration (10 mg/kg) to the control and feed restricted calf.

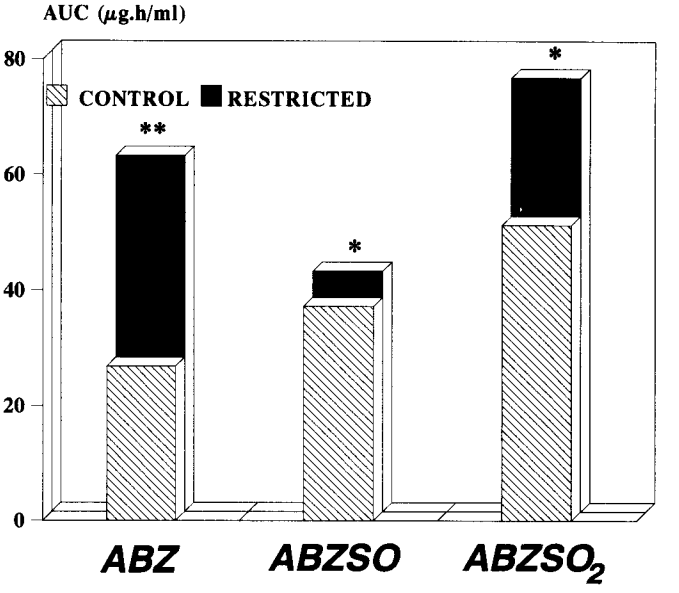

Figure 5. Comparative area under the curves (*AUC*) in abomasal fluid for albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$), obtained after intraruminal administration of albendazole (10 mg/kg) to the control and feed restricted calf ($n = 4$).

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Table 2. Pharmacokinetic parameters for albendazole (ABZ), albendazole sulphoxide (ABZSO) and albendazole sulphone ($ABZSO_2$) in abomasal fluid, obtained after the intraruminal administration of albendazole (10 mg/kg) to the control and feed restricted calf.

Drug/metabolites	Group	C_{max} (μg/ml)	T_{max} (h)	$T_{1/2_{el}}$[a] (h)	Ratio *AUC* abomasum/plasma
ABZ	Control	1·79 ± 0·11	13·5 ± 2·27	6·70 ± 0·43	na
	Restricted	4·58 ± 0·15*	12·0 ± 0·00**	7·67 ± 0·26**	
ABZSO	Control	2·16 ± 0·20	14·0 ± 2·00	42·6 ± 5·37	3·64
	Restricted	3·20 ± 0·60	12·0 ± 0·00	33·7 ± 1·47*	2·30
$ABZSO_2$	Control	2·89 ± 0·20	16·5 ± 2·48	33·3 ± 3·93	2·25
	Restricted	3·53 ± 0·14*	24·0 ± 0·00**	24·9 ± 4·31	1·95

na, not applicable.

C_{max}, peak plasma concentration; T_{max}, time of the peak plasma concentration; $T_{1/2_{el}}$, elimination half-life.

Values are presented as mean ± SEM ($n = 4$). Values are statistically different from those of the control group at: *$p < 0·05$; **$p < 0·01$.

[a] $T_{1/2_{el}}$, for ABZSO and $ABZSO_2$ were calculated using a triexponential pharmacokinetic model.

in the feed-restricted compared with control calf. Elevation of serum FFA concentrations is also an important aspect in the adaptation to different nutritional conditions resulting in decreased glucose availability. FFA concentrations in plasma may be used to indicate the adequacy of feed energy supply because the concentrations of FFA increase if energy supply is insufficient. FFA concentration also reflects changes in metabolic turnover in adipose tissues, reflecting the substrate supply to β-oxidation in tissues (Riis 1983). Thus, animals subjected to a dietary restriction in the current study were an adequate model to characterize the influence of nutritional alterations on ABZ disposition kinetics.

Albendazole kinetic behaviour in cattle

As previously reported after administration of either ABZ (Prichard *et al.* 1985) or its pro-drug, netobimin (Lanusse *et al.* 1991), ABZ parent compound was not detected in plasma at any time post-treatment in the present experiment in cattle. A first-pass oxidation in the liver would account for ABZ not being found in jugular blood (Lanusse and Prichard 1993). However, the parent molecule was detected in abomasal fluid over 48 h post-treatment (figure 4). The early detection of ABZ and its metabolites in abomasal fluid and plasma (metabolites) may confirm both a very fast absorption process, and that some oxidation occurs in the gastrointestinal (GI) tract before absorption.

ABZSO and $ABZSO_2$ were the only molecules detected in plasma after ABZ treatment in both experimental phases. They were rapidly depleted from plasma, resulting in short elimination half-lives (table 1). Peak plasma concentrations of ABZSO and $ABZSO_2$ were reached at 11·3 and 18 h post-treatment, respectively (table 1) and they were followed by well-defined concentration declining phases. However, whereas plasma concentration fell to undetectable levels (30–36 h post-treatment), the profile of these metabolites in the abomasum showed an 'extra' slow elimination phase that extended to 120 h post-treatment. The concentration–time plots for ABZSO and $ABZSO_2$ in abomasal fluid were best fitted using a triexponential equation, which resulted in significantly longer elimination half-lives for these metabolites in abomasum compared with plasma (tables 1 and 2).

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Influence of nutritional status on albendazole disposition

The overall disposition kinetics of ABZ and its metabolites were markedly modified in the feed-restricted calf compared with controls. The plasma disposition of the anthelmintically active ABZSO metabolite resulted notably changed in the calves subjected to a period of undernutrition (figure 2). A significantly higher ABZSO peak plasma concentration reached at a delayed T_{max} was obtained in the restricted compared with controls (table 1). However, a delayed ABZSO elimination with extended detection in plasma (figure 2) was the most evident kinetic alteration observed in the calves in a poor nutritional condition, which resulted in significantly longer $T_{1/2_{el}}$ and MRT in comparison with those obtained for the control group. This delayed elimination of ABZSO from the bloodstream correlated with the significantly higher AUCs and $AUMC$s obtained for this metabolite in calves with a reduced nutrient intake, compared with those of the control group (table 1).

$ABZSO_2$ is an inactive metabolite formed in a P450-mediated liver microsomal sulphonation reaction (Souhaili-El-Amri *et al.* 1988). The disposition of $ABZSO_2$ may be rate limited by the formation and disposition of ABZSO (Lanusse *et al.* 1993a). The modifications to the kinetics of $ABZSO_2$ observed in feed restricted animals showed a similar pattern to those described for ABZSO (figure 3). A significantly delayed C_{max} and prolonged $T_{1/2_{el}}$, resulting in significantly higher plasma AUC for $ABZSO_2$, were obtained in the restricted compared with control calf (table 1). These pharmacokinetic changes for $ABZSO_2$ seem to reflect the modifications to the disposition of the first metabolite in the sequence, ABZSO, induced by the nutritional deficiency of the feed restricted animals.

It is well established that animals deprived of food obtain at least 80% of their energy demands from fat and the rest from protein (Giesecke 1983). FFAs are long-chain fatty acids bound to albumin as a carrier. They represent the transport form of adipose tissue fatty acids to the liver, muscle and other organs in need of energy from the body stores (Giesecke 1983). A major site of FFA uptake in ruminants is the liver, which transforms them to triacylglycerol and lipoproteins for transport to extrahepatic organs (Martin 1987). The liver can also oxidize them completely to CO_2 or incompletely to ketone bodies and acetate. Up to 70% of the FFA taken up by the liver is converted into β-OHB under catabolic conditions (Katz and Bergman 1969). Ketone body production permits transformation of FFA into a soluble substrate, which, unlike fatty acids, is independent of carriers both in plasma (albumin) and in the cells (carnitine). This is of a remarkable physiological relevance. The poor nutritional status of the feed-restricted cattle under our experimental conditions was clearly evidenced by their increased FFA and β-OHB serum profiles. These nutritionally induced metabolic alterations resulted in an enhancement of the AUCs for both ABZ metabolites in plasma, which correlated with the significantly longer $T_{1/2_{el}}$ and MRT obtained for these metabolites in the animals subjected to undernutrition in comparison with control animals. Finally, these changes on disposition kinetics could reflect an impairment on the hepatic biotransformation and clearance of ABZ and/or its metabolites as a consequence of massive FFA mobilization from adipose tissue and overproduction of β-OHB in the liver mitochondria.

The delayed elimination of ABZ metabolites from the bloodstream correlated with the higher concentration profiles of these molecules found in the abomasal fluid of the animals subjected to a dietary restriction (table 2). Interestingly, while ABZ was not detected in plasma, a significantly higher concentration profile of the parent

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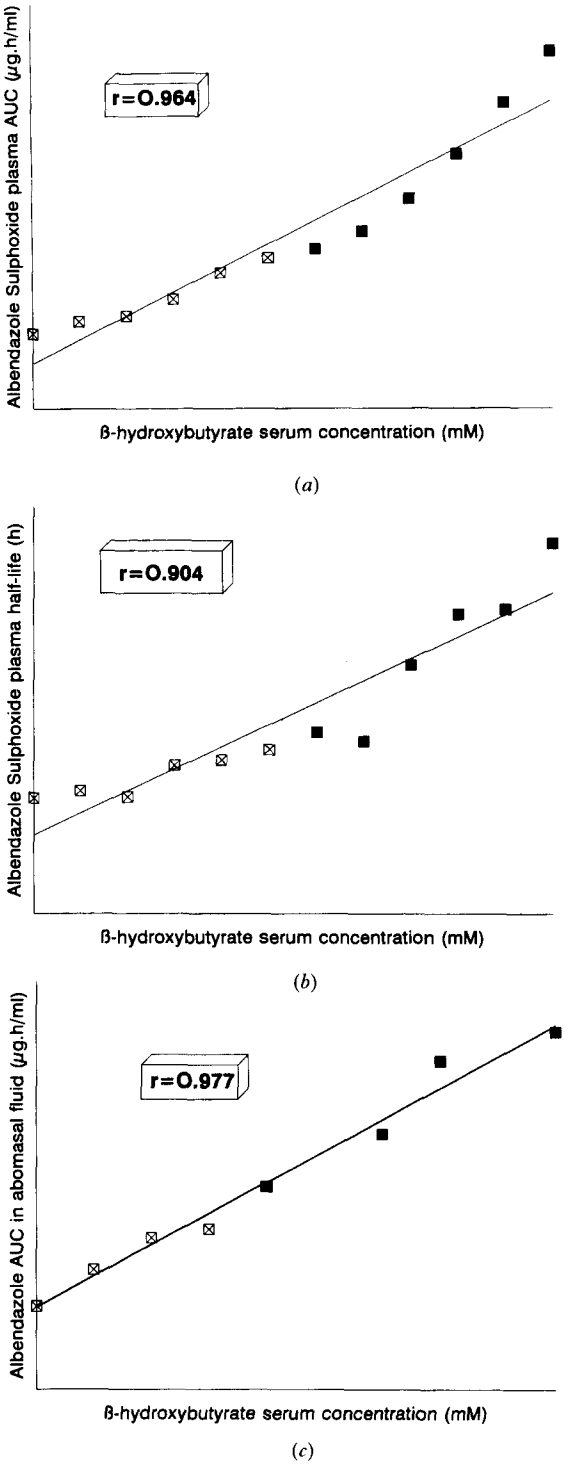

Figure 6. Relationship between β-hydroxybutyrate (β-OHB) serum concentrations and (*a*) albendazole sulphoxide plasma area under the curve (*AUC*) ($n = 12$), (*b*) albendazole sulphoxide plasma half-life ($n = 12$), (*c*) albendazole *AUC* in abomasal fluid ($n = 8$), in the control (⊠) and feed-restricted (■) calf. *r* is the correlation coefficient.

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molecule was observed in the abomasal fluid of the restricted animals (figure 4). The *AUC*s for ABZ and its metabolites in abomasal fluid were significantly higher in the animals subjected to a reduced feed intake (figure 5). ABZ metabolites are reversibly exchanged between plasma and GI compartments in a distribution process driven by a plasma/GI tract pH gradient (Lanusse *et al.* 1993a). This phenomenon facilitates a strong ionic trapping effect, which would account for the higher concentration profiles of ABZSO and $ABZSO_2$ found in abomasum (pH = 1–2) compared to plasma (pH = 7·4). It has also been shown that the GI microflora reduces ABZSO to form the ABZ parent drug (Lanusse *et al.* 1992). Distribution of ABZSO from plasma to ruminal fluid and ionic-trapping in this fluid may facilitate the microflora-mediated reduction of ABZSO to ABZ. Such a phenomenon may have accounted for the increased concentration profile of ABZ parent molecule found in the abomasum of the animals subjected to undernutrition, which showed delayed plasma depletion of the sulphoxide molecule compared with control animals. Thus, the increased concentration profiles of ABZ metabolites recovered in the abomasal fluid of the feed restricted animals reflect the higher plasma concentration profiles found in these animals compared with calves in the control group.

Nutritional variations affect metabolism directly by determining the availability of exogenous substrates for cellular processes and indirectly through stimulation of neuro-endocrine factors regulating metabolic turnover (Riis 1983). Nutritionally induced modifications to the pattern of endo- and xenobiotic biotransformation have been described elsewhere (Catz 1990). Minerals (calcium, copper, magnesium, selenium, zinc), vitamins (C, E, B) and protein deficiencies have been associated with a decrease in the total concentration of P450 with reduced activity of this enzymatic pathway, and/or decreased phase II conjugative activities (Neumann and Zanoni 1988, Burrows and Egerton 1989). Metabolism of BZD anthelmintics and closely related compounds has been shown to be catalysed by the enzymatic system of hepatic microsomal mixed-function oxidases (Short *et al.* 1988, Gottschall *et al.* 1990). ABZ and other available BZD thioether anthelmintics are metabolized to their respective sulphoxide and sulphone entities by liver microsomal oxidation. Sulphoxidation of ABZ into ABZSO *in vitro* has been shown for rat (Fargetton *et al.* 1986), sheep (Galtier *et al.* 1986), and cattle (Lanusse *et al.* 1993b) liver microsomes. A portion of ABZSO undergoes a second, slower and irreversible oxidative step to form $ABZSO_2$ (sulphonation) (Souhaili-El-Amri *et al.* 1988). Two distinct microsomal enzymatic pathways have been proposed for this sequential ABZ oxidation; the flavin-containing monooxygenase system (FMO) may be responsible for the sulphoxidation reaction to form ABZSO in a NADPH-dependent process (Galtier *et al.* 1986, Lanusse *et al.* 1993b), whereas the P450 system is primarily involved in the formation of $ABZSO_2$ (Souhaili-El-Amri *et al.* 1988).

The results presented here clearly show that the poor nutritional status of the feed-restricted animals induced marked modifications to the pattern of ABZ biotransformation and to its resultant disposition kinetics. At the moment we can only speculate on the possible site/s of interference of the nutritionally induced biochemical changes on the sequence of ABZ liver biotransformation in cattle. The microsomal oxidation of ABZ into ABZSO (sulphoxidation) is highly dependent on the NADPH concentration (Lanusse *et al.* 1993b). *In vitro*, virtually no sulphoxidase activity was observed in the absence of NADPH, and ABZSO production by sheep and cattle liver microsomes increased with an increase in the concentration of NADPH (Lanusse *et al.* 1993b). During both starvation and underfeeding in cattle

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the hepatic concentrations of NADPH and ATP have been shown to decrease to about 60 and 40% respectively of their normal values in animals fed *ad libitum* (Riis 1983). The decreased availability of NADPH in the liver during periods of undernutrition has been related to a shift from acetate production to ketogenesis (Riis 1983). Such a phenomenon may have also accounted for the overproduction and increased serum concentration of β-OHB found in the restricted calves in the present trial. It is likely, therefore, that both the decreased energy (ATP) production and the reduced NADPH availability in the liver cell may have contributed to the decrease of the rate of ABZ biotransformation and the delay in the elimination of the parent drug and its metabolites in the animals subjected to undernutrition. Furthermore, high FFA concentrations reaching the liver may act as an uncoupling agent in the hepatocyte mitochondria and thus affect ATP generation in a direct manner (Martin 1987).

Regardless of the identification of the specific metabolic site/s where ABZ biotransformation is affected by dietary induced metabolic and homeostatic alterations, it is evident that the nutritional condition of the treated animals influences the disposition kinetics of ABZ in cattle. The results reported herein are a further contribution to the understanding of different factors affecting the kinetic behaviour of anthelmintic drugs in ruminants. The practical implications of these findings in parasite control and in the pattern of tissue residue of these molecules in food-producing animals are being further investigated.

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