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Anthelmintic residues in goat and sheep dairy products

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Abstract

A multiresidue method (LC-MS/MS) for determination of wide range of anthelmintics was developed. The method covered benzimidazoles: albendazole (and metabolites), cambendazole, fenbendazol (and metabolites), flubendazole (and metabolites), mebendazole (and metabolites), oxibendazole, thiabendazole (and metabolites), triclabendazole (and metabolites); macrocyclic lactones: abamectin, doramectin, emamectin, eprinomectin, ivermectin, moxidectin; salicylanilides: closantel, ioxynil, nitroxynil, oxyclosamide, niclosamide, rafoxanid and others: clorsulon, derquantel, imidocarb, monepantel (and metabolites), morantel, praziquantel, and pyrantel. The method was used to examine the potential presence of anthelmintics in goat and sheep milk and dairy products from the Polish market. A total of 120 samples of milk, yoghurt, cottage cheese, cream cheese, and curd were analysed. None of the samples were found positive above CCα (1-10 µg/kg) except for one cottage cheese in which traces of albendazole sulfone were detected (5.2 ug/kg) and confirmed. The results of the study showed negligible anthelmintic residues in the goat and sheep milk and dairy products and confirm their good quality.

Keywords: anthelmintics, albendazole, milk, dairy products, Poland.

Introduction

In recent times, a growing interest in goat and sheep milk and dairy products has been observed. This is due to the unique properties of this type of food. Compared with cow milk, goat milk has a better ratio of amino acids preferred from the standpoint of infant nutrition. Goat milk proteins are faster and more easily digested. Goat milk casein does not contain αS1 fraction, which enables children with celiac disease to consume the milk without the danger of allergic disorders. These benefits have been noticed by the experts of the European Agency Food Safety Authority (EFSA), who gave a positive opinion on the use of protein from goat milk in the infant diet (6).

The demand for these products results in intensive breeding and enforces the appropriate care for the animals' welfare. The specificity of goat and sheep rearing in Poland (grazing in open areas) entails the exposure of the animals to parasitic infections. Since the invasion of parasites causes substantial economic losses, antiparasitic drugs are frequently used in veterinary medicine. The use of such drugs in farm animals is associated with the presence of their residues in tissues and milk, which may have an adverse effect on the health of consumers.

The fight against parasitic infections in small ruminants is a challenge for both breeders and veterinarians. A growing concern lies in the increase in drug-resistant parasites (7), which is caused by the shortage of registered medicines for sheep and goats, the lack of new drugs, as well as errors in the use of these drugs by breeders. In addition, the use of drugs which are not approved for small ruminants, neglecting the rules of good agricultural practice, may be also observed. Due to the small size of regional production of sheep and goat milk only single milk samples are tested in the national residue control programme, while dairy products (*e.g.* cheese, yogurt, etc.) are not covered by the above control.

The results of the previous studies showed a different depletion of antiparasitic drug residues in sheep and goat milk. Ivermectin and moxidectin (belonging to the avermectins), used as antiparasitic medication in sheep, were detected in milk 25 and 35 d after treatment, respectively (11), and doramectin 30 d after administration (12). Eprinomectin (avermectin) passes into milk to a very limited extent, and quickly fades, so there is no need to establish withdrawal period (13). The authors pointed to the persistence of antiparasitic drug residues during milk processing and the production of dairy products, including cheeses (8). Levamisole and oxyclozanide were stable during technological processes and their concentration in cheeses was 3 and 10 times higher, respectively, than in milk (17). Similar results were obtained when the transition of eprinomectin to various types of goat cheese was investigated (2).

Due to the fact that drug residues in food may pose a potential threat to consumers' health, the European Community has established a law requiring a systematic and uniform control of all the above-mentioned groups of veterinary drugs in food in all member countries. The results of this control in the EU countries have also brought interesting data on contamination of food by antiparasitic drugs. In 2011, in all EU countries, 0.21% non-compliant tissues samples of sheep and goats and 0.18% of milk samples containing residues of antiparasitic drugs above the permissible limit have been found. (5). These data indicate that food from sheep and goats may contain residues of antiparasitic drugs at a level similar to that of antibacterial agents residues.

The aim of this study was to verify the presence of anthelmintic drug residues in the samples of goat and sheep milk and dairy products collected in Poland.

Material and Methods

Samples and sampling. The samples were purchased from the local and domestic manufacturers (one type of sample from one manufacturer). Goat milk (15 samples of pasteurised milk) and dairy products: yoghurt (10 samples), cottage cheese (25 samples), cream cheese (30 samples), and curd (25 samples) were purchased in the Mazowieckie, Małopolskie, Wielkopolskie, Lubelskie, and Podkarpackie provinces. Twenty samples of sheep cheese were purchased in the Podkarpackie provinces. The samples were stored at 2-10°C during the transport to the laboratory and during storage before analysis.

Sample preparation. The samples were analysed according to a method described by Kinsella *et al.* (15) and modified in our laboratory (14). In brief, the samples (2 g of milk and cheese) were spiked with internal standard solution and extracted with 10 mL of acetonitrile in the presence of 2 g of ammonium acetate. The samples were centrifuged (4500 rpm, -10°C) and 1 mL of each extract was transferred to 2 mL Eppendorf tube with 150 mg of magnesium sulphate and 50 mg of C18 bulk-sorbent. The tubes were vortex-mixed and centrifuged (14800 rpm). Then, 0.5 mL of extract was transferred to autosampler vial and 0.050 mL was injected on LC-MS/MS system.

LC-MS/MS determination. The method allows to determine the following anthelmintic residues in milk and dairy products: benzimidazoles: albendazole (and metabolites), cambendazole, fenbendazol (and metabolites), flubendazole (and metabolites), mebendazole (and metabolites), oxibendazole, thiabendazole (and metabolites), triclabendazole (and metabolites); macrocyclic lactones: abamectin, doramectin, emamectin, eprinomectin, ivermectin, moxidectin; salicylanilides: closantel, ioxynil, nitroxynil, oxyclosamide, niclosamide, rafoxanid; and others: clorsulon, derquantel, imidocarb, monepantel (and metabolites), morantel, praziquantel, and pyrantel.

Anthelmintics were separated on Agilent Zorbax C18 column (50 × 4.6 mm, 1.8 μm) with 20 min gradient of acetonitrile and 0.025 M ammonium acetate (pH 5.0) and analysed by ABSciex API 4000 mass spectrometer with ionisation mode switching. The method performance was positively validated according to the requirements described in the Commission Decision 2002/657/EC (4): linearity, precision (repeatability and within-laboratory reproducibility), recovery, decision limit (CCα), and detection capability (CCβ) were calculated. The method allowed to determine anthelmintic residues above 1-10 μg/kg, depending on the analyte.

Results

A total of 120 milk and dairy product samples were tested for forty anthelmintic residues. None of the samples were found positive above CCα (1-10 μg/kg) except for one goat cottage cheese in which traces of albendazole sulfone were detected (Fig. 1). The presence of the residues was confirmed by the analysis of two characteristic transitions (282/240 and 282/208) and comparison of signal intensity (peak areas) ratio with blank cheese sample spiked with albednazole sulfone. Ion ratio fit within the limits (+/- 20%) described in the Commission Decision 2002/657/EC (4). Retention time of the analyte peak was positively compared with the retention time of internal standard (albedazole sulfone-d3).

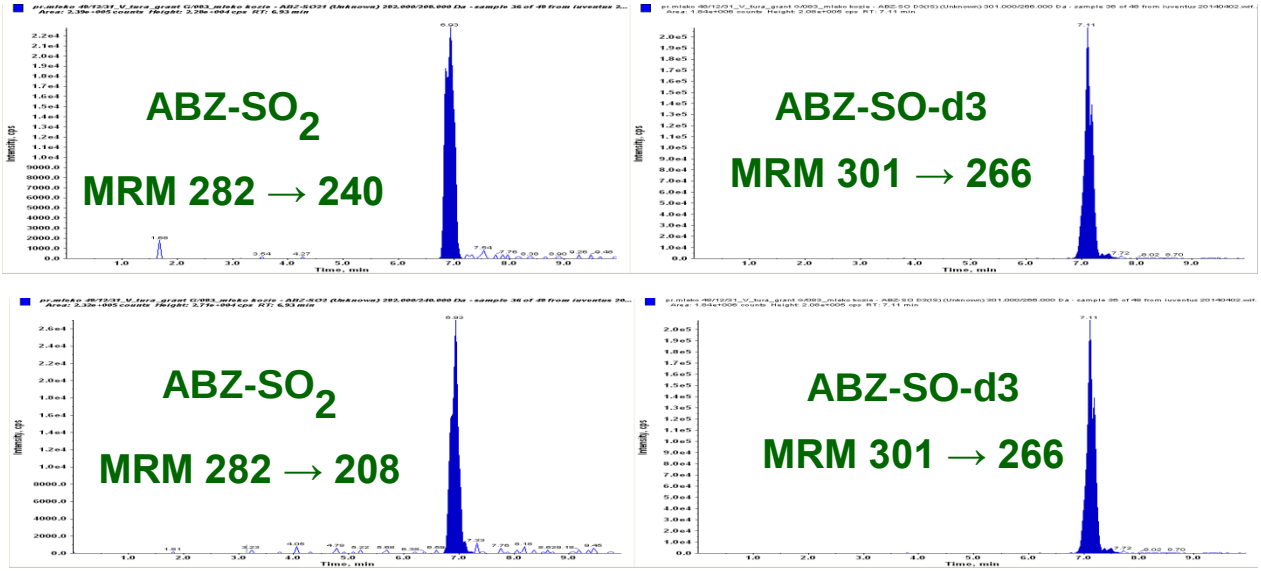

Fig. 1. The ion chromatograms of the incurred goat milk containing albendazole sulfone (5.2 µg/kg). The chromatograms present two transitions (MRMs, multiple reaction monitoring) of albendazole sulfone (m/z = 282) in comparison to ion chromatogram of a labelled internal standard (albendazole sulfone – d3)

Discussion

According to the authors' best knowledge, it was the first study regarding the wide range of anthelmintic residues in the goat and sheep milk and dairy products in Europe. The results showed a very small percentage of detectable residues in goat and sheep milk and dairy products (0.83%). They correlated with the results of official residue control in Europe (7). In recent years, only a few authors have published results for anthelmintics in milk, and their study was based on the newly developed methods for sample preparation and LC-MS/MS determination.

Aguilera-Luis *et al.* (1) found traces of fenbendazole (<3 µg/kg) in one out of ten milk samples collected from the supermarkets in Spain. Chen *et al.* (3) used the HPLC-DAD method for determination of 11 benzimidazoles and metabolites in 50 milk samples obtained from local dairy farmers in China. No detectable residues of analytes were found in any of the samples. In Brasil, Furlani *et al.* (9) detected five avermectins (ivermectin, abamectin, doramectin, eprinomectin, and moxidectin) in 342 samples of milk (135 samples of UHT milk and 104 samples of pasteurised milk) and yoghurt (104 samples). One sample of pasteurised milk was positive for moxidectin (0.29%). Gomez-Prerez *et al.* (10) presented rare data about residues of veterinary drugs in cheese. The LC-MS/MS method covered 17 veterinary drugs (macrolides, sulfonamides, and anthelmintics). Thirteen samples collected from the stores located in Almeria (Spain) were analysed. Thiabendazole was detected in two cheese samples (blue cheese and goat cheese) (traces around 0.7 µg/kg).

An important exception from the above publications was reported by Tsiboukis *et al.* (16). The authors examined 123 milk samples from the Greek market for albendazol and fenbendazole using HPLC-DAD method. They found a high percentage (27.6%) of positive samples, and in 14 samples (11.4%) the residues exceeded the maximum limits. Such high results confirm that in specific environmental and breeding conditions the residues of anthelmintics in milk products can be an important issue in food safety area.

The results of the study showed negligible residues in goat and sheep milk and dairy products in the Polish market and confirm their good quality.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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