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Research Note

The Fate of Eprinomectin in Goat Milk and Cheeses with Different Ripening Times Following Pour-On Administration

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ABSTRACT

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The distribution of eprinomectin in goat milk and cheeses (cacioricotta, caciotta, caprilisco) with different ripening times following a pour-on administration at a single dose rate (500 µg/kg of body weight) and a double dose rate (1,000 µg/kg of body weight) to goats with naturally occurring infections of gastrointestinal nematodes was studied. Milk residues of eprinomectin reached a maximum of 0.55 ± 0.18 µg/kg and 1.70 ± 0.31 µg/kg at the single and double doses, respectively. The drug concentrations decreased progressively until the fifth day after treatment, when they were less than the detection limit at both dose rates. The eprinomectin levels measured in all cheese types (both treatments) were higher than those recovered in milk at all the sampling times. In caciotta cheeses, the eprinomectin residues levels were constantly higher than other cheeses. With the exception of cheeses made with milk the first day after treatment, eprinomectin concentrations were nearly constant up to the fourth day then decreased by the fifth and sixth days after treatment. In all cases, at both the single and double dosages, the maximum level of eprinomectin residues in goat milk and cheeses remained below the maximum residual level of 20 µg/liter permitted for lactating cattle.

Few anthelmintics have been cleared for use in lactating animals where the milk is destined for human consumption (5). Eprinomectin (4'-[epiacetyl amino]-4'-deoxy-avermectin) is a member of the avermectin class of compounds marketed as a pour-on endectocide for use in cattle at a dose rate of 500 µg/kg of body weight (20, 21). It has been found highly effective in cattle against adult and immature nematodes, including the inhibited larval stages of *Ostertagia ostertagi* (8, 13, 18, 21, 22, 23) and in sheep against gastrointestinal nematodes of naturally infected animals (9, 16). In addition, recent results (10) show that the activity of eprinomectin applied topically to goats with naturally occurring infection of the gastrointestinal nematodes *Teladorsagia circumcincta*, *Haemonchus contortus*, *Trichostrongylus colubriformis*, and *Oesophagostomum venulosum* is significantly higher at the double dose rate of 1,000 µg K⁻¹g than at the single dose rate of 500 µg/kg. Eprinomectin is known to be subjected to minimal partitioning into milk of lactating cattle. The resulting negligible milk residues make its use possible in lactating dairy cattle (21). As far as goats are concerned, the pharmacokinetic profiles of eprinomectin have been obtained following topical (1, 12) and subcutaneous administration (15).

The aims of the present study were to investigate the

fate of eprinomectin in goat milk and to evaluate to what extent eprinomectin levels may be present in cheeses with different ripening times following a pour-on administration at the single and double dose rates to pastured goats with naturally occurring infection of gastrointestinal nematodes.

MATERIALS AND METHODS

Study animals. For the field trial, a total of 50 female Siriana lactating goats, aged between 2 and 4 years and weighing between 40 and 50 kg, were used. The animals were kept under field conditions during the entire experimental period. The pasture (60 ha) was permanent and hilly, and the predominant forage species were *Galium* spp., *Cicorium* spp., *Dactylis glomerata*, and *Lolium perenne*. No moving of animals was performed immediately before or during the study. No supplementary feeding was given, and water was supplied in a drainage ditch that surrounded the pasture.

The goats, according their body weight, were divided in two treatment groups of 25 animals each. One group was treated with an eprinomectin single dose (E1 group) the other with an eprinomectin double dose (E2 group). Each treatment group was maintained on a separate but comparable pasture, thus avoiding physical contact among goats of different treatment groups.

Treatments. The treatments were performed on 10 June 2003 (day 0). Eprinomectin (Eprinex, Merial- Italia) was given in a pour-on administration as a 0.5% solution at the dose rate of 500 µg/kg of body weight (1 ml/10 kg of body weight) to animals in the E1 group and at the dose rate of 1,000 µg/kg of body weight (2 ml/10 kg of body weight) to animals in the E2 group. The dosages

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were calculated on the basis of the animal body weight on day 0. The formulation was applied topically with a syringe along the midline of the back from the withers to the tail head. All animals were dry at the time of the treatments, and rain did not occur until 42 days after treatment. All treated animals were intermittently observed for adverse reactions for 4 h after treatment and then daily until the end of the trial. At these observation times, the application sites were visually examined for any significant hair and/or skin changes. Dairy goats were milked twice a day, and the milk production measured before and throughout the trial ranged from 500 to 1,200 ml/day.

Milk and cheese samples. Samples of milk were collected from each animal before treatments and at 24, 48, 72, 96, 120, and 144 h after treatment. The total daily production was collected and kept at 4°C. An aliquot was stored at -18°C until analysis. The remaining part of the milk was processed to produce three different kinds of cheeses named cacioricotta, caciotta, and caprilisco, with ripening periods of 20, 40, and 80 days, respectively. The summarized processing techniques of each cheese are described herein and in the article by Rubino et al. (19). For cacioricotta, raw milk was heated to 85 to 90°C and then cooled to 38°C, at which point a paste of kid rennet was added. On coagulation, the curd was broken with a wooden stick to granules the size of rice grains. Next, the curd was gathered on the bottom of the vat, put into small wicker baskets, and set to dry on a tilted wooden table. Salt was rubbed on the cheese once a day for 3 days. After 7 days, the forms were dipped into fresh water for 24 h, dried, and aged 20 days on wooden tables. The moisture content was 59%. For caciotta, raw milk was heated to 36 to 38°C and a paste of kid rennet added. On coagulation, the curd was broken with a wooden stick to granules the size of nuts. After this, the techniques are similar to those described for cacioricotta, except for the ripening time of 40 days. The moisture content of caciotta was 30%. For caprilisco, the processing technique was similar to that of cacioricotta and caciotta cheeses with the following differences: the use of liquid kid rennet and the partial cooking of the curd to 40 to 42°C, the curd was broken to granules of the size of corn kernels, and the ripening time was 80 days. The moisture content of caprilisco was 30%. For each cheese type and for each sampling time, 6 samples were analyzed in duplicate; thus, the total number of samples was 126. Results are means and standard deviations.

Analytical procedures. Water, methanol, and acetonitrile (high-performance liquid chromatography grade) were obtained from Carlo Erba (Milan, Italy). Trifluoroacetic anhydride and N-methylimidazole of analytical grade were purchased from Aldrich (St. Louis, Mo.). The **Solid Phase Extraction (SPE)** columns were C8 cartridges Bond Elut, 500 mg (Carlo Erba).

Drug extraction and derivatization. The analytical standard of eprinomectin was obtained from Merck and Co. (Rahaway, N.J.). A standard stock solution of eprinomectin (1 mg/ml) was prepared in methanol and was stored at -20°C. A working standard solution (0.2 µg/ml) was prepared from the standard stock solution on the day of use. Milk (5 ml) and cheese (2 g) samples were extracted with acetonitrile (15 ml) and cleaned-up by SPE on C8 cartridges preconditioned with acetonitrile, water, and triethylamine as previously described (4). Residues were eluted with acetonitrile. The eluate was collected in a clean test tube and evaporated to dryness under nitrogen at 60°C.

A 225-µl portion of methylimidazole-acetonitrile (2 + 7, vol/vol) was added to the test tube, which was vortexed for 2 min. Successively, a similar portion (225 µl) of trifluoroacetic anhy-

dride-acetonitrile (2 + 7, vol/vol) was added, and the tube was vortexed for 1 min. Finally, a 50-µl portion of glacial acetic acid was added, and the tube was vortexed for 1 min (11). The derivatized sample extract was filtered through a 0.45 µm filter into a vial and injected (100 µl) into the high-performance liquid chromatography system.

Chromatographic conditions. Milk and cheese residues of eprinomectin were analyzed using a high-performance liquid chromatography system that consisted of a Jasco (Tokyo, Japan) instrument equipped with a ternary pump (PU-90), an autosampler (AS-2055 PLUS), and a fluorescence detector (FP 210). **The separation was performed on a stainless-steel analytical Novapak C 18 HPLC column (3.9 mm) (Waters, Massachusetts, USA).** The column temperature was maintained at 37°C. The mobile phase, which consisted of methanol plus 1% triethylamine in acetonitrile plus 1% phosphoric acid in water (61 + 30 + 9.0, vol/vol/vol), was pumped at 1 ml/min. Excitation and emission wavelengths were 365 and 470 nm, respectively.

Method validation. A standard mixture was analyzed at 2.5, 12.5, 25, 50, and 100 µg/liter to check the linearity. These mixtures were obtained from a working solution of 0.2 µg/ml and were evaporated and derivatized as described herein. A calibration curve was constructed by plotting peak area (y) versus standard concentration (x). Recovery was measured by comparison of the peak areas from spiked milk with the peak areas that result from direct injections of standards in methanol.

Five-gram portions of negative control goat milk were weighed in 50-ml extraction tubes to determine accuracy. A 50-µl portion of a 0.2-, 0.3-, and 0.4-µg/ml standard solution was added to give fortification levels of 2, 3, and 4 µg/kg, respectively. Fortified samples were extracted, cleaned, and determined as described earlier. The precision of the method, expressed as within-day repeatability, was determined by analyzing samples spiked with eprinomectin standard solution. The specificity of the method was confirmed by analysis of blank samples. No interfering peak eluted at the same retention time of eprinomectin. The detection limit and the limit of quantification calculated according to AOAC International guidelines (3) criteria were 0.045 µg/liter and 0.080 µg/kg, respectively.

RESULTS AND DISCUSSION

During the study, the eprinomectin formulation (at the dose rate of both 500 and 1,000 µg/kg of body weight) was well tolerated by all the treated animals with no adverse reactions to the treatments. The analytical method used to extract, derivatize, and quantify the milk and cheese eprinomectin concentrations was shown to be adequate. The regression analysis of the data obtained from the standard solutions showed that the eprinomectin responses were linear over the range of the concentrations examined (1 to 100 µg/liter). The correlation coefficient (r^2) obtained exceeded 0.99, demonstrating a good linearity. The overall eprinomectin recoveries were 88.2 and 89.4% for milk and cheese, respectively. Intra-assay variations ranged from 9.0 (milk) to 10.0% (cheese).

Time concentration profiles after pour-on eprinomectin administrations of 500 and 1,000 µg/kg of body weight in goat milk and dairy products are shown in Figures 1 through 4. Milk residues of eprinomectin reached maximum peak levels of 0.55 ± 0.18 µg/kg and 1.70 ± 0.31 µg/kg after application of the dosage suggested from the

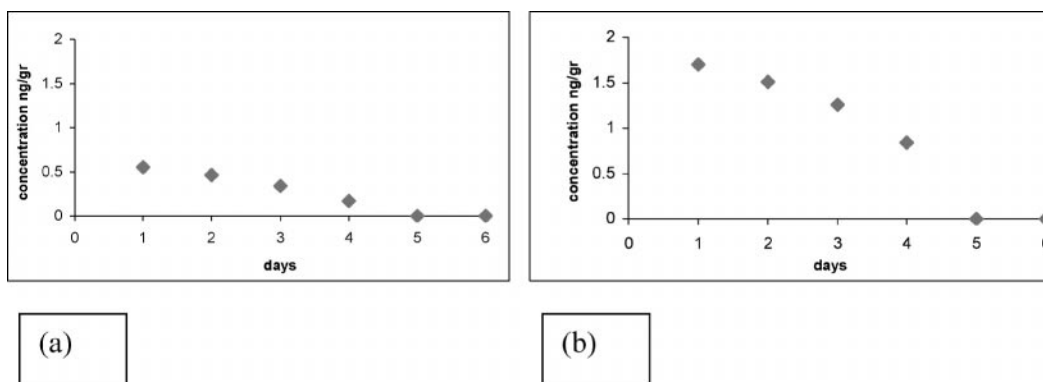


FIGURE 1. Concentration-time profile of eprinomectin in the milk of goats following pour-on administration of the drug at 500 $\mu\text{g}/\text{kg}$ of body weight (a) and 1,000 $\mu\text{g}/\text{kg}$ of body weight (b).

manufacturer for cattle and of the double dosage, respectively. The drug concentrations decreased progressively until the fourth day after treatment, when they were less than the detection limit at both dose rates (Fig. 1).

The eprinomectin levels measured in all cheese types (both treatments) were higher than those recovered in milk at all sampling times. The concentrations of drug residues were similar in all cheeses made with milk sampled on the first day after treatment. Concentrations ranged from 9.71 ± 0.9 to 10.9 ± 1.0 ng/g at the dose rate of 500 $\mu\text{g}/\text{kg}$ and 19.21 ± 10.8 to 20.75 ± 1.6 $\mu\text{ng}/\text{g}$ at the dose rate of 1,000 $\mu\text{g}/\text{kg}$ for cacioricotta (20-day ripening period) and caciotta (40-day ripening period), respectively. These concentrations were the eprinomectin maximum peak levels. From the second day on, in cacioricotta and caprilisco the residue concentrations decreased progressively to 2.65 ± 0.4 and 0.53 ± 0.07 ng/g for the single dose and 6.37 ± 0.9 and 1.2 ± 0.3 ng/g for the double dose, respectively (Figs. 2 and 4). In caciotta cheeses, the eprinomectin residue levels were consistently higher than those in the other cheeses, and, in both doses, they were constant up to the fourth day and then decreased on the fifth and sixth days following treatment (Fig. 3).

Previous studies have reported the pharmacokinetics of eprinomectin in dairy cattle (2) and in lactating and non-lactating goats (1, 12). Eprinomectin concentrations detected in milk following a pour-on administration at the lower dose were different from those observed in dairy cattle (2)

but similar to those obtained in buffalo (unpublished data) and goats (12). In bovine milk, the eprinomectin concentration was 0.89 ± 0.4 ng/ml at the first sampling period, and the maximum concentration was 5.14 ± 2.5 ng/ml. In the present experiment, the eprinomectin peak concentration was 10-fold lower (0.55 ng/ml), and residues were not detected after day 4 after treatment.

The residues detected after administration of eprinomectin at the dose rate of 1,000 $\mu\text{g}/\text{kg}$ of body weight were similar to those reported by Dupuy et al. (12) following administration of the same pour-on dose. The milk partitioning process was controlled by several factors, including physicochemical properties of the drug, membrane interactions, and animal species.

In lactating animals, probably due to decrease of body fat during the lactation (14), the drug is eliminated more rapidly. Previous studies have shown that, in lactating goats at a dose of 0.5 mg/kg of body weight, the mean residence time, a parameter that integrates all the steps of the drug fate in the body, was lower (2.6 days) than in lactating cows (3.99 days) (1, 12), thus affecting drug efficacy.

Eprinomectin residues in cheeses seem to show a different behavior in relation to many factors. As observed by other authors, this compound, because of its lipid solubility, increases with the decrease of the water content. Studies performed in buffalo and cow's milk (6) show that eprinomectin residues are found primarily in the curd and the concentration factor is inversely related to the manufactur-

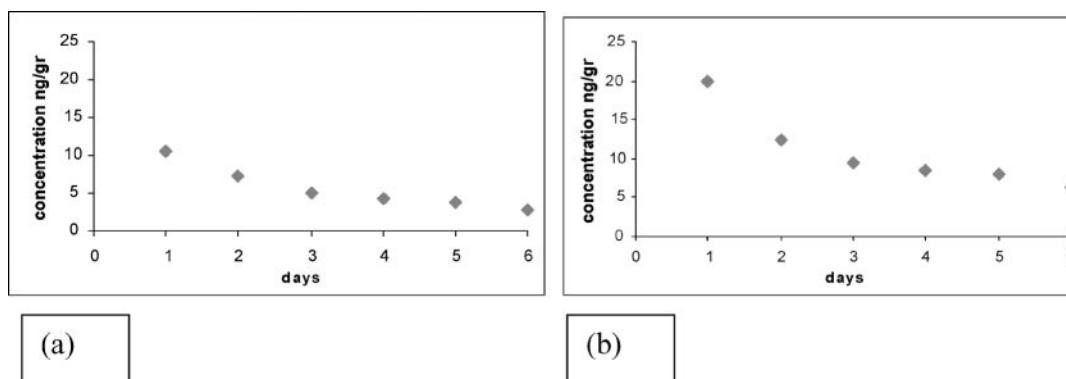


FIGURE 2. Concentration-time profile of eprinomectin in the cacioricotta cheese (20-day ripening period) following pour-on administration of the drug at 500 $\mu\text{g}/\text{kg}$ of body weight (a) and 1,000 $\mu\text{g}/\text{kg}$ of body weight (b).

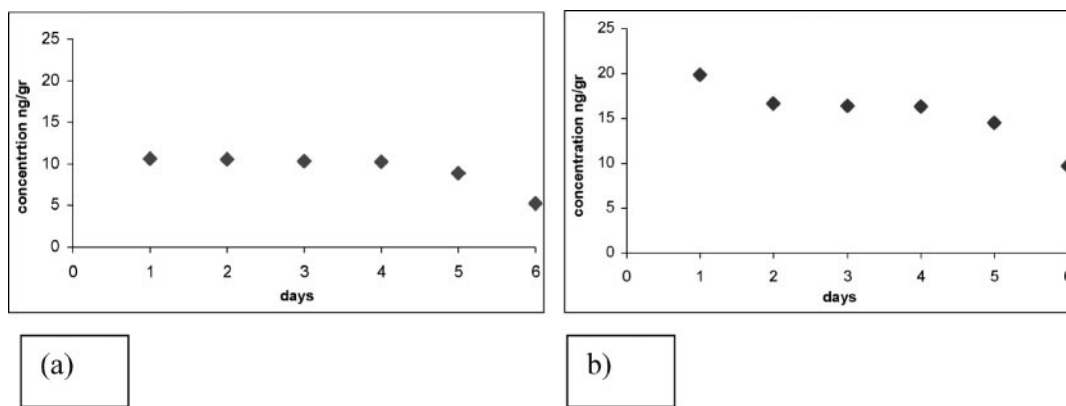


FIGURE 3. Concentration-time profile of eprinomectin in the caciotta cheese (40-day ripening period) following pour-on administration of the drug at 500 µg/kg of body weight (a) and 1,000 µg/kg of body weight (b).

ing yield. That is the reason for higher concentrations in cheeses than milk. In cheeses made from milk sampled after 6 days after treatment with eprinomectin, concentrations were nondetectable at both doses used.

The data obtained in this study show that in caciotta and caciotta cheeses, with the exception of the first day after treatment, eprinomectin concentrations increase as the activity water and the yield decrease. In contrast, the eprinomectin levels in caprilisco, the cheese with the longest aging time and with a manufacturing yield of 11%, were similar or lower than the other two types of cheeses. A possible explanation for this observation could be the long ripening time (almost 80 days) during which eprinomectin may have undergone a degradation process.

From a parasitological point of view, recent results of a field trial conducted on the same goats of the present study demonstrated that the activity of eprinomectin applied topically to goats with naturally occurring infection of *T. circumcincta*, *H. contortus*, *T. colubriformis*, and *O. venulosum* was significantly higher at the dose rate of 1,000 µg/kg of body weight than at the dose rate of 500 µg/kg of body weight (10). In fact, 1 week after the treatment, the control group goats had a doubling in egg counts, which remained unchanged during the trial, whereas the single dose group had lowered egg counts by 90% and the double

dose by 99%. These results are similar to those obtained by Chartier et al. (5) in goats experimentally infected by gastrointestinal nematodes.

The findings of the present study show that the use of eprinomectin at the dose rate of 1,000 µg/kg of body weight, the dose with the higher antiparasitic activity, did not pose a residue problem for goat milk and dairy products whatever their ripening time. In fact, the peak levels of the milk and cheeses after administration of eprinomectin at this dosage were always below the maximum residual level of 20 µg/liter fixed by European Union (EU) for bovine milk (13). For reason and because of improved efficacy, a double dose of eprinomectin for lactating goats is recommended. This would also be of great interest to dairy goat farmers, since the use of anthelmintics at dosages lower than those required for high efficacy may cause a build-up of anthelmintic resistance (10).

In conclusion, the findings of the present study demonstrate that there is no hazard arising from ingestion of milk or cheeses from goats treated with eprinomectin. This has important implications in terms of public health and trade of goat milk and milk products, considering the increasing of human consumption of goat milk and cheeses and the fact that goat milk has biochemical properties theoretically favoring its nutritional value for humans (17).

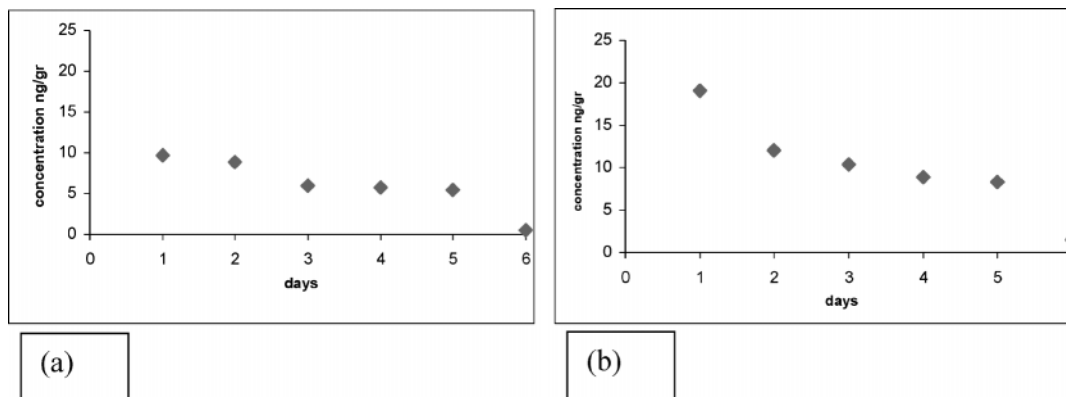


FIGURE 4. Concentration-time profile of eprinomectin in the caprilisco cheese (80-day ripening period) following pour-on administration of the drug at 500 µg/kg of body weight (a) and 1,000 µg/kg of body weight (b).

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