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Milk Excretion of Ivermectin and Moxidectin in Dairy Sheep: Assessment of Drug Residues during Cheese Elaboration and Ripening Period

FERNANDA A. IMPERIALE,*,† MARGARITA R. BUSETTI,§ VICTOR H. SUÁREZ,§ AND CARLOS E. LANUSSE†

Laboratorio de Farmacología, Núcleo FISFARVET, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Campus Universitario, 7000 Tandil, Argentina, and Unidad Regional en Sanidad Animal, INTA-Estación Experimental Agropecuaria de Anguil, CC 11, 6326, Anguil, La Pampa, Argentina

Ivermectin (IVM) and moxidectin (MXD) are broad-spectrum endectocide antiparasitic drugs extensively used in food-producing animals. The patterns of IVM and MXD excretion in milk were comparatively characterized following their subcutaneous administration (200 μg·kg^{-1} of body weight) to lactating dairy sheep. The relationship between milk excretion and plasma disposition kinetics of both compounds was characterized. A pool of milk collected from all of the animals in each experimental group was used for cheese elaboration. IVM and MXD residual concentrations were assessed during the cheese-making process and ripening period. IVM and MXD concentrations were measured in plasma, milk, and milk product (whey, curd, and cheese) samples using an HPLC-based methodology with fluorescense detection. IVM and MXD were extensively distributed from the bloodstream to the mammary gland, and large quantities, particularly of MXD, were excreted in milk. Residual concentrations of both compounds were recovered in milk up to 30 (IVM) and 35 (MXD) days post-treatment. The total fraction of the administered dose excreted in milk for MXD was significantly higher than that of IVM. During cheese production, the highest residual concentrations of both molecules were measured in the curd. Thirty-four percent of the total drug residue measured in the pooled milk collected from treated sheep was lost during the cheese-making process. The lowest residual concentrations were measured in the whey. IVM and MXD concentrations in the elaborated cheese tended to increase during the ripening period, reaching the highest residual level at 40 days of cheese maturation. The long persistence of milk residual concentrations of MXD and IVM in lactating dairy sheep and the high concentrations found in cheese and other milk-related products should be seriously considered before recommendation of the extralabel use of these antiparasitic drugs in dairy animals.

KEYWORDS: Endectocide drugs; ivermectin; moxidectin; milk residues; cheese; curd; whey; dairy sheep

INTRODUCTION

Sheep milking for dairy production is relatively new in many areas of the world. Sheep dairying has achieved international relevance as an alternative production system. Currently, due to the intensification of milk production in small ruminants, there has been an increase in the frequency of mastitis in dairy sheep, and a main concern with drug residues in milk is based on the use of antibiotic therapy for mastitis treatment (*1*). The intensification of milk production has been also associated with an enhanced vulnerability to parasitic infections. Gastrointestinal parasitism (*2*) and sarcoptic mange infections (*3*) have been demonstrated to cause a reduction in milk yield in lactating dairy animals. Although different management strategies are used to prevent or minimize production losses, the use of antiparasitic drugs is still the main control measure available against parasitism in lactating dairy sheep. The use of strategic anthelmintic treatments during the lactation period has been correlated with a significant enhancement in the volume of milk produced in dairy sheep (*3*−*5*).

The presence of drug residues in milk supplies may have public health implications and is perceived by consumers as undesirable. Moreover, some active substances or their metabolites may produce changes in the milk, which may adversely affect its suitability for processing (cheesemaking). For instance, following the treatment of fasciolosis with the antiparasitic drug niclofolane in dairy cattle, a marked prolongation of the ren-

* Corresponding author (telephone 54-2293-447108; fax 54-2293-422357; e-mail fernanda@vet.unicen.edu.ar).
† Universidad Nacional del Centro de la Provincia de Buenos Aires.
§ Unidad Regional en Sanidad Animal.

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neting and hardening time of the curd was observed with changes in the structure of the final milk-derived product (*6*).

The avermectins and milbemycins are structurally related macrocyclic lactone antiparasitic drugs. Both groups are included under the term "endectocides" due to their antiparasitic activity against endo- and ectoparasites, and they are used worldwide for parasite control in livestock (*7*). The plasma and milk kinetic behavior of ivermectin (IVM) and moxidectin (MXD) has been investigated in lactating (*8*) and nonlactating sheep (*9*, *10*). The extralabel use of some endectocide compounds in dairy animals is well-known, and some recent work refers to the issue (*11*). Only topical formulations of eprinomectin and moxidectin are currently approved for use in dairy cattle without a required withdrawal time in milk. The maximum residue limit (MRL) value for MXD (40 $\mu g \cdot kg^{-1}$) in cow's milk was determined by the European Union and included in Annex I—Council Regulation (EEC) No. 2377/90 (*12*). The MRL value for IVM (10 $\mu g \cdot kg^{-1}$) in cow's milk was determined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (*13*). However, milk MRLs for endectocide compounds in dairy sheep have not been established, although it seems to be highly necessary due to the recent worldwide development of the sheep dairy industry.

The work reported here was designed to assess the comparative pattern of IVM and MXD excretion in lactating dairy sheep following subcutaneous administration and to evaluate the pattern of IVM and MXD residues during milk processing for cheese elaboration. Drug residual concentrations were assessed during the ripening period (40 days) of semihard sheep's milk cheese.

MATERIALS AND METHODS

Experimental Animals, Treatment, and Sampling. Twelve (12) female Pampinta (three-quarters East Friesian and one-quarter Corriedale) dairy sheep weighing between 67 and 92 kg were used. The experimental animals were clinically healthy and in the mid-late lactation period. They were kept under field conditions, grazing on pasture, and had free access to drinking water during the whole experimental period. The health of the animals was closely monitored prior to and throughout the trial. Dairy sheep were milked once a day with a milking machine, and milk production was measured prior to and throughout the trial. The average milk production during the trial was 1039 ± 50.8 mL/day.

The animals were allocated into two groups of six animals. Each animal in each group was treated with either IVM or MXD subcutaneous (sc) immediately after milking was completed. IVM was given by sc injection in the shoulder area (group A) as a 1% injectable solution (Ivomec, Merial Inc., Sao Paulo, Brazil; lot PR 108/97). MXD was given by sc injection in the shoulder area (group B) as a 1% injectable solution (Cydectin, Fort Dodge, Sanidad Animal S.A., Argentina; lot 205). Both formulations are commercially available, and they were given at a dosage of 200 $\mu g \cdot kg^{-1}$ of body weight (bw). Neither pain nor irritation was observed at the site of injection at any time after treatment. Blood samples were taken from the jugular vein in heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) prior to treatment and at 8 and 12 h and 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30, and 35 days post-treatment. Milk samples were collected prior to treatment and at 1, 4, 8, and 12 h and 1, 2, 3, 4, 5, 7, 9, 11, 15, 20, 25, 30, and 35 days post-treatment. At each time point, a milk sample was collected by hand milking before the complete mechanical milking of each sheep. The blood samples were centrifuged at 2000*g* for 20 min, and the recovered plasma was transferred to vials. Milk and plasma samples were frozen at −20 °C until analyzed.

On days 1, 3, 5, 10, 15, and 25 post-treatment the total milk production of each animal of the experimental group ($n = 6$) was collected into the pool and processed according to the cheese elaboration protocol under use in the INTA Anguil Dairy Sheep Experimental Unit (Anguil, Argentina). During the process of cheese elaboration (three cheeses at each sampling point) with milk obtained from each group, the solid (curd) and liquid (whey) samples were collected in vials and frozen at −20 °C until analyzed. The elaborated sheep cheeses were ripened during 40 days. Cheese samples were taken at different ripening times (1, 20, and 40 days). Cheeses were round in shape, 9 cm in diameter and 4−6 cm in height at the center, weighing between 300 and 400 g. Samples were taken from the cheese center, halfway from the center to the ring, and next to the rind portion. The cheese slices (20 g) were minced and mixed and kept as a pooled sample in appropriate vials. Samples were frozen at −20 °C until analyzed.

Analytical Procedures. The extraction procedures and chromatographic conditions to quantify IVM and MXD in fortified and experimental samples (plasma, milk, whey, curd, and cheese) were carried out following modifications of a previously described method (*14*). Detailed information on the chromatographic procedures, including the use of abamectin (ABM) as internal standard to quantify IVM and MXD and the extraction of both analytes from these biological matrices, is given below.

Drug Extraction and Derivatization. Pure reference standards of IVM (97.5% purity), ABM (97.4% purity), and MXD (91.8% purity) were used to validate the HPLC method. Standard solutions of IVM and MXD were prepared by successive dilutions in methanol from the parent stock solution (1 $mg \cdot mL^{-1}$) and stored at 4 °C. The fortified and experimental samples (plasma, milk, whey, curd, and cheese) were added with 100 μL of ABM as internal standard (100 $ng \cdot mL^{-1}$).

Liquid samples (plasma, milk, and whey) were extracted using the following procedure. Acetonitrile (1 mL) and deionized water (0.25 mL) were added to each tube containing 1 mL of liquid sample and mixed for 15 min. After mixing, the batch of tubes containing the liquid samples was centrifuged at 2000*g* for 15 min. The tubes containing the milk sample were placed in an ultrasonic bath for 8 min prior to their centrifugation. The precipitate obtained from the MXD milk samples was re-extracted with 1 mL of acetonitrile as above-described.

Solid samples (curd and cheese) were extracted using the following procedure. Acetonitrile (1 mL) was added to each tube containing 1 g of solid sample. After thorough mixing for 15 min, the batch of tubes containing the sample was put into an ultrasonic bath for 8 min. After that, the batch of tubes (solid sample) was centrifuged at 2000*g* for 15 min. The precipitates obtained from the curd and cheese samples with MXD were re-extracted twice as described above. Water (a volume equal to that of acetonitrile) was incorporated into the supernatant obtained from all of the liquid and solid samples. These samples were then applied to a conditioned Supelclean LC 18 cartridge (Supelco, Bellefonte, PA). After washing with 1 mL of water followed by 1 mL of water/methanol (4:1, v/v), the cartridges were dried for 5 min and the sample was eluted with 1.5 mL of methanol and collected. The eluate was evaporated to dryness under a gentle stream of nitrogen at 60 °C in a water bath, and the dry residue of the elution was dissolved with 100 μL of *N*-methylimidazole (Sigma, St. Louis, MO) solution in acetonitrile (1:1, v/v) and 150 μL of trifluoroacetic anhydride (Sigma) solution in acetonitrile (1:2, v/v) (*15*). After the reaction took place, an aliquot (100 μL) of this solution was injected directly into the chromatographic system.

Chromatographic Conditions. Concentrations of IVM and MXD were analyzed using a Shimadzu LC-10 AS HPLC system (Shimadzu Corp., Kyoto, Japan), which included a fluorescence detector (Shimadzu, RF 551, Shimadzu Corp.) set at an excitation wavelength of 365 nm and an emission wavelength of 475 nm. The mobile phase of acetic acid (0.2% in water, v/v), methanol, and acetonitrile (4:40:56 v/v/v for IVM and 5:40:55 v/v/v for MXD) was pumped at a flow rate of 1.5 $mL \cdot min^{-1}$ through a Selectosil C_{18} (5 μm, 250 × 4.60 mm) reverse phase column (Phenomenex, Torrance, CA) kept in an oven at 30 °C. Compounds were identified by comparison with retention times of pure reference standards. The area under the peaks was calculated using the integrator software (Class LC 10 Software 1.2, Shimadzu Corp.) of the HPLC system.

Method Validation. A complete validation of the analytical procedures for the extraction and quantification of IVM and MXD in each matrix was performed before the analysis of experimental samples from

the specific trial was begun. Calibration lines in the ranges of 0.1−100 ng·mL^{-1} or ng·g^{-1} for IVM and 0.1−500 ng·mL^{-1} or ng·g^{-1} for MXD were plotted using the peak area ratios between each analyte and the internal standard. The data were analyzed for linearity using a linear least-squares regression analysis, and using the Run Test and ANOVA to determine if the data differed from a straight line. The absolute recovery of the drugs under study was measured by comparison of the peak areas from spiked samples with the peak areas resulting from direct injections of standards in methanol. The recoveries of IVM and MXD from sheep plasma, milk, whey, curd, and cheese were obtained at 0.1, 0.25, 1, 5, 10, and 50 ng·mL^{-1} or ng·g^{-1}, using three replicates for each drug concentration. The interday precision of the extraction and chromatographic procedures was evaluated by processing four replicate aliquots of pooled liquid and solid samples containing known amounts of IVM or MXD (2 and 20 ng·mL^{-1} or ng·g^{-1}) on different working days. The accuracy of the analytical method was estimated by the differences between observed and calculated concentrations, and it is expressed as the percentage of relative error (%RE). The accuracy was estimated for all of the matrices under study at the IVM and MXD concentrations of 0.1, 2, and 20 ng·mL^{-1} or ng·g^{-1} with three determinations for each concentration value. The coefficient of variation (CV) for recovery and interday precision of the method were calculated (*16*). The limit of quantification (LOQ) was defined as the lowest concentration that can be measured with acceptable precision (CV < 20%) and accuracy (±20%) (*17*).

Drug Quantification and Pharmacokinetic and Statistical Analyses of the Data. Drug concentrations in experimental samples (liquid and solid) were determined by HPLC calculating the ratio between the areas under the peaks of IVM or MXD and ABM using the CR10 software and interpolating these areas on the calibration lines prepared for each biological matrix. The statistical program (Instat 3.0, Graph Pad Software Inc., San Diego, CA) was used for linear regression analyses and linearity tests.

The milk and plasma concentration versus time curves obtained after treatment in each individual animal was analyzed with the PK Solution 2.0 (Ashland, OH) computer program. Pharmacokinetic variables were determined using a noncompartmental method. The peak concentration (C_{max}) and time to peak concentration (t_{max}) were read from the plotted concentration−time curves in each individual animal. The terminal half-life ($t_{1/2,el}$) was calculated as ln $2/\lambda_z$, where λ_z is the elimination rate constant. The λ_z was determined by performing regression analysis using at least five points of the terminal phase of the concentration−time plot. The areas under the concentration−time curves (AUC) were calculated by the trapezoidal rule (*18*) without extrapolation to infinity. The $AUC_{milk/plasma}$ ratios were estimated for each drug treatment by using the partial AUC values obtained between 0.33 days and the time when the last quantifiable concentration was measured, since the first plasma data point was obtained at 0.33 days. The percentage of total dose excreted in milk for each individual animal was estimated using the values of drug concentration at each sampling time interval and the volume of milk production during the experiment. The IVM and MXD milk and plasma estimated kinetic variables are reported as mean ± standard error of the mean (SEM). The Mann−Whitney test was used to estimate the differences between kinetic parameters obtained in milk and in plasma. Values lower than $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The results of the complete validation of the analytical method developed to measure IVM and MXD in plasma, milk, and milk-derived products are summarized in **Table 1**.

Typical chromatograms of IVM and MXD blank and fortified cheese samples are shown in **Figure 1**. Retention times were 9.4 min for ABM (used as internal standard) and 14.0 min for IVM, respectively (**a**). Retention times were 11.5 min for ABM (used as internal standard) and 8.1 min for MXD, respectively (**b**). The mean correlation coefficients for drug standards prepared in different milk-related matrices ranged between 0.996 and 0.998.

Table 1. Validation Parameters of the HPLC Method Used To Measure Ivermectin and Moxidectin Concentrations in Plasma, Milk, and Milk-Derived Products from Dairy Sheep[a]

matrix	ivermectin	moxidectin
plasma		
LOQ (ng·mL^{-1})	0.1	0.1
accuracy (%)	≤8.00	≤10.0
mean recovery (%)	87.3	82.0
CV (%)	4.82	5.02
interday precision (CV%)	5.52	2.86
milk		
LOQ (ng·mL^{-1})	0.1	0.1
accuracy (%)	≤7.33	≤9.67
mean recovery (%)	89.0	92.0
CV (%)	5.89	3.79
interday precision (CV%)	4.11	4.12
whey		
LOQ (ng·mL^{-1})	0.1	0.25
accuracy (%)	≤6.67	≤3.09
mean recovery (%)	97.1	80.0
CV (%)	2.20	4.47
interday precision (CV%)	4.80	4.80
curd		
LOQ (ng·g^{-1})	0.25	0.25
accuracy (%)	≤10.8	≤10.8
mean recovery (%)	86.0	89.3
CV (%)	3.17	12.2
interday precision (CV%)	5.74	6.62
cheese		
LOQ (ng·g^{-1})	0.25	0.25
accuracy (%)	≤6.40	≤11.8
mean recovery (%)	87	78.4
CV (%)	9.13	10.3
interday precision (CV%)	5.16	10.3

[a] LOQ, limit of quantification defined as the lowest concentration that can be measured with acceptable precision (CV < 20%) and accuracy (±20%) ($n = 4$); accuracy, defined as the closeness of the measured value in plasma, milk, whey, curd, and cheese to the true value (concentrations of 0.1, 2, and 20 ng·mL^{-1} or ng·g^{-1}); mean recovery, mean percentages of IVM and MXD recoveries from fortified plasma, milk, whey, curd, and cheese samples (concentrations range between 0.1 and 50 ng·mL^{-1} or ng·g^{-1}) ($n = 3$); CV, coefficient of variation for the recovery assays; interday precision, CV for the interday precision studies ($n = 4$).

The results obtained in this validation procedure ensure that a reliable method for the detection of IVM and MXD residues in milk-related matrices is available.

Both parent IVM and MXD were detected in plasma between 8 h and 25 (IVM) or 35 (MXD) days post-treatment and in milk between 1 h and 30 (IVM) or 35 (MXD) days after sc administration to dairy sheep. Milk residues increased progressively to reach peak concentrations of 13.05 ng·mL^{-1} (IVM) and 183.5 ng·mL^{-1} (MXD) at 3.80 and 3.75 days post-treatment, respectively. The milk concentration profiles measured after sc administration of IVM and MXD are compared in **Figure 2**. The concentrations of MXD measured in milk were higher than those obtained for IVM at all sampling times. The values of IVM concentration in milk were between 2- and 4-fold higher than those obtained in plasma (**Figure 3a**). The highest milk−plasma partitioning ratios were obtained for MXD (between 14- and 18-fold) in dairy sheep (**Figure 3b**). The mean MXD milk residual concentration measured at 35 days post-treatment was as high as 17.8 ng·mL^{-1}. Similarly, MXD systemic availability measured as plasma AUC value was significantly higher than that obtained for IVM after sc treatment in dairy sheep.

The AUC values for IVM and MXD subcutaneously administered were 2.55-fold (IVM) and 18.5-fold (MXD) higher in milk than those observed in plasma. The large systemic

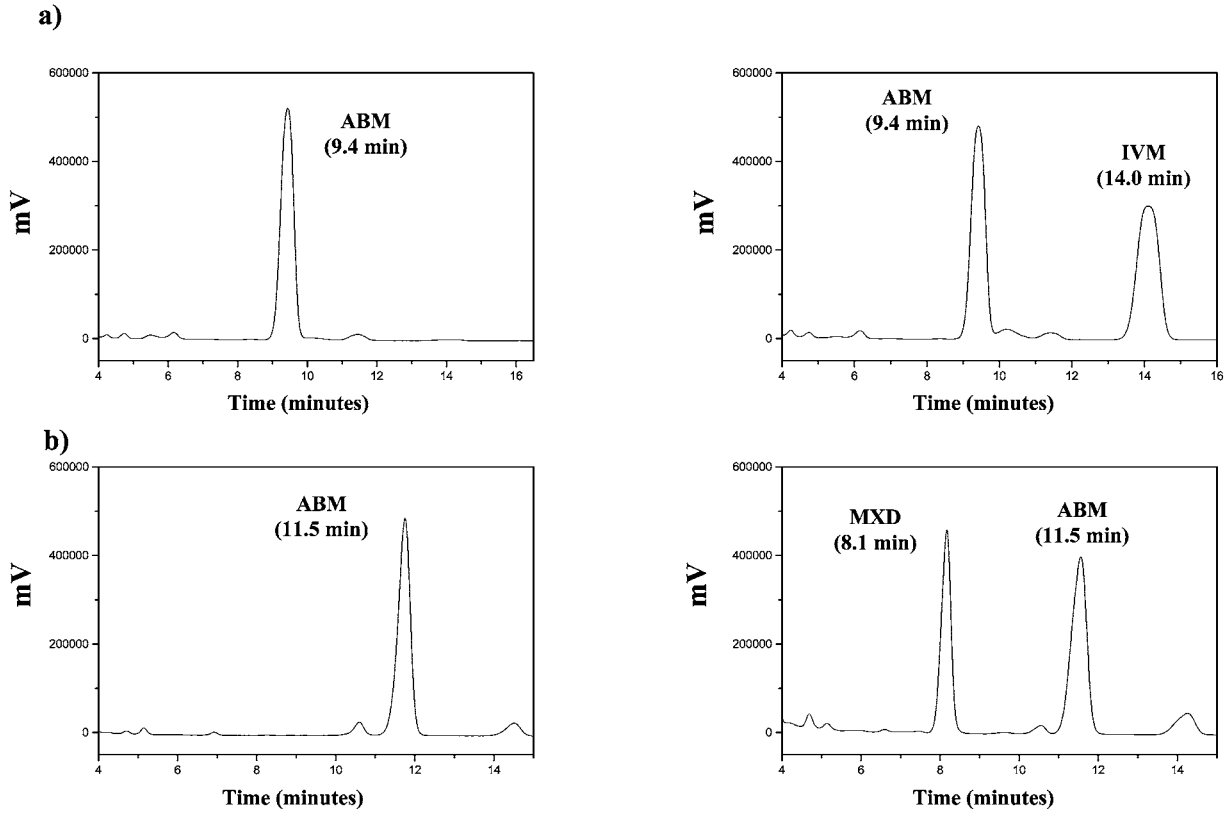

Figure 1. Typical chromatographic identification of ivermectin (IVM) (**a**) and moxidectin (MXD) (**b**) using abamectin (ABM) as internal standard in blank (left panel) and fortified (10 ng·g^{-1}) (right panel) cheese samples.

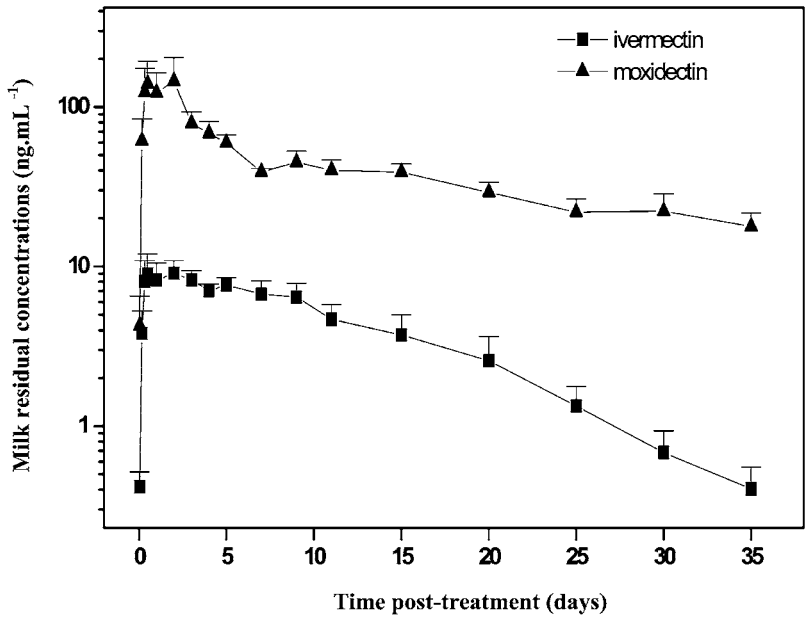

Figure 2. Comparative mean (± SEM) ($n = 6$) residual concentration profiles of ivermectin (IVM) and moxidectin (MXD) in milk obtained after their subcutaneous administration (200 μg·kg^{-1}) to lactating dairy sheep.

availability observed for MXD accounted for the large AUC values measured in milk. The extensive distribution of both analytes from the bloodstream to milk was clearly reflected in the AUC ratios between milk and plasma (**Table 2**). The percentages of IVM and MXD excreted in milk after sc treatment were 0.81 and 8.17% of the total dose, respectively. The enhanced MXD systemic availability compared to that observed for IVM after the sc administration corresponded with the amount of drug excreted in milk. The total percentage of dose recovered in milk for MXD was significantly higher ($P <$ 0.01) than that obtained for IVM. Consistent with the plasma kinetic results, MXD showed the highest percentage of the dose excreted in milk (**Table 2**).

The results reported here indicate that milk excretion is an important route of elimination for lipophilic drugs such as IVM and, particularly, for MXD. A linear correlation between milk IVM concentrations and fat/solid milk contents has beendemonstrated (*19*). A similar correlation may be expected for MXD due to its extremely high lipophilicity, which accounts for its great excretion in milk. Additionally, an increase in milk

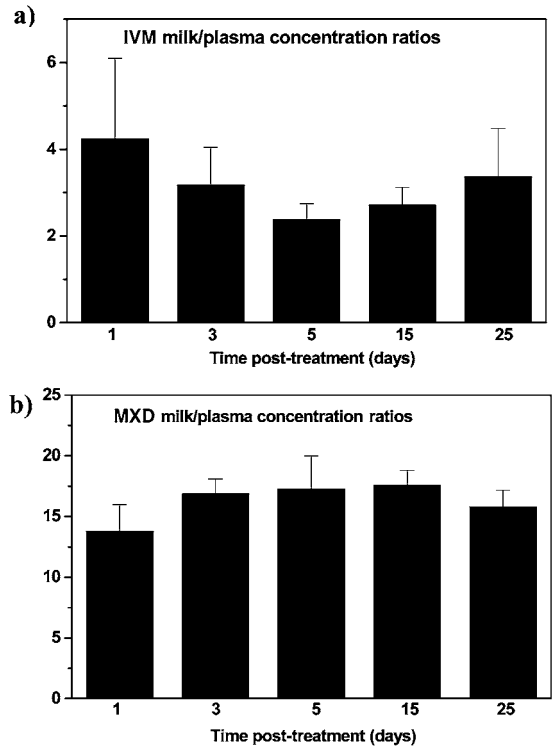

Figure 3. Milk/plasma concentration ratios (mean ± SEM) obtained at different times after subcutaneous administration of ivermectin (IVM) (**a**) and moxidectin (MXD) (**b**) in lactating dairy sheep.

Table 2. Mean (±SEM) Kinetic Variables Describing the Disposition of Ivermectin and Moxidectin from Milk and Plasma Following Its Subcutaneous Administration at 200 $\mu g \cdot kg^{-1}$ in Lactating Dairy Sheep ($n = 6$)[a]

kinetic variable	ivermectin	moxidectin
milk		
t_{max} (days)	3.80 ± 2.28	3.75 ± 2.28
C_{max} (ng·mL^{-1})	13.05 ± 2.12	183.5 ± 58.9 (**)
$t_{1/2,el}$ (days)	4.95 ± 0.69	21.7 ± 4.25 (**)
AUC (ng·day·mL^{-1})	126 ± 22.4	1426 ± 115.4 (**)
ratio $AUC_{milk/plasma}$	2.55 ± 0.38	18.5 ± 1.19 (**)
dose fraction recovered in milk (%)	0.81 ± 0.18	8.17 ± 0.84 (**)
plasma		
t_{max} (days)	2.43 ± 1.07	2.08 ± 1.78
C_{max} (ng·mL^{-1})	5.33 ± 0.74	14.1 ± 3.06 (*)
$t_{1/2,el}$ (days)	3.73 ± 0.35	16.6 ± 2.83 (**)
AUC (ng·day·mL^{-1})	49.4 ± 5.40	79.7 ± 7.48 (**)

[a] Mean kinetic variables obtained for IVM are statistically different at $P < 0.05$ (*) or $P < 0.01$ (**) from those obtained after MXD administration. t_{max}, time to peak concentration; C_{max}, peak milk or plasma concentration; $t_{1/2,el}$, elimination half-life; AUC, area under the concentration vs time curve from 0 h to the last sampling at which the drug was measurable (without extrapolation to infinity); ratio $AUC_{milk/plasma}$, ratio between AUC values obtained in milk and plasma from 0.33 day to the last sampling at which the drug was measurable.

fat content (from 6.7 to 8.5%) was observed during the lactation period in the current trial. The extensive distribution of both antiparasitic drugs from the bloodstream to milk and their high residual concentrations in milk was clearly reflected in the residue values measured in the dairy products under evaluation (cheese and related products).

The manufacture of traditional ripened semihard cheese results in a product in which ~75−80% of the total milk proteins are retained in the cheese curd, whereas 20−25% are lost in the whey. Values within those ranges were obtained in the cheese-making process carried out in the trial reported here, which assured a correct cheese elaboration procedure. IVM and MXD milk residual concentrations were detected at all days of sampling in the milk collected from each experimental group, which was derived for cheese elaboration. The concentration values of parent MXD in pooled milk were between 7.6- and 22-fold higher than those obtained for IVM at the different sampling days (1, 3, 5, 10, 15, and 25 days). During milk processing a high proportion of parent IVM and MXD was found in the curd, which agrees with the higher fat content of this milk-derived product. The ratio between drug concentrations measured in curd and milk ranged from 2.4 ± 0.17 (MXD) to 2.8 ± 0.23 (IVM). However, a lower proportion of these lipophilic analytes ended in the whey (whey/milk ratio = 0.16 ± 0.01) due to the high water content of this milk subproduct (**Figure 4**). Unfortunately, the low number of determinations did not permit to statistical validation of the differences observed among the various milk-derived products. However, a well-defined tendency was observed at all of the sampling collection times as can be visualized in **Figure 4**.

Similarly, the concentration of IVM residue in mozzarella cheese was 4-fold higher than that in buffalo milk (*20*); however, contradictory results were obtained with less lipophilic compounds such as albendazole sulfoxide and sulfone, metabolites of the anthelmintic albendazole, where a high proportion of both metabolites was found in whey, owing to the polarity of these compounds. This high concentration of drug in whey is also undesirable because this dairy product is used as a farm animal feed and as an ingredient in human dietetic food (*21*).

From the total amount of drug residue found in the pooled milk, 62 (IVM) and 66% (MXD) remained in the elaborated cheese after 1 day of ripening. The loss of drug during the cheese-making procedure (34%) was similar to that previously reported for IVM (*16*). Between 13 (IVM) and 16% (MXD) of the drug residue loss during cheese elaboration was recovered in whey. The remaining drug residues could be lost during the cheese-making procedure. However, it has been shown that neither thermal treatment (*22*, *23*) nor lactic acid fermentation (*19*) modified IVM stability.

Concentrations of IVM and MXD gradually increased during the ripening period (40 days) of the semihard cheese elaborated in this trial. At different days of sampling (days 1, 20, and 40) during the ripening period, the proportion of both analytes gradually increased (between 2.9- and 3.4-fold) compared to their residual concentrations in the milk used to elaborate that cheese. The highest residual concentrations for IVM and MXD were obtained after 40 days of cheese maturation (**Table 3**). A loss of weight of the final product due to water loss (between 22 and 25%) from cheese during the ripening period was observed. This water loss and the enhancement of solid contents in the ripened cheese may have accounted for the high concentrations of IVM and MXD found in cheese at 40 days of maturation. A linear correlation between the percentages of water loss and the IVM and MXD concentrations measured in cheese during maturation was observed ($r > 0.90$).

Although high residual concentrations for both IVM and MXD were found in milk, the cheese-making process (renneting time, rate of firming, and consistency of the curd) was unaffected. This is in accordance with results previously reported, where the high concentrations of both analytes present in bovine milk did not affect the acid fermentation process in vitro (*24*). Hence, the extralabel use of IVM and MXD in dairy sheep does not seem to be a risk for milk processing. However, the impact of endectocide drug residues in milk and its derived

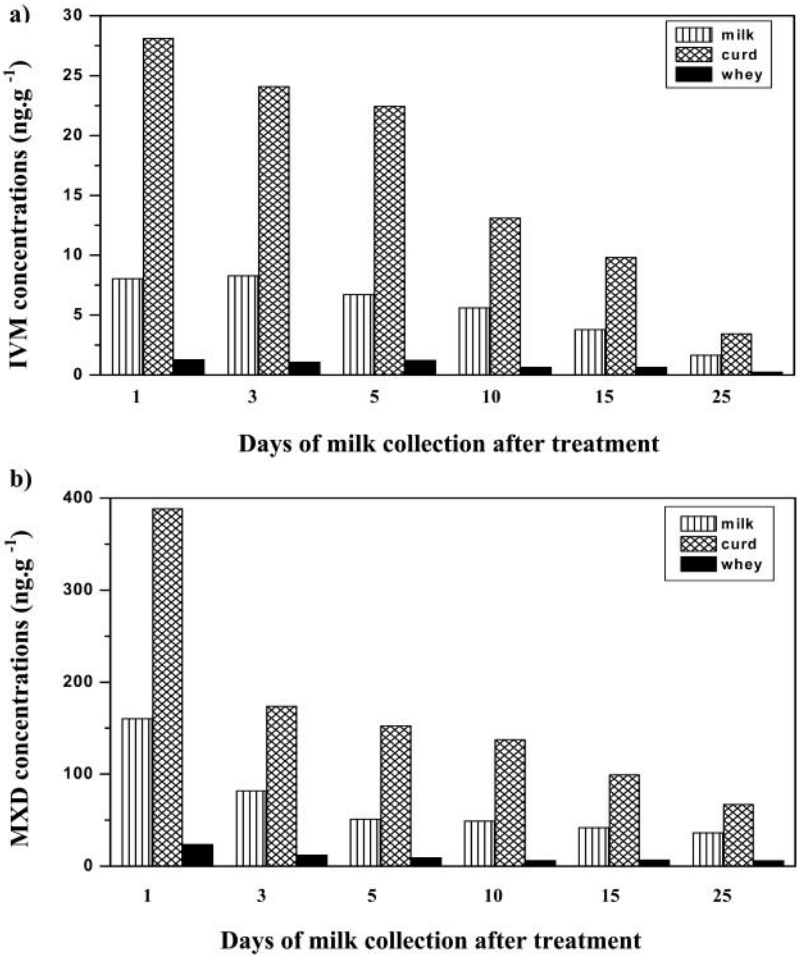

Figure 4. Relative distribution of ivermectin (IVM) (**a**) and moxidectin (MXD) (**b**) residues in milk, curd, and whey during the cheese-making process. Values express mean drug concentrations ($ng \cdot g^{-1}$) obtained in milk and derived products during cheese elaboration using milk collected at different times post-treatment.

Table 3. Ivermectin and Moxidectin Residual Concentrations in Pooled Milk Collected from Dairy Sheep ($n = 6$) at Different Days after Treatment (Subcutaneous Administrations at 200 $\mu g \cdot kg^{-1}$) and in Ripened Cheese (40 Days) Elaborated with the Same Pooled Milk

	IVM concn ($ng \cdot g^{-1}$)		MXD concn ($ng \cdot g^{-1}$)	
days of milk collection after treatment	pooled milk	ripened cheese	pooled milk	ripened cheese
1	8.07	28.7	161	516
3	8.32	31.9	82.2	298
5	6.74	30.2	51.5	243
10	5.63	23.2	49.6	170
15	3.82	8.40	42.4	146
25	1.69	2.90	36.9	67.0
mean $ratio_{cheese/milk}$	3.3		3.4	

dairy products on consumers' health is yet unknown. However, when the acceptable dietary intake (ADI) value for IVM and MXD determined by the European Union (Annex I—Council Regulation) (EEC) and the estimated daily cheese intake determined by the EU Scientific Co-operation Programme (SCOOP) (*25*) are considered, the consumption of IVM and MXD in cheese could reach 3 (IVM) and 17% (MXD) of the established ADI, assuming that the consumed cheese was elaborated with milk containing the observed maximum residual concentrations. The scientific evidences shown here indicate that IVM and MXD residues in cheese are 3-fold higher than those measured in the milk used for its elaboration. These should be considered to establish legislation addressed to protect consumer's safety. At the present time it is imperative to estimate a withdrawal period after extralabel drug use in dairy sheep. The characterization of the relationship between milk residue disposition of IVM and MXD in dairy sheep and residual concentrations in dairy products (cheese) reported here may be useful to determine the maximum residue level acceptable in cheese and other milk-derived products for these widely used endectocide compounds.

ABBREVIATIONS USED

IVM, ivermectin; MXD, moxidectin; ABM, abamectin; sc, subcutaneous; HPLC, high-performance liquid chromatography; SEM, standard error of the mean; CV, coefficient of variation; LOQ, limit of quantification; JECFA, Joint FAO/WHO Expert Committee on Food Additives; ADI, acceptable dietary intake.

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