

Research

Salbutamol Residue in Plasma and Urine of Balinese Calves after Single-Dose Administration

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ABSTRACT

Salbutamol, a short-acting β_2 -adrenergic receptor agonist, due to illegal use in animal feed, is very dangerous for the safety of animal origin products. The purpose of this study is to determine the residue of salbutamol in plasma and urine of balinese calf after a single-dose administration. Three calves were given of feed fortification of salbutamol (10 mg/kg body weight). The salbutamol concentrations were measured in the plasma and urine. Samples were extracted with perchloric acid and purified by cation exchange solid phase extraction (SPE), then analyzed by High-Performance Liquid Chromatography (HPLC) with a photodiode array detector using an RP C18 column with a mixed mobile phase of purified water pH 3.0 (adjusted with phosphoric acid) and acetonitrile (90:10, v/v). The highest salbutamol concentration in the plasma sample was 0.28 ± 0.15 $\mu\text{g/mL}$ at 12 hours and decreased after 24 hours after feeding. The highest concentration of salbutamol in urine was 15.85 ± 4.42 $\mu\text{g/mL}$ at 18 hours and decreased gradually starting at 24 hours. This result concluded that salbutamol residues mostly excreted in the urine, and the highest salbutamol residues in plasma samples formed faster but also decreased more rapidly than residues formed in urine. The residue formed in plasma is lower than that in the urine.

Keywords: HPLC, plasma, salbutamol, balinese calf, urine

ABSTRAK

Salbutamol, adrenoseptor β_2 -selektif kerja pendek, karena penggunaan ilegal dalam pakan ternak, sangat berbahaya bagi keamanan produk asal hewan. Penelitian ini bertujuan untuk mengukur kadar residu salbutamol yang terbentuk dalam plasma dan urin pedet sapi bali setelah pemberian salbutamol dosis tunggal. Tiga ekor pedet diberi pakan mengandung salbutamol (10 mg/kg bobot badan). Konsentrasi salbutamol diukur dari sampel plasma dan urin. Sampel diekstraksi dengan asam perklorat dan dipurifikasi dengan *solid phase extraction (SPE) cation exchange*, kemudian dianalisis secara kromatografi cair kinerja tinggi (KCKT) dengan detektor *photodiode array* menggunakan kolom RP C18 dengan fase gerak campuran *purified water* pH 3.0 (d disesuaikan dengan asam fosfat) dan asetonitril (90:10, v/v). Konsentrasi salbutamol tertinggi sampel plasma sebesar 0.28 ± 0.15 $\mu\text{g/mL}$ pada jam ke-12 dan menurun pada jam ke-24. Konsentrasi tertinggi urin sebesar 15.85 ± 4.42 $\mu\text{g/mL}$ pada jam ke-18, dan menurun secara bertahap mulai jam ke-24. Disimpulkan bahwa residu salbutamol sebagian besar dieksresikan di urin dan residu tertinggi salbutamol pada sampel plasma lebih cepat terbentuk namun juga lebih cepat menurun dibandingkan residu yang terbentuk pada urin. Residu yang terbentuk pada plasma lebih rendah dibandingkan yang terbentuk pada urin.

Kata kunci: KCKT, plasma, salbutamol, sapi bali, urin

INTRODUCTION

Beta-agonist have been illegally used to improve carcass conditions and performance of livestock production. Meat products from animals treated by β -agonist with long plasma half-lives, high oral bioavailability, may have high oral potencies of β -agonist in humans. On the other hand, β -agonist with short plasma half-lives, low oral bioavailability, have low oral potencies in consumers as the shorter plasma half-life leads to much lower residues in edible tissue and a much lower risk to consumers (Smith, 1998). The residue of β_2 -agonists in the tissues, organs, and processed products accumulating in the human body can have adverse effects on human health (Du *et al.*, 2013; Zhang *et al.*, 2017). Numerous literature has reported various potential hazards and adverse effects of β_2 -agonists, including cardiovascular effects, and affect the central nervous system (Hung *et al.*, 2010). However, now a day public concern is more focused on clenbuterol than salbutamol, so there is a possibility that salbutamol illegally used as a substitute for clenbuterol (Granja *et al.*, 2017; Tang *et al.*, 2018).

Salbutamol, a short-acting β_2 -adrenergic receptor agonist, is widely used to treat respiratory diseases acting as tocolytic and bronchodilator agents in animals and humans (Yan *et al.*, 2016). The administration of salbutamol in high doses (≥ 10 fold than the therapeutic dose) can cause repartition effects, feed efficiency, increase body weight gain, and lean carcasses, by converting nutrients from fat deposition to production muscle tissue (Lei *et al.*, 2008; Marchant-Forde *et al.*, 2012; Hung *et al.*, 2010).

Various methods for detecting residue of β_2 -agonists have developed, namely immunoassay (Pleadin *et al.*, 2013), chemiluminescent (Gao *et al.*, 2014), molecularly imprinted polymer (Tang *et al.*, 2018) and high-performance liquid chromatography (HPLC) with ultraviolet detectors (Du *et al.*, 2013; Yan *et al.*, 2016), or fluorescence detectors (FLD) (Hu *et al.*, 2010). Among these methods, HPLC is one of the most widely used methods.

Several studies have reported the elimination (depletion) of salbutamol in plasma and urine in the calf (Hung *et al.*, 2010), adult dairy cows (Zhang *et al.*, 2016), and Chinese Simmental beef cattle (Zhang *et al.*, 2017). However, the use of Balinese (*Bos javanicus*) cattle (domestic subspecies originated in Indonesia) (Mohamad *et al.*, 2009) for study, its elimination has never been reported. This research is a preliminary study, conducted to detect levels of salbutamol in plasma and urine of balinese calves

after a single dose of feed fortification, using HPLC with a photodiode array detector.

MATERIALS AND METHODS

Animal ethic approval

All procedures in this study have approved by the Institutional Animal Care and use Committee (IACUC) of Indonesian Agency for Agriculture Research and Development, Ministry of Agriculture No. Balitbangtan/BBLitvet/RM/02/2019.

Chemicals

Salbutamol standard (99.5%) was purchased from Sigma Aldrich (USA). All other chemicals were commercially available from Merck (Darmstadt, Germany), except ultrapure water was provided from Millipore (MA, USA) and acetonitrile grade purchased from Tedia (USA). Solid-phase extraction (SPE) cation exchange PCX Plexa columns (60 mg, 3 mL) (Agilent, USA).

Animal and Sampling Procedures

The experiment was conducted three healthy male Balinese (*Bos javanicus*), with 110, 128, and 100 kg in weight at the beginning of the treatment were used for this study. All calves were fed concentrate fortified by a single dose of 10 mg of salbutamol/kg BW in the morning feeding.

Blood samples were collected at 2, 6, 8, 12, and 24 hours after administration, and urine samples at 6, 12, 24, 48, 72, 96, 120 and, 144 hours, after the drug was administered. Blood samples were taken from the jugular vein into a blood tube containing heparin anticoagulant, then centrifuged at 3000 rpm for 10 minutes to separate the plasma. Plasma and urine samples are stored at -20°C before analyzed.

Preparation of plasma and urine samples

Analysis of plasma and urine samples referred to the method developed by Zhang *et al.* (2017) with modification. A 1 mL aliquot of plasma or urine samples were placed in a 15-mL centrifuge tube, 4 mL 0.5% perchloric acid in water was added, and the sample was mixed on a vortex mixer for 1 min. After centrifugation for 10 min at 4000 rpm, the supernatant was loaded onto a solid phase extraction PCX column that had been activated with 3 mL of methanol and 3 mL of water, washed with 3

mL of 2% formic acid and 3 mL of methanol: acetonitrile (50:50, v/v), and finally eluted with 5 mL of 5% ammonium in methanol: acetonitrile (50:50, v/v). The eluent was evaporated to dryness, under nitrogen gas at 40°C, then reconstituted in 1 mL for plasma samples, and 1 mL for urine samples of a mixture of purified water pH 3.0 (adjusted with orthophosphoric acid) and acetonitrile (9:1, v/v), ready to be injected onto the HPLC.

Equipment and Chromatographic Condition

Analysis of salbutamol residues in plasma and urine samples was carried out using the HPLC Shimadzu LC-20AD, which was equipped with a Lab-solution data analysis system, as well as a photodiode array detector at 225 nm (Shimadzu, Japan). The analytical separation process was carried out using an XTerra C18 column (150 mm x 4.6 mm, i.d 5 µm) (Waters, USA). The mobile phase used was a mixture of purified water pH 3.0 (adjusted with orthophosphoric acid) and acetonitrile (9:1, v/v) with a flow rate of 0.7 mL/min. The sample injection volume was 20 µL.

RESULTS

Different mobile phase mixtures with different pH have been experimented to obtain separation peak with excellent resolution and reasonable retention times. During the optimization process, blank and positive samples were injected to get a good separation between the matrix sample peak and the salbutamol peak. A mixture of purified water with pH 3.0 (adjusted using phosphoric acid) and acetonitrile (90:10, v/v) was used to obtain the optimal separation of salbutamol peak with good retention time. The analysis time was relatively short, and salbutamol detected at a minute of 3.9. Figure 1 shows the chromatogram of salbutamol standard. The limit detection (LOD) and limit quantitation (LOQ) were evaluated as the amount required to achieved signal to noise ratio of 3:1 and 10:1, respectively. LOD and LOQ were 0.008 and 0.012 µg/mL for plasma samples and 0.026 and 0.036 µg/mL for urine samples, respectively.

Figures 2, shows the increase of the salbutamol concentrations residue in plasma at 12 hours, and decreasing at 24 hours after administration. Salbutamol

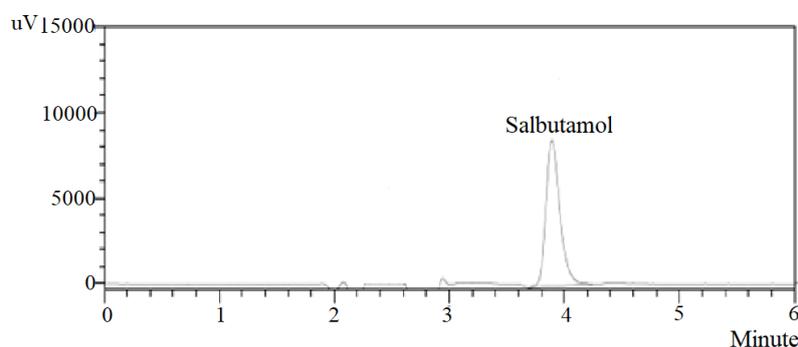


Figure 1 Chromatograms of salbutamol standard 1.0 µg/mL

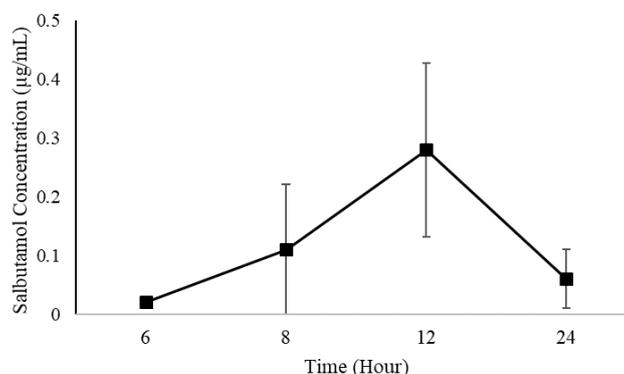


Figure 2 Salbutamol residues in plasma samples collected after single-dose drug administration

concentrations in plasma at 2 hours after administration were below the limit quantification, then at 6, 8, 12, and 24 hours after administration, the average of salbutamol concentration in plasma were 0.02, 0.11, 0.28 and 0.06 $\mu\text{g/mL}$.

The increase of salbutamol concentration also occurs in the urine sample shown in Figures 3. The highest concentration occurred 18 hours after administration, then decreased in 24, 48, 72, 96, 120, and 144 hours with consecutive concentrations. The peak concentration of salbutamol in urine samples occurred 18 hours after administration of 15.85 $\mu\text{g/mL}$, then decreased in 24, 48, 72, 96, 120 and 144 hours with consecutive concentrations of 5.12, 2.8, 0.4, 0.23, 0.07 and 0.014 $\mu\text{g/mL}$.

DISCUSSION

Our findings show the same patterns compared to the study conducted on heifers (Zhang *et al.*, 2016), who showed that the highest in unhydrolyzed plasma occurred at 12 h and decreased subsequently. Distribution of salbutamol in pigs shows a high drug concentration in serum 5 hours after the last administration of salbutamol, and the half-life of salbutamol absorption after oral administration is about 4.01 ± 0.2 h (Suppadit, 2005). The study of Pou *et al.* (1992) reported salbutamol concentration in plasma veal calves did not detect on day 4, after the last administration of milk fortification (10 mg/day) for ten days.

The study from Zhang *et al.* (2016) in heifers, shows that the highest salbutamol concentration from urine samples was at 8 hours and did not show the data at 12 and 18 hours after administration. The steady condition that occurred from these studies was similar and started at 72 h. Pou *et al.* (1992) reported that immunoreactive salbutamol was detected in the urine sample taken in the evening after morning administration. In this study, the salbutamol concentration occurred at the urine samples at 18 hours and show signal suppression. This increase could be related to the presence of metabolites. However, salbutamol metabolites could not be measured since no hydrolysis procedure performed.

According to our study, the salbutamol had a long detectable period for six days (Figure 3). The study on unhydrolyzed samples of dairy heifers urine, salbutamol detected below the limit of quantitation (0.2 ng/mL) on 48 h and 120 h, respectively, after single-dose oral administration through phosphate-buffered saline (Zhang *et al.*, 2016). Similar to this study, salbutamol begins to reach eliminate phase after 48 h, and decreases until 144 h after administration. Research conducted by Montrade *et al.* (1995) showed that salbutamol concentration decreased rapidly in urine and also from body tissues, after half-life, except kidney and liver.

The salbutamol variation of concentrations in plasma and urine samples from three individual cattle may be due to the consumption of feed from each Balinese calves (Figures 3), which did not accustom to consume concentrate but grass instead.

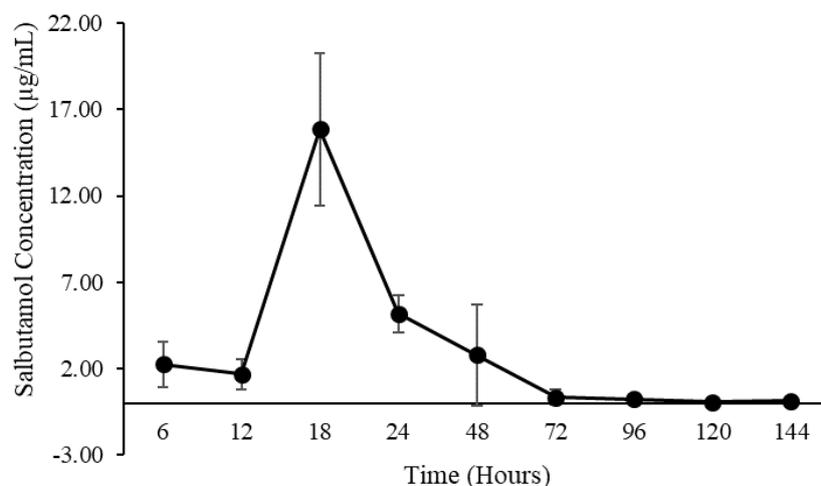


Figure 3 Salbutamol residue in urine samples collected after single-dose drug administration

Beyene (2015) suggested that diet can affect the bioavailability of a drug. Further explained that animals receiving feed containing fresh green herbage had lowered bioavailability of the drug, also the drug uptake and pharmacodynamics also affected by gut contents. Decheng *et al.* (2015) observed that the variability in rates also might be related to the different times of sample collection, which implemented to the difficulties in collecting, especially the plasma samples.

Plasma and urine samples in food-producing animals, generally considered to be used as monitoring samples of the illegal use of β -agonists. The findings show that the plasma concentrations of salbutamol during the treatment period are lower compared with urine samples and rapidly decreases after administration. Therefore, our findings indicate that for preslaughter monitoring, urine is more suitable as a target of the illegal use of salbutamol.

The salbutamol residue in plasma samples is formed rapidly but also decreased faster than the residue formed in urine. The residue concentration in plasma is lower than urine.

“All authors declare that there are no conflicts of interest”.

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