Moxidectin and ivermectin metabolic stability in sheep ruminal and abomasal contents

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The oral administration of macrocyclic lactones to sheep leads to poorer efficacy and shorter persistence of the antiparasitic activity compared to the subcutaneous treatment. Gastrointestinal biotransformation occurring after oral treatment to ruminant species has been considered as a possible cause of the differences observed between routes of administration. The current work was addressed to evaluate on a comparative basis the *in vitro* metabolism of moxidectin (MXD) and ivermectin (IVM) in sheep ruminal and abomasal contents. Both compounds were incubated under anaerobic conditions during 2, 6 and 24 h in ruminal and abomasal contents collected from untreated adult sheep. Drug concentrations were measured by high-performance liquid chromatography with fluorescence detection after sample clean up and solid phase extraction. Neither MXD nor IVM suffered metabolic conversion and/or chemical degradation after 24-h incubation in ruminal and abomasal contents collected from adult sheep. Unchanged MXD and IVM parent compounds represented between 95.5 and 100% of the total drug recovered in the ruminal and abomasal incubation mixtures compared with those measured in inactive control incubations. The partition of both molecules between the solid and fluid phases of both sheep digestive contents was assessed. MXD and IVM were extensively bound (>90%) to the solid material of both ruminal and abomasal contents collected from sheep fed on lucerne hay. The results reported here confirm the extensive degree of association to the solid digestive material and demonstrates a high chemical stability without evident metabolism and/or degradation for both MXD and IVM in ruminal and abomasal contents.

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INTRODUCTION

Antiparasitic compounds are extensively used in veterinary medicine to control a variety of ecto- and endoparasites affecting domestic animals. They are administered by different routes according to the target animal species, including subcutaneous, intramuscular, oral, intraruminal and topical routes. The animal species to be treated, the target parasites, the chosen antiparasitic compound and its pharmaceutical formulation are among the factors to be considered to select the administration route. The macrocyclic lactones (ML), moxidectin (MXD) and ivermectin (IVM) are potent ecto-endoparasiticidal compounds from the milbemycin and avermectin families, respectively (Takiguchi *et al.*, 1980; Fisher & Mrozik, 1989). MXD and IVM are commercially available as injectable and pour-on formulations for use in cattle. Oral and subcutaneous preparations are the most widely used for parasite control in sheep and goats (McKellar & Benchaoui, 1996).

The oral administration of IVM to sheep leads to poorer efficacy and shorter persistence of the antiparasitic activity compared with the subcutaneous treatment (Borgsteede, 1993). Prichard *et al.* (1985) reported a lower IVM plasma availability obtained after the intraruminal administration to sheep compared with the parenteral treatment. These authors suggested the metabolism and/or degradation of the drug in the ruminal environment. Although the stability of IVM in ruminal fluid was demonstrated some years later (Bogan & McKellar, 1988; Andrew & Halley, 1996), degradation of IVM (Ali & Hennessy, 1996) and doramectin (Hennessy *et al.*, 2000) in the ruminant gastrointestinal tract has not been ruled out.

Moxidectin and IVM differ in some of their physicochemical properties, which is reflected on their formulation flexibility,

comparative pharmacokinetic behavior in cattle (Lanusse *et al.*, 1997) and patterns of efficacy and persistence of antiparasitic activity (Armour *et al.*, 1985; Shoop *et al.*, 1995; Vercruysse *et al.*, 1997). A markedly longer residence time for MXD compared with IVM, has also been observed after oral treatment in sheep (Imperiale *et al.*, 2004; Molento *et al.*, 2004). The liver metabolism of both compounds has been characterized in different animal species (Chiu *et al.*, 1987; Afzal *et al.*, 1994; Zulalian *et al.*, 1994). However, only limited information on IVM ruminal stability is available (Andrew & Halley, 1996) and the comparative gastrointestinal metabolism of MXD and IVM has not been investigated, in spite of the fact that the disposition of these ML compounds in the ruminant digestive tract is consider relevant to their overall kinetic behavior and pattern of efficacy. To gain some further insight on the subject, the goal of the current work was to investigate on a comparative basis the *in vitro* metabolism of MXD and IVM in sheep ruminal and abomasal contents. Additionally, the relative distribution of both parent molecules between the fluid and particulate phases of both digestive contents was determined. The outcome of the work reported here is complementary to the available body of information on the gastrointestinal disposition of the ML in ruminants (Steel, 1993; Ali & Hennessy, 1996; Lifschitz *et al.*, 1999, 2000; Hennessy *et al.*, 2000; Hennessy, 2001) and contributes to the comprehension of the fate of these compounds in ruminant species.

MATERIALS AND METHODS

Experimental animals. Collection of ruminal and abomasal contents

Three healthy untreated Corriedale sheep (40–50 kg) were used as a source of ruminal and abomasal contents. Experimental animals were located in boxes and fed *ad libitum* with high quality lucerne hay for 10 days before starting the trial to standardize the feeding condition. Water was provided *ad libitum*. The management of experimental animals was in agreement with institutional and internationally accepted welfare guidelines (American Veterinary Medical Association (AVMA), 2001). Sheep were slaughtered by captive bolt and ruminal and abomasal contents collected, filtered through a hydrophilic gauze to remove rough material, kept at 38 °C and immediately processed for incubation.

Evaluation of ruminal and abomasal metabolism

The gastrointestinal metabolism of the analytes was studied using the technique described by Virkel *et al.* (1999, 2002). Either MXD or IVM dissolved in 20 μL of methanol were added to 2 mL of each gastrointestinal content (ruminal and abomasal) to reach a final concentration of 1 nmol/mL. Each incubation mixture with ruminal content was gently gassed with pure N_2 for 5 min at 38 °C. The incubations ($n = 9$) were carried out in a shaking water bath at 38 °C under anaerobic conditions for 2, 6 and 24 h. An aliquot of each incubation (1 mL) were immediately frozen at −20 °C until high-performance liquid chromatography (HPLC) analysis.

Unfortified ruminal and abomasal content samples ($n = 4$) were incubated as blank samples. Control samples of boiled ruminal content ($n = 6$) were prepared and incubated under the same conditions. Abomasal content samples spiked with MXD and IVM were immediately extracted without incubation and used as a control ($n = 6$). The compound albendazole sulphoxide (ABZSO), which is extensively sulphoreduced to albendazole (ABZ) by the ruminal microflora (Lanusse *et al.*, 1992), was used to corroborate the metabolic activity (viability) of the ruminal content (positive control). Six replicates of ruminal content spiked with 14 nmol/mL of ABZSO (dissolved in 20 μL of methanol) were incubated under anaerobic conditions during 24 h. Additionally, the substrate was also incubated for 1 h with an aliquot of ruminal content ($n = 6$) collected 23 h before (which was kept at 38 °C in anaerobiosis).

Incubation assays: partitioning between fluid and solid contents

Moxidectin and IVM were incubated in ruminal and abomasal content during 2, 6 and 24 h as described above. Once the incubation period was over, the partitioning of MXD and IVM between fluid and solid contents of each digestive fluid, was determined following the technique described by Hennessy *et al.* (1994). Briefly, 1 mL of each incubation vial was centrifuged at 18 000 ***g*** for 15 min to separate the solid (particulated) and fluid phases of the ruminal and abomasal contents. The particulate samples were re-suspended in distilled water. Both solid and fluid content samples were frozen at −20 °C until HPLC analysis.

Drug extraction and chromatographic analysis

Moxidectin and IVM concentrations were measured by HPLC with fluorescence detection using automated solid phase extraction, following a procedure previously described by Alvinerie *et al.* (1993) and Lifschitz *et al.* (1999, 2000). A solid phase extraction of fortified and experimental samples was performed after thawing at room temperature. A sample aliquot (100 μL) was combined with 0.5 mL of water and 0.5 mL of acetonitrile and mixed for 20 min (Multi Tube Vortexer; VWR Scientific Products, Wilmington, NC, USA) sonicated for 10 min (Transsonic 570/H; Lab Line Instruments Inc., Melrose Park, IL, USA), and the solvent-sample mixture was centrifuged at 2000 ***g*** for 15 min. The supernatant was manually transferred into a tube and the procedure repeated once. The pooled supernatants obtained were then placed on the appropriate rack of an Aspec XL autosampler (Gilson, Villiers Le Bell, France). Automatic sample preparation was performed as follows: (a) the cartridge (Supelclean^R LC_{18} cartridge, 100 mg, 1 mL; Supelco INC., Bellefonte, PA, USA) positioned on the holder, was first conditioned with 2 mL of methanol and 2 mL of water, (b) ruminal/abomasal samples (supernatants) were applied to the cartridge. The cartridge was then washed with 1 mL of water followed by 1 mL of water–methanol (75:25 v/v), then 1.5 mL

of methanol was applied to the cartridge and the elute was collected. After automated solid phase extraction, the elute was evaporated to dryness under a gentle stream of dry nitrogen at 60 °C in a water bath. Reconstitution was done using 100 μL of a solution of N-methylimidazole (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1:1). Derivatization was initiated by adding 150 μL trifluoroacetic anhydride (Sigma Chemical Co., St Louis, MO, USA) solution in acetonitrile (1:2) (De Montigny *et al.*, 1990). After completion of the reaction, an aliquot (100 μL) of each sample was injected directly into the chromatograph. The chromatographic conditions included a mobile phase of acetic acid (0.2% in water)-methanol-acetonitrile (4:40:56 and 7:40:53 v/v/v for IVM and MXD respectively) pumped at a flow rate of 1.5 mL/min through a reverse phase C_{18} column (Selectosil; Phenomenex, Torrance, CA, USA) (5 μm, 4.6 mm × 250 mm). Fluorescence detector (Spectrofluorometric detector RF-10; Shimadzu Corporation, Kyoto, Japan) was set at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. MXD and IVM concentrations were determined by using the Class LC 10 Software version 1.2 (Shimadzu Corporation) on an IBM compatible AT computer. The analytes (MXD and IVM) were identified with the retention times of the pure reference standard. Calibration lines between 0.1 and 1 nmol/mL were prepared using least squares linear regression analysis, and correlation coefficients (r) and coefficient of variations (CV) calculated. A complete validation of the analytical procedures for extraction and quantification of MXD and IVM from ruminal and abomasal contents was performed before starting the analysis of experimental samples. Linearity was established to express the concentration–detector response relationship, as determined by injection of spiked MXD and IVM in gastrointestinal contents. The departure of the calibration curves from linearity was determined using the ANOVA test (Instat 3.0; Graph Pad software Inc., San Diego, CA, USA). Drug recovery was estimated by comparison of the peak area from spiked ruminal and abomasal standards at different concentrations, with the peak areas resulting from direct injections of standards in methanol. The limit of drug detection was established by injection and HPLC analysis of blank digestive contents and measurement of the baseline noise at the time of retention of the MXD and IVM peaks. The mean baseline noise plus six standard deviations was defined as the theoretical quantification limit. The precision of the method was estimated by processing replicate aliquots ($n = 4$) of pooled gastrointestinal content samples containing known MXD and IVM concentrations (0.1, 0.5 and 1 nmol/mL) and calculating the CV. The accuracy of the method was defined as the closeness of the measured value to the true value and was measured at the same fortification levels that precision.

Albendazole sulphoxide and its related products formed after the incubation of the positive ruminal content control samples, were extracted and analyzed by HPLC following the techniques described by Alvinerie and Galtier (1984) and Lanusse *et al.*, (1992), respectively. Briefly, an aliquot of ruminal samples (0.5 mL) were mixed with 2.5 mL of ethyl acetate and shaked for 20 min. The extraction were repeated once and the combined ethyl acetate extracts were evaporated and redissolved in 300 μL of mobile phase. Fifty microliters of each extracted sample were injected into a HPLC system (Shimadzu Corporation) fitted with a C_{18} column (Selectosil; Phenomenex) (5 μm, 4.6 mm × 250 mm) and UV detector (SPD-10 A UV detector) reading at 292 nm. The solvent system was acetonitrile/ammonium acetate (0.025 M) gradient in the following proportions: 27/73 (initial conditions) 50:50 (from 5 to 12 min) and 27/73 (from 13 to 16 min), with a flow rate of 1.2 mL/min.

The percentages of unchanged parent drug (MXD and IVM) were determined by comparison between the concentrations measured in the experimentally incubated samples and those in ruminal or abomasal control samples. Concentrations of MXD and IVM determined in the fluid and solid phases of gastrointestinal contents were expressed as a percentage (mean ± SEM) of the total drug measured. The departure from linearity of the calibration curves was determined using ANOVA test. Data were statistically compared by Student t-test (Instat 3.0; Graph Pad software Inc.). A value of $P < 0.05$ was considered significant.

RESULTS

The analytical methodology required to identify and quantify MXD and IVM in ruminal and abomasal contents was successfully developed and validated. The linear regression lines for MXD and IVM showed correlation coefficients between 0.990 and 0.998. The lack of fit test showed that there was not a significant departure from linearity. The extraction recoveries of MXD and IVM from ruminal and abomasal contents were >72% at the different concentrations analyzed. The limit of quantification was established at 0.001 nmol/mL. The precision of the analytical methods showed coefficients of variation ranging from 1.23 to 14.5% for both compounds. Accuracy of the method developed to quantify MXD and IVM in digestive contents showed only a slight deviation from true values (between 0.60 and 17%). The main validation parameters for IVM and MXD in both gastrointestinal contents are summarized in Table 1. The mean percentages of recovery for ABZ and ABZSO from the ruminal content were 95 and 80%, respectively. The CV (precision) determined for both compounds ranged between 2 and 8%.

Table 1. Percentage of recovery, precision and accuracy determined for ivermectin (IVM) and moxidectin (MXD) in ruminal and abomasal content

Validation Parameters	Ruminal content		Abomasal content	
	IVM	MXD	IVM	MXD
Recovery (%)	72–80	75–83	73–77	75–78
Precision (CV)	3.61–5.98	4.65–14.5	1.23–13.7	2.54–6.79
Accuracy (%)	0.60–15.0	1.60–12.6	1.50–16.0	1.00–17.0

The validation parameters were evaluated by processing replicates ($n = 4$) of IVM and MXD ruminal and abomasal contents at 0.1, 0.5 and 1 nmol/mL.
CV, coefficient of variation.

The viability and metabolic capacity of the ruminal microflora during 24-h postcollection of ruminal content was corroborated by incubation of positive control samples. ABZSO was extensively sulphoreduced to ABZ in fresh ruminal content immediately after its collection. The amount of ABZ formed after 24 h of incubation represented 83% of the total analytes recovered from the incubation mixture. A 60-min long incubation of ABZSO with ruminal content collected 23 h before (kept under anaerobic conditions) showed a metabolic conversion that represented a 64% of the total products recovered. The metabolic activity determined in the positive control ruminal content samples is shown in Fig. 1.

Both MXD and IVM were metabolically stable in ruminal and abomasal contents at the different incubation times assayed. Metabolic conversion and/or chemical degradation were not observed neither in ruminal nor in abomasal contents obtained from adult sheep. Chromatographic peaks that would represent metabolite products formed during the incubation of MXD and IVM in both gastrointestinal contents were not observed. The concentrations of MXD and IVM parent molecules recovered after their incubation in ruminal and abomasal contents ranged between 95.5 and 100%, compared with those measured after their incubation in boiled inactive control samples. The percentages of unchanged parent drug determined after the incubation of MXD and IVM in ruminal and abomasal contents are shown in Figs 2 & 3.

There was a singular partition of the ML molecules in both sheep gastrointestinal contents. Significantly higher concentrations (between 90 and 99% of the total drug recovered) of MXD and IVM were measured in the solid particulate phase of both digestive contents compared with the fluid phase. Any marked effect of the incubation time on the relative distribution of both molecules between phases of the gastrointestinal content was observed. In the ruminal content, the percentage of drug bound to the particulate material was significantly higher for MXD ($P < 0.05$) compared to IVM at all incubation times. However, there were not differences between drug molecules on the binding to the abomasal particulate phase. The amount of drug measured in the solid phase of ruminal and abomasal contents expressed as the percentage of total drug (solid and fluid phases) for both anthelmintic compounds under study, are shown in Tables 2 & 3.

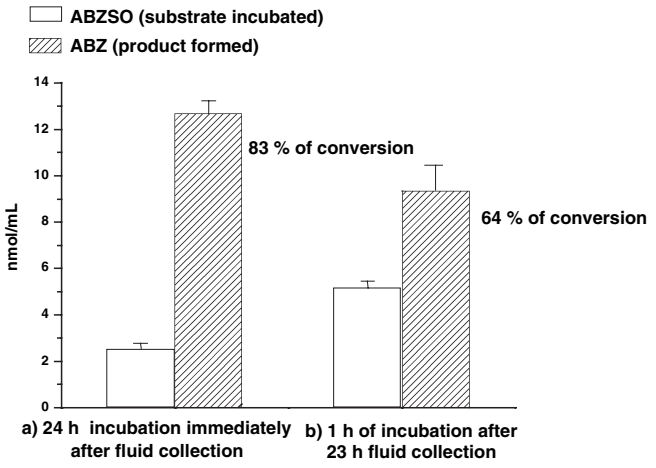

Fig. 1. Evaluation of the metabolic activity (viability) of sheep ruminal content immediately after (a) and 23 h after (b) of its collection (kept at 37 °C under anaerobiosis). Albendazole sulphoxide (14 μM) was incubated in the ruminal content during either 24 h (immediately after its collection) (a) or only over 1 h (23 h after fluid collection) (b).

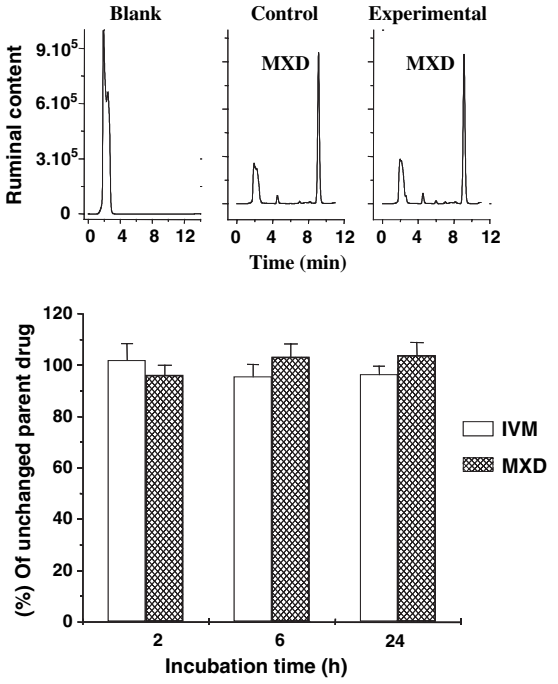

Fig. 2. Metabolic stability of moxidectin (MXD) and ivermectin observed after their incubation (between 2 and 24 h) in sheep ruminal content under anaerobic conditions. The results are expressed as the percentage of unchanged parent drug recovered from the incubation assays compared with those determined in the incubations with metabolically inactive ruminal content (control) (mean ± SEM) ($n = 9$). The insert shows typical chromatograms determined after 24-h incubation of unfortified blank (left panel), MXD-spiked in inactive control (middle panel) and MXD-spiked in experimental metabolically active (right panel) ruminal content samples.

DISCUSSION

The gastrointestinal disposition of different anthelmintic molecules given orally to ruminants is influenced by several drug, formulation and host-related factors. Fasting, level of feed intake, type of diet, the spontaneous closure of the oesophageal groove, and the degree of binding to particulate material of digesta, are among some of the factors affecting the gastrointestinal absorption of benzimidazole anthelmintics in sheep and cattle (Taylor *et al.*, 1992; Lanusse & Prichard, 1993; Hennessy *et al.*, 1994; Ali & Hennessy, 1995; Sánchez *et al.*, 1997), modifying the available concentrations of the active drug required to exert the anthelmintic action in systemic tissues. There is an important evidence that shows an active gastric secretion

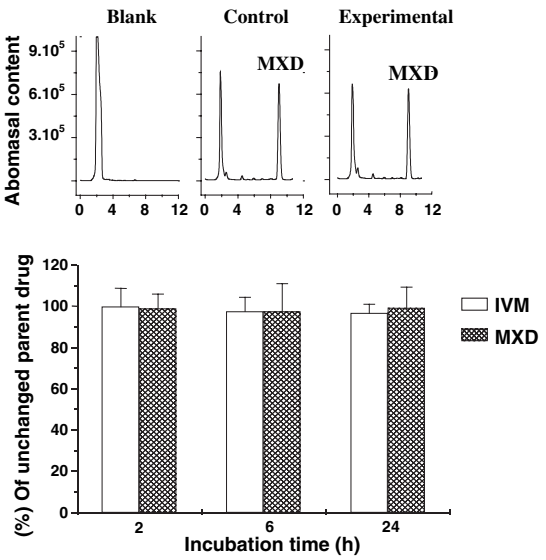

Fig. 3. Metabolic stability of moxidectin (MXD) and ivermectin observed after their incubation (between 2 and 24 h) in sheep abomasal content. The results are expressed as the percentage of unchanged parent drug recovered from the incubation assays compared with those determined in the incubations with control abomasal content (mean ± SEM) ($n = 9$). The insert shows typical chromatograms determined after 24-h incubation of unfortified blank (left panel), MXD-spiked in control (middle panel) and MXD-spiked in experimental (right panel) abomasal content samples.

Table 2. Percentage (%) of total moxidectin (MXD) and ivermectin (IVM) associated to the particulate (solid) phase of the sheep ruminal content after 2-, 6- and 24-h incubation under anaerobic conditions

Incubation time (h)	Particulate phase of ruminal content	
	% of total MXD*	% of total IVM*
2	96.9 ± 0.70†	90.8 ± 0.79
6	98.3 ± 0.31†	92.3 ± 1.00
24	98.6 ± 0.17†	95.1 ± 0.57

Values are expressed as mean ± SEM (nine determinations).
*The percentages of total MXD and IVM associated to the particulate phase of ruminal content at all incubation times were significantly higher than those determined in the fluid phase ($P < 0.05$).
†Values determined for MXD were significantly higher than those for IVM ($P < 0.05$).

Table 3. Percentage (%) of total moxidectin (MXD) and ivermectin (IVM) associated to the particulate (solid) phase of the sheep abomasal content after 2-, 6- and 24-h incubation under anaerobic conditions

Incubation time (h)	Particulate phase of abomasal content	
	% of total MXD*	% of total IVM*
2	99.6 ± 0.09	99.4 ± 0.12
6	99.1 ± 0.65	92.3 ± 0.11
24	98.9 ± 0.46	99.4 ± 0.09

Values are expressed as mean ± SEM.
*The percentages of total MXD and IVM associated to the particulate phase of abomasal content at all incubation times were significantly higher than those determined in the fluid phase ($P < 0.05$).

process and plasma/saliva exchange for the bezimidazoles compounds orally administered (Ali & Hennessy, 1995; Cristofol *et al.*, 2001). However, it seems likely that the ML are not involved in a relevant active secretion into the abomasum. After the subcutaneous administration of IVM, MXD and DRM, high drug concentrations were determined in the abomasal mucosal but only traces of these compounds were detected in the abomasal content (Bogan & McKellar, 1988; Lifschitz *et al.*, 1999, 2000). A low amount of [^{3}H]-doramectin in abomasal digesta was collected in sheep after its intravenous administration, that reflect the minimal gastric secretions of ML (Hennessy *et al.*, 2000).

Although the liver is the main site for the biotransformation of xenobiotic compounds, drug metabolism also takes place in extra-hepatic tissues such as the gastrointestinal tract, which may contribute to reduce the systemic availability of orally administered compounds (Rowland, 1986; Vynckier & Debackere, 1993). The anthelmintics netobimin (an albendazole prodrug) and ABZSO have been shown to suffer extensive biotransformation in ruminal (Virkel *et al.*, 1999) and intestinal (Lanusse *et al.*, 1992) contents collected from sheep and cattle. Those metabolic conversions are mediated by the digestive microflora and both the type of diet (Virkel *et al.*, 1999) and the antibacterial effect of monensin (Virkel *et al.*, 2004), have demonstrated to markedly affect the bioconversion activity of the ruminal content. The metabolic capacity of sheep ruminal content over 24-h postcollection was clearly demonstrated in the current *in vitro* trial using the same experimental conditions reported by Virkel *et al.* (1999). The well-known reduction of ABZSO to ABZ in the ruminal fluid (Lanusse *et al.*, 1992) was used as a positive control to test the metabolic viability of the ruminal microflora. The concentration of ABZ formed after the incubation of ABZSO in ruminal fluid collected 23 h before represented 64% of the total products recovered (Fig. 1), which corroborates the high metabolic efficiency of the ruminal content experimentally used in the current trial.

The stability of the ML in the gastrointestinal tract was controversial for several years. Prichard *et al.* (1985) suggested the conversion of IVM in the ruminal fluid to a less potent anthelmintic molecules such as the monosaccaharide and/or aglycone derivatives. However, the *in vitro* stability of a radiolabeled IVM in ruminal fluid was suggested some time later (Andrew & Halley, 1996). As postulated by Ali and Hennessy (1996), the low IVM concentrations measured by Prichard *et al.* (1985) in sheep ruminal fluid could be explained by a strong binding of the drug to particulate ruminal material. Additionally, IVM binding to different plastic and glassware surfaces used for the *in vitro* assays (Tway *et al.*, 1981) may also contribute to explain the *in vivo* results reported by those authors. The incubation assays reported here confirm the high metabolic stability of IVM, not only in sheep ruminal content but also in the acid environment of the abomasal content after 24 h of incubation. The current work also demonstrates the lack of MXD metabolism in both gastrointestinal contents. After 24 h of incubation in ruminal content with demonstrated metabolic capacity, there were no changes in the concentrations of neither

MXD nor IVM compared with those determined in the control samples incubated with metabolically inactive ruminal content (Fig. 2). The hydrolysis of the IVM molecule to monosaccaharide and aglycone derivatives in the acidic conditions of the abomasum was not ruled out by earlier reports (Ali & Hennessy, 1996). An *in vivo* study designed to evaluate the kinetics of doramectin in cannulated sheep, demonstrated that the parent compound represents <50% of total [^{3}H] residues measured in abomasal fluid, which would suggest that some degradation of doramectin in rumen and/or abomasum may have occurred (Hennessy *et al.*, 2000). Although doramectin gastrointestinal metabolism was not evaluated in the current work, both MXD and IVM were metabolically stable in the abomasal content without formation of any metabolic product under the experimental conditions of the trial reported here (Fig. 3). Consequently, a first-pass metabolic effect associated with metabolism by the gastrointestinal microflora would not be relevant for the LM after their oral administration to sheep.

The association of orally-administered anthelmintic compounds with the particulate phase of the gastrointestinal content was earlier described for oxfendazole (Ali & Hennessy, 1995) and IVM (Steel, 1993; Ali & Hennessy, 1996) in sheep fed on a lucerne/wheaten (50:50) ration twice a day. In spite of the fact that sheep in our trial were fed *ad libitum* on a different type of diet (lucerne hay), similar percentages of adsorption to the particulate material of rumen and abomasal contents were determined for MXD and IVM. A slightly higher degree of binding to solid material was observed for MXD (96.9–98.6%) compared with IVM (90.8–95.1%) in ruminal content. Similar binding values (>97%) has been reported for MXD in rumen particulate material, using radiolabeled drug (Hennessy, 2001). The relative distribution between solid and fluid phases of the abomasum was similar for both drugs. Values ranging between 98.9 and 99.6% of the total amount of drug recovered were associated to the particulate phase.

Avermectins and milbemycins differ on some structural and physicochemical properties, which accounts for the differences observed on the pharmacokinetic behavior and persistence of the antiparasitic activity of MXD and IVM. Extended plasma (Lanusse *et al.*, 1997) and target tissues (Lifschitz *et al.*, 1999, 2000) residence times were observed for MXD compared with IVM, likely due to its higher lipophilicity. Furthermore, a greater hepatic metabolism was reported for MXD compared with that observed for most of the avermectin-type compounds (Chiu & Lu, 1989; Afzal *et al.*, 1994; Lanusse *et al.*, 1997). Despite these pharmacokinetic and/or metabolic differences between MXD and IVM in ruminants, both compounds showed a similar metabolic stability in the gastrointestinal contents and an analogous degree of association to the particulate material from both rumen and abomasum (Figs 2 & 3, Tables 2 & 3).

The oral administration of the ML in sheep has been shown to result in lower systemic availability of the parent compounds compared with the subcutaneous treatment (Marriner *et al.*, 1987; Imperiale *et al.*, 2004; Lespine *et al.*, 2004). Overall, the systemic drug exposure expressed as the area under concentration vs. time curve (*AUC*) determined after the subcutaneous administration of MXD and IVM in sheep was between 2.80 and 5.50-fold greater than that determined after oral administration (Imperiale *et al.*, 2004; Lespine *et al.*, 2004). Considering the well established high correlation occurring between IVM and MXD plasma profiles and those achieved at the tissues of parasite location (Lifschitz *et al.*, 1999, 2000), the lower plasma concentrations determined after oral administration of these ML, may explain the lower efficacy against ectoparasites (Benz *et al.*, 1989; McKellar & Benchaoui, 1996) and the shorter duration of the antiparasitic activity against gastrointestinal parasites (Borgsteede, 1993), observed following drench treatments in sheep. Whereas a 10-day post-treatment persistent antiparasitic effect against gastrointestinal worms was collected after subcutaneous administration of IVM, no persistent activity was determined after the oral treatment at the same dosage (Borgsteede, 1993). Thus, if a metabolic degradation in the gastrointestinal tract is not the cause of the low systemic availability observed after oral administration in sheep, the high association of MXD and IVM to the particulate material of digesta appears as a relevant factor modulating the ML absorption process, which would confirm the argument earlier postulated by Ali and Hennessy (1996). In addition, the ML are known substrates of the transmembrane transport protein P-glycoprotein (P-gp) (Schinkel *et al.*, 1996; Pouliot *et al.*, 1997), which may also reduce the amounts of IVM and/or MXD absorbed after oral administration. The presence of P-gp in the intestine constitutes a barrier limiting the absorption of administered orally (Kwei *et al.*, 1999). The higher C_{max} (>83%) determined after the co-administration of IVM with verapamil (a well known P-gp modulator) in sheep compared with the treatment with IVM alone, may reflect an increase in the absorption of the antiparasite compound from the gastrointestinal tract in the presence of the P-gp modulator agent (Molento *et al.*, 2004). To facilitate the overall understanding of the disposition of the ML from the digestive tract, the main factors limiting the systemic

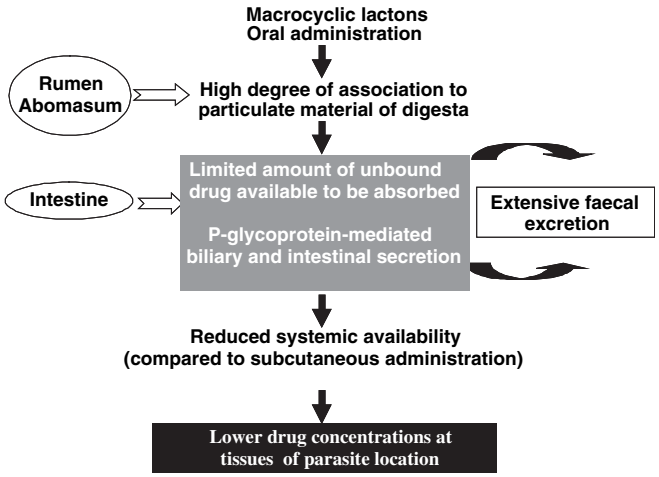

Fig. 4. Schematic representation of the factors affecting the gastrointestinal disposition of the macrocyclic lactones after their oral administration to sheep, which would account to explain their lower systemic availability compared to the parenteral treatment.

availability of these compounds following oral treatment in sheep are summarized in Fig. 4. In conclusion, both compounds were extensively associated to the solid phase of the digestive contents. A high chemical stability without evident metabolism and/or degradation was demonstrated for both MXD and IVM in ruminal and abomasal contents obtained from sheep. Collectively, the findings reported here complement previously available data on the topic and contribute to further understand the kinetic behavior for these extensively used antiparasitic compounds.

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