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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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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 ± 70.1 μg/kg, 112.8 ± 28.7 μg/kg, 152.0 ± 23.6 μg/kg, respectively), decreasing to lower than the maximum residue limit (MRL, 100 μg/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.

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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nearly three treatments per year [1,2]. Among benzimidazole drugs, the most widely used are albendazole (ABZ), febendazole, oxfebendazole, 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 ($ABZSO_2$), and albendazole 2-aminosulfone (NH_2-$ABZSO_2$)]. 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 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.). 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, $ABZSO_2$, and NH_2-$ABZSO_2$) 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), $ABZSO_2$ (35394), and NH_2-$ABZSO_2$ (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, $ABZSO_2$, and NH_2-$ABZSO_2$ 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, $ABZSO_2$, and NH_2-$ABZSO_2$ 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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Table 1 – Albendazole and albendazole metabolite residues in goat milk (μg/kg) during the first 7 days post treatment.

Compound	Residues of albendazole metabolites, ± SD and ranges						
	Days post treatment						
	1	2	3	4	5	6	7
ABZ	ND	ND	ND	ND	ND	ND	ND
ABZSO	260 ± 70.1 (105–665)	10.1 ± 1.2 (16–47)	5.6 ± 1.7 (8–9)	2.2 ± 1.4 (ND–8)	ND	ND	ND
$ABZSO_2$	112.8 ± 28.7 (37–248)	25.6 ± 14.9 (20–130)	14.8 ± 5.2 (ND–42)	6.1 ± 4.2 (ND–30)	ND	ND	ND
NH_2-$ABZSO_2$	152.0 ± 23.6 (82–251)	15.9 ± 2.23 (15–24)	12.2 ± 3.1 (14–31)	4.9 ± 2.9 (ND–20)	ND	ND	ND
Σ Metabolites	526.4 ± 104.1 (224–1,164)	51.7 ± 16.3 (51–201)	32.6 ± 8.1 (22–82)	13.2 ± 7.5 (ND–58)	ND	ND	ND

ABZ = albendazole; ABZSO = albendazole sulfoxide; $ABZSO_2$ = albendazole sulfone; NH_2-$ABZSO_2$ = 2-aminosulfone; Σ Metabolites = sum of all metabolites; ND = nondetectable; SD = standard deviation.

treatment for ABZSO (KW = 47.77, $p < 0.001$), $ABZSO_2$ (KW = 41.70, $p < 0.001$), and NH_2-$ABZSO_2$ (KW = 55.15, $p < 0.001$) and the sum of its metabolites (KW = 34.28, $p < 0.001$). The analysis of the Bonferroni inequality test indicates that the mean concentrations of ABZ metabolites were higher for the 1st day post treatment than those for the other days ($p < 0.05$).

Regarding the effect of antiparasitic drug residues after ABZ treatment on the microbial tests for screening antibiotics in goat milk, only one positive result was obtained for Delvotest SP-NT MCS and Eclipse 100 tests on the 1st day after treatment. Conversely, when milk samples were analyzed by the BRT MRL test, 18 positive results were obtained during the 6 days post treatment (Figure 1).

As shown in Figure 1, the highest frequency of positive outcomes (33%) was reached on the 3rd day post treatment when the ABZ metabolites showed lower concentrations in milk than on Days 1 and 2 post treatment. However, on Days 5 and 6 after ABZ administration, a large number of positive results were observed (22.2 and 17%, respectively); however, no marker residues of ABZ were detected.

4. Discussion

The animal factor has an effect of the large range variation in the ABZ metabolite concentrations as indicated in Table 1.

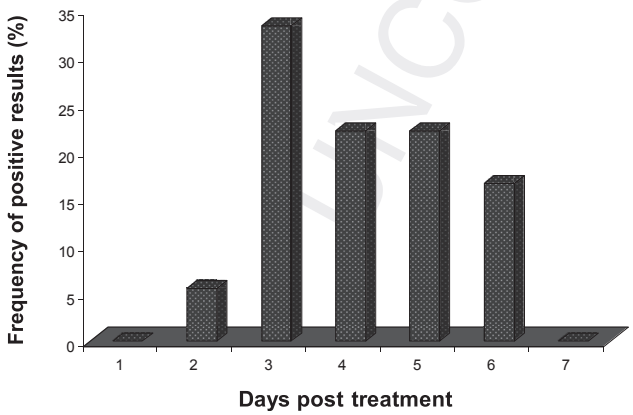

Figure 1 – Frequency of positive results in the BRT MRL test in goat milk samples during the 1st week after treatment of the goats with albendazole. BRT MRL = brilliant black reduction test maximum residue limit.

The possible cause of the high variability could be due to the fact that the dose administered to each goat dose was the same (18 mL), which corresponds to the highest weight of animals (65 kg). Considering that the milk production of each goat is different (range, 700–3600 mL), the amount of ABZ residues in milk has a wide range.

The rapid oxidation of the parent compound of ABZ after an antiparasitic treatment (Table 1) was also noted by other authors in ewe's [12] and goat's milk [13]. In sheep milk (oral dose, 12.5 mg/kg b.w.), De Liguoro et al [12] reported a concentration of 3896 μg/kg and 902 μg/kg of ABZSO and $ABZSO_2$, respectively at 12 hours post treatment, decreasing to 62 μg/kg and 106 μg/kg at 48 hours. The only metabolite found at 36 hours was NH_2-$ABZSO_2$ (89 μg/kg). From the 3rd day, no ABZ residues were detected. The larger amounts of drug residues found in sheep milk could be explained by the dose administered (12.5 mg/kg vs. 7.5 mg/kg b.w., respectively), and by their higher fat content [14] since ABZ is a lipophilic anthelmintic drug [15].

Cinquina et al [13], in milk from Saanen goats treated with an oral dose of ABZ (3.75 mg/kg b.w.), observed residues of 1100 μg/kg for ABZSO, 480 μg/kg for $ABZSO_2$, and 29 μg/kg for NH2-$ABZSO_2$ at 24 hours. After 48 hours of treatment, the quantity of ABZSO (25 μg/kg) and $ABZSO_2$ (43 μg/kg) decreased, whereas that of NH_2-$ABZSO_2$ increased to 54 μg/kg. From the 3rd day, no ABZ residues could be detected. These authors obtained a higher drug concentration and a shorter elimination period than those reported herein, probably related to the ABZ dose (3.75 vs. 7.5 mg/kg b.w, respectively).

The positive results obtained in the BRT MRL test could be associated to the methodological differences between the microbial tests (BRT MRL, Mueller Hinton agar, and Billiant Black as redox indicator; Delvotest SP-NT MCS and Eclipse 100, Plate Count Agar and bromocresol purple as pH indicator) and other test characteristics (pH, spores, etc.). Moreover, the occurrence of positive results in the BRT MRL test could also be related to alterations of the immune system of the animals, producing substances to which this method is more sensitive than the other tests, although there is only limited information on this aspect. For instance, levamisole (benzimidazole drug) acts unspecifically, restoring the cellular and humoral immune response in animals [16]. In bovines, levamisole stimulates the differentiation of T lymphocytes and their response to antigens, increasing the activity of macrophages and neutrophils [17]. In addition, the stress in dairy livestock has been associated to changes in some physiological and

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biochemical indicators [18] that could be related to the presence of inhibitory substances in milk [19] able to affect the microbial test responses.

In conclusion, the oral ABZ treatment in dairy goats produces concentrations of drug residues in milk lesser than MRL from the 3rd day post administration. A high percentage of false-positive results was only observed in the BRT MRL tests even when ABZ metabolites were no longer detected in goat milk.

Conflicts of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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