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Short Communication

Determination of tylosin excretion from sheep to assess tylosin spread to agricultural fields by manure application

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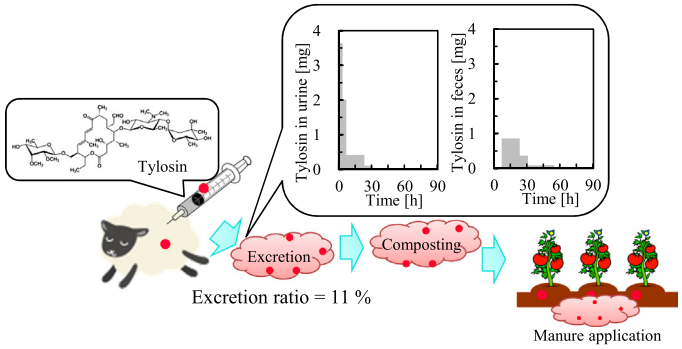
HIGHLIGHTS

- The excretion ratio of tylosin from sheep was obtained.
- Tylosin was excreted in urine and feces for four days.
- The total excretion ratio was 11% in average.
- Our results provide useful knowledge to prevent antibiotic spread to agricultural fields.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibiotics administered to livestock are partly excreted with urine and feces. As livestock excrement is used as manure on agricultural fields, soil may be contaminated by excreted antibiotics, potentially resulting in the development of antibiotic-resistant bacteria. Therefore, it is necessary to determine the amount of antibiotic administered to livestock that could spread to agricultural fields through manure application. This study reveals the excretion ratio of tylosin from sheep. After developing an analysis procedure for tylosin in urine and feces from sheep, a tylosin excretion study was performed with two sheep. Tylosin was excreted in urine and feces for four days, after which its concentrations dropped below the limits of quantification (urine: 0.5 μg/kg, feces: 2.4 μg/kg). The total excretion ratio was 11% on average. The results of our study can provide useful knowledge for treating excrement in order to prevent the spread of antibiotics to agricultural fields through manure application.

1. Introduction

Antibiotics are used as veterinary drugs for curing or preventing diseases. More than 90 types of antibiotics are administered to livestock in Japan (Ministry of Agriculture, Forestry and Fisheries of Japan, 2015). After an antibiotic has been administered to an animal, a portion of it

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is excreted with the urine and feces (Chiesa et al., 2015; Widyasari-Mehta et al., 2016). Since livestock excrement is used as manure in agricultural fields, soil may be contaminated by excreted antibiotics, potentially resulting in the development of antibiotic-resistant bacteria (Tang et al., 2015; Zhao et al., 2017). Therefore, it is essential to determine the amount of antibiotic given to livestock that could spread to agricultural fields through manure application.

An analytical procedure, including a method of extraction from manure, has been developed for antibiotics, such as tetracyclines and sulfonamides, which are widely used (Chiesa et al., 2015; Martínez-Carballo et al., 2007; Solliec et al., 2014). Several antibiotics were detected in manure. A total of nine antibiotics were detected in broiler manure (Ho et al., 2014), a group of tetracyclines in pig manure (Widyasari-Mehta et al., 2016), and a group of sulfonamides in pig, chicken, and turkey manure (Martínez-Carballo et al., 2007). Furthermore, although many different antibiotics are used worldwide, high recovery analytical procedures for antibiotics in manure have not yet been developed.

Although limited data is available on antibiotic concentrations in manure, studies on the excretion ratios of antibiotics administered to livestock have not yet been published. The excretion ratio is essential for assessing the environmental risk due to antibiotics administered to livestock. Several researchers have investigated the biodistribution of antibiotics, the time dependence of antibiotic concentrations in plasma for effective treatment (Anadón et al., 1996), and the antibiotic concentration in meat required to ensure the safety of a food product (Blasco et al., 2007; Kaale et al., 2008). However, no studies have been published on the time dependence of antibiotic concentrations in the urine and feces of livestock and the excretion ratios of antibiotics administered to livestock.

The objective of this study is to obtain the urinary and fecal excretion ratio of tylosin, which is a veterinary antibiotic commonly used worldwide for a variety of animals. Three tylosin compounds are used in Japan: tylosin, tylosin phosphate, and tylosin tartaric acid. The average annual use of tylosin was 173–392 kg for beef cattle, 191–411 kg for cows, and 212–259 kg for pigs from 2011 to 2014 (Ministry of Agriculture, Forestry and Fisheries of Japan, 2015). Tylosin was used for dairy cows, beef cattle, and pigs, while tylosin phosphate and tylosin tartaric acid were used for pigs, poultry, and laying hens.

The procedure for determining antibiotics in manure investigated by Blackwell et al. (2004) is not suitable for tylosin. Loke et al. (2000) succeeded in analyzing tylosin in pig manure by high performance liquid chromatography with high precision, and the limit of detection was 0.4 mg/L. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has recently become the main analytical technique for antibiotics due to its accurate compound identification and low detection limit. However, clean-up treatment of the extracted sample by solid phase extraction (SPE) is required to remove the matrix from the samples before the analyte sample can be injected for LC-MS/MS. Not only the clean-up treatment but also procedures for extraction from excrement needed to be developed for LC-MS/MS analysis.

This study investigated the clean-up conditions for urine and feces samples, and the experimental conditions for extracting tylosin from feces. After developing the method, the excretion ratio was obtained for two sheep, which were used as model animals for ruminants such as dairy cows and beef cattle.

2. Materials and methods

2.1. Reagents

Tylosin is a macrolide antibiotic that can be used to treat mycoplasma and respiratory infections, and dysentery in pigs. Its chemical structure is shown in Fig. 1. Previous studies reported the pKa values of tylosin as 3.31 ± 0.30 and 7.50 ± 0.13 (Qiang and Adams, 2004), 7.1 (Kan and Petz, 2000; Wollenberger et al., 2000), and 7.73 (McFarland et al., 1997). A stock solution (2 mg/L) was prepared from a tylosin standard (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in methanol and stored at 4 °C. This was used to create a standard curve for quantitative analysis. Tylosin Chu 200 [KS] (Kyoritsu Seiyaku Corp., Tokyo, Japan) was used to inject to the test animals.

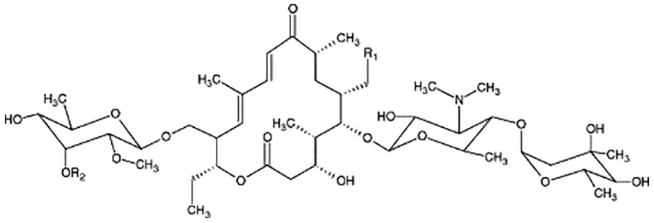

Fig. 1. Chemical structure of tylosin (Jacobsen et al., 2004).

2.2. Urine and feces samples

All animal experiments were performed at the Agricultural Research Center, NARO. The procedures were approved by the Animal Use and Care Committee of the Agricultural Research Center, NARO (No. 28-18).

Urine and feces samples were taken from two Suffolk sheep to which tylosin had never been administered. Each animal was placed in a metabolic cage with a feces-urine separator. Urine and feces samples were collected individually from the separator and immediately stored at 4 °C prior to testing.

A simulated tylosin-containing urine sample was prepared by mixing about 10 g of urine and 1 mL of tylosin solution (20 μg/L) in a 300 mL conical flask. The sample was diluted with 200 mL of deionized water. The diluted sample had a pH of around 9; phosphoric acid (Blackwell et al., 2004) was added to adjust this to 7–8. Finally, the sample was used for the following SPE clean-up process.

2.3. Extraction procedure for feces sample

An extraction procedure was investigated based on methods reported by Martínez-Carballo et al. (2007). The experiment was performed at least three times. The feces were homogenized with a mixer. Simulated tylosin-containing feces was obtained by mixing 2 g of the homogenized feces and 1 mL of 20 μg/L tylosin solution in a 50-mL glass sedimentation tube and leaving the mixture to stand for about 10 min. Subsequently, 10 mL of McIlvaine extraction buffer (pH 5, 6, 7, or 8) (Blackwell et al., 2004) was mixed with the sample. The tube was placed in an ultrasonic bath for 10 min and then centrifuged at 3000 rpm for 5 min in a centrifugal separator (Sakuma, M201-1UD, Japan). The supernatant was decanted into a 200-mL conical flask. The extraction process was repeated twice. Each supernatant was diluted with 200 mL of deionized water for the following SPE clean-up process.

2.4. SPE clean-up of urine sample and feces extract

Two SPE cartridges were used: Oasis HLB Plus (225 mg) (Waters, Massachusetts, USA), which was used in previous studies on tylosin (Kolz et al., 2005; Aust et al., 2008) and is suitable for samples over a wide pH range, and Oasis WCX Plus (225 mg) (Waters, Massachusetts, USA), which is used for basic compounds. The HLB cartridge was used for urine and feces samples at all pH values, while the WCX cartridge was used for urine samples at all pH values and feces samples at pH 7 and 8.

For urine samples, SPE cartridges were conditioned with 5 mL of methanol and equilibrated with deionized water. For feces samples, SPE cartridges were conditioned with 5 mL of methanol and equilibrated with 5 mL of McIlvaine buffer diluted 20 times with deionized water. The pH of the McIlvaine buffer was the same as that used for