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Original Article

Albendazole residues in goat's milk: Interferences in microbial inhibitor tests used to detect antibiotics in milk



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ARTICLE INFO

Article history:

Received 14 April 2016

Received in revised form

27 July 2016

Accepted 22 August 2016

Available online 10 November 2016

Keywords:

antiparasitic agents

albendazole residues

goat's milk

screening tests

ABSTRACT

Albendazole (ABZ) residues in goat's milk and their effect on the response of microbial inhibitor tests used for screening antibiotics were evaluated. A total of 18 Murciano-Granadina goats were treated with ABZ and individually milked once a day over a 7-day period. ABZ quantification was performed by high performance liquid chromatography. The ABZ parent drug was not detected. The maximum concentration of its metabolites (ABZ sulfoxide, ABZ sulfone, and ABZ 2-aminosulfone) was reached on the 1st day post treatment ($260.0 \pm 70.1 \mu\text{g/kg}$, $112.8 \pm 28.7 \mu\text{g/kg}$, $152.0 \pm 23.6 \mu\text{g/kg}$, respectively), decreasing to lower than the maximum residue limit (MRL, $100 \mu\text{g/kg}$) on the 3rd day post treatment. Milk samples were also analyzed by microbial tests [Brilliant Black Reduction Test (BRT) MRL, Delvotest SP-NT MCS and Eclipse 100], and only one positive result was found for Delvotest SP-NT MCS and Eclipse 100. However, a high occurrence of positive outcomes was obtained for BRT MRL during 6 days post treatment, whereas ABZ residues were not detected from the 4th day post administration, suggesting that factors other than the antiparasitic agent might affect the microbial test response.

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1. Introduction

Gastrointestinal nematodes (GN) remain one of the main constraints in ruminant production as they can cause a reduction in skeletal growth, live-weight gain, and milk yield.

The prevention control of GN infestations in goats is based on the use of anthelmintic drugs at regular intervals. The main group of anthelmintics used for the prevention and control of GN in animals is benzimidazoles.

Some authors indicate that goat farmers essentially use benzimidazoles (> 80%) in the control for herds at a rate of

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<http://dx.doi.org/10.1016/j.jfda.2016.08.007>

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nearly three treatments per year [1,2]. Among benzimidazole drugs, the most widely used are albendazole (ABZ), febendazole, oxfendazole, and thiabendazole [3].

ABZ is effective against GN, including migrating larval stages and lungworms [4]. Moreover, it is metabolized by rapid oxidation into different metabolites [albendazole sulfoxide (ABZSO), albendazole sulfone (ABZSO₂), and albendazole 2-aminosulfone (NH₂-ABZSO₂)]. Due to their embryotoxicity and teratogenicity in a variety of animal species [5], their residue levels require careful monitoring in animal products. Maximum residue limit (MRL) for ABZ and its metabolites has been established at 100 µg/kg for milk of all ruminants [6].

To guarantee that the presence of veterinary drugs in milk does not represent a health problem, numerous controls to screen antimicrobial substances are carried out at different phases of the goat milk production chain [7]. Microbial inhibitor tests are widely used in control laboratories for screening antimicrobials in milk as they are easy to perform, inexpensive, and have a broad spectrum detection capability. However, these methods are nonspecific, and milk components, somatic cell count, and substances such as detergents and antiparasitic agents, might cause false positive results in antibiotic-free milk samples [8–10].

The aim of this study was to estimate the effect of oral ABZ treatment in dairy goats on the microbial screening tests response and determine the presence of residues of this drug in milk.

2. Methods

2.1. Animals, ABZ treatment, and milk sampling

Eighteen healthy lactating Murciano-Granadina goats in midlactation [body weight (b.w.), 55–65 kg] from the experimental farm of the Institute of Animal Science and Technology of Universitat Politècnica de València (UPV) were used.

A pre-experiment was performed 3 days prior to ABZ treatment to verify that milk samples from each animal had negative results in microbial inhibitor tests. Goats were treated with a single oral dose (18 mL) of a commercial ovine formulation of ABZ (7.5 mg/kg b.w. of ABZ) frequently used in goats (Ovidax, Fatro Ibérica S.L., Barcelona, Spain). The withdrawal period of Ovidax is 4 days for sheep milk.

The animals were individually milked once a day (8:00 AM), and the sampling was performed along a 7-day period. Milk samples obtained were analyzed in triplicate by microbial inhibitor tests. Furthermore, aliquots of milk were frozen (–80°C) for the high performance liquid chromatography quantification for ABZ and its metabolites.

2.2. ABZ and metabolite quantification

Extraction procedures, standards, and chromatographic conditions to quantify ABZ and its metabolites (ABZSO, ABZSO₂, and NH₂-ABZSO₂) were performed, according to the protocol established by Moreno et al [11] with some minor modifications detailed below. The standards of ABZ (A4673), ABZSO (35395), ABZSO₂ (35394), and NH₂-ABZSO₂ (32181) were provided by Sigma-Aldrich (Sigma-Aldrich Química, S.A.). The

stock and working solutions were prepared in methanol (HPLC-grade, J.T.Baker).

Previously conditioned Sep-Pak vac 1 cc C18 cartridges (Waters Corporation) were used. Next, 50 µL of each milk sample was analyzed using a Waters HPLC system (Waters Corporation) consisting of two pumps (Mod. 515, Waters), an autosampler (Mod. 717 plus, Waters), and an ultraviolet visible detector (Mod. 2489, Waters). A reversed-phase SunFire C18 column (Waters, 4.6 mm × 150 mm, 5 µm) was used for separation at a constant temperature of 35°C with a flow rate of 1 mL/min using acetonitrile and ammonium acetate buffer (0.025M, pH 5) as the mobile phase. The detection of ABZ/metabolites was performed at a wavelength of 290 nm.

Detection limits (LOD) of ABZ, ABZSO, ABZSO₂, and NH₂-ABZSO₂ were 7.9 µg/kg, 5.1 µg/kg, 11.0 µg/kg, and 1.0 µg/kg, respectively, whereas the quantification limits (LQD) were 19.6 µg/kg, 7.7 µg/kg, 26.0 µg/kg, and 2.4 µg/kg, respectively. The recovery of ABZ/metabolites in milk ranged between 80 and 98%.

2.3. Microbial inhibitor tests

Goat's milk samples were analyzed by three microbial inhibitor tests, Brilliant Black Reduction Test MRL (BRT MRL, AiM, Analytik in MilchProduktions-und Vertriebs-GmbH), Delvotest SP-NT MCS (DSM Food Specialties), and Eclipse 100 (ZEULAB S.L.). Negative (antimicrobial-free goat milk) and positive (antimicrobial-free goat milk spiked with 4 µg/kg of benzylpenicillin) controls were included on each plate. The tests were used according to each manufacturer's instructions, and the indicator color changes were assayed by visual interpretation, classifying milk samples as "positive" when the color remained purple/blue and "negative" when the color changed to yellow.

2.4. Statistical analysis

The nonparametric Kruskal–Wallis (KW) test was used to analyze statistical differences ($p < 0.05$) between the ABZ concentration and its metabolites with respect to days post treatment. The Bonferroni test was performed to establish the differences along the days post administration. Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, VA, USA) was used for statistical analyses.

3. Results

The concentration and range corresponding to the elimination of ABZ and its metabolites during the 7 days post treatment are shown in Table 1. The parent compound, ABZ, was not detected in any milk sample. The maximum concentration of ABZSO, ABZSO₂, and NH₂-ABZSO₂ was observed on the 1st day and subsequently decreased until the 4th day. From the 3rd day, the sum of all metabolites was lower than the MRL established for ABZ (100 µg/kg). Thus, the withdrawal period of 4 days established for sheep could be applied to dairy goats.

The nonparametric KW test applied to the first 4 days of this study showed significant differences for the concentrations of metabolites in the milk recorded at different days post

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