

## **Salbutamol Residues in Swine Tissues and Body Fluids after Feeding**

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### ***Abstract***

Salbutamol residues in swine tissues and fluids were investigated. Tissue, serum and urine samples were collected from 33 pigs after 14 days of oral administration of 3 ppm salbutamol. The concentrations of salbutamol were determined by enzyme-linked immunosorbent assay and further confirmed by capillary electrophoresis. The highest urinary concentration of salbutamol was 145.12 ng/ml at 18 hour and remained detectable for 30 days after the last feeding. Salbutamol was undetectable in serum samples. The highest concentrations of salbutamol in liver, kidney, lung, heart, brain and muscle were 70.42 ng/g, 31.88 ng/g, 26.06 ng/g, 6.76 ng/g, 3.41 ng/g and 2.97 ng/g, respectively. Stomach and large intestine retained salbutamol residue for 11 days which is longer than those in the liver, lung, brain (4 days) and other tissues (2 days). The data shown in this study will be helpful for the screening of salbutamol residues and regulations on its illegal use in pigs.

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**Keywords:** Residue, Salbutamol, swine, tissue, urine

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## บทคัดย่อ

### การตอกค้างของสาร Salbutamol ในเนื้อยื่อ และ ของเหลว ในสุกร หลังจากได้รับสาร Salbutamol โดยการผสมในอาหาร

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การศึกษานี้เป็นการตรวจสอบเพื่อหาสารตอกค้างของ Salbutamol ในเนื้อยื่อและของเหลวที่เก็บจากสุกร โดยเนื้อยื่อ ชีรั่ม และน้ำปัสสาวะถูกเก็บจากสุกร จำนวน 33 ตัว หลังจากที่ สุกรได้รับ Salbutamol บริมาณ 3 ppm โดยการผสมในอาหาร เป็นเวลา 14 วัน ทำการตรวจหาความเข้มข้นของ Salbutamol ด้วยวิธี Enzyme-linked immunosorbent assay และตรวจสอบช้าโดยวิธี Capillary electrophoresis ผลพบว่าสาร Salbutamol เข้มข้นมากที่สุดในตัวอย่างน้ำปัสสาวะมีค่า เท่ากับ 145.12 ng/mlหลังจากได้รับ salbutamol 18 ชั่วโมง และยังคงสามารถตรวจพบหลังจากระยะเวลาผ่านไปแล้ว 30 วัน สาร Salbutamol ตรวจไม่พบในตัวอย่างชีรั่ม สาร Salbutamol ตรวจพบและเข้มข้นมากที่สุดในตับ ไต ปอด หัวใจ สมอง และกล้ามเนื้อ พบริมาณเท่ากับ 70.42 ng/g, 31.88 ng/g, 26.06 ng/g, 6.76 ng/g, 3.41 ng/g และ 2.97 ng/g ตามลำดับ สาร Salbutamol ตกค้างใน กระเพาะ และ ลำไส้ใหญ่ เป็นเวลา 11 วัน ชีง ยาวนานกว่า ในตับ ปอด และ สมอง (ตกค้าง 4 วัน) และเนื้อยื่อส่วนอื่นๆ ตกค้าง 2 วัน ข้อมูลที่ได้จากการศึกษานี้จะเป็นประโยชน์ใน ตรวจสอบสารตอกค้างของ Salbutamol และ การควบคุมการใช้ Salbutamol อย่างผิดกฎหมายในสุกร

**คำสำคัญ:** สารตอกค้าง ชาลบูทามอล สุกร เนื้อยื่อ น้ำปัสสาวะ

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## Introduction

$\beta_2$ -agonists or  $\beta_2$ -adrenergic agonists have been illegally used to improve production performance and carcass condition of livestock. Pharmacologically, these compounds have been found to exert a repartitioning activity causing an increase in muscle accretion and decrease in fat deposition (Mersmann, 1998).  $\beta_2$ -agonists have been reported to enhance meat-producing performance in different livestock species such as cattle, pigs, sheep and poultry (Garssen et al., 1995; Hansen et al., 1997<sup>a</sup>; Hansen et al., 1997<sup>b</sup>; Mersmann 1998) at dosages approximately 10-fold greater than the therapeutic dose (Lei et al., 2008). However, the residues of  $\beta_2$ -agonists may present health risk to public health. In Europe, food poisoning cases of  $\beta_2$ -agonists have been associated with the consumption of both beef liver and non-liver beef meat. The symptoms of patients were nervousness, tachycardia, muscle tremors, myalgia and headache (Mastro et al., 1995; Salleras et al., 1995). In public health reports to date, clenbuterol was the compound among the  $\beta_2$ -agonists to cause food poisoning (Martinez-Navarro, 1990; Pulce et al., 1991; Mastro et al., 1995; Salleras et al., 1995). However, in addition to clenbuterol, there was an indication that salbutamol had continually been detected during routine screening in Taiwan (Lei et

al., 2008).  $\beta_2$ -agonists, including salbutamol, terbutaline, clenbuterol and ractopamine have been prohibited in food-producing animals since 2006, according to Veterinary Drugs Control Act., Enzyme-linked immunosorbent assay (ELISA) has been used for screening of  $\beta_2$ -agonists in liver, eye and body fluids in cattle and sheep (Bucknall et al., 1993; Sauer et al., 1993; Sawaya et al., 2000). Also, capillary electrophoresis has been applied in the separation and determination of  $\beta_2$ -agonists in swine feed (Chen et al., 2008). Data on salbutamol residues in livestock are still limited. This study investigated the detectable periods and levels of salbutamol in swine tissues and body fluids after feeding for a 14-day period.

## Materials and Methods

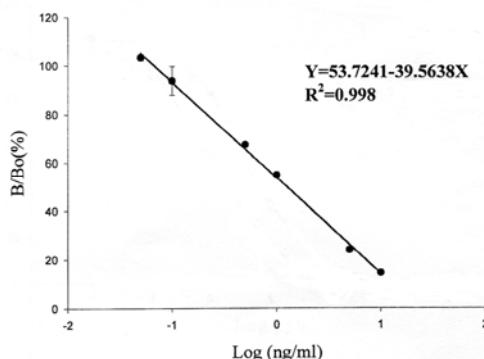
**Experimental animals and sample collection:** A total of 33 sows of approximately 25 kg were housed in individual pens (4x6 m<sup>2</sup>) and were maintained with antibiotic- and salbutamol-free feed. When body weight of the pigs reached approximately 80 kg, thirty pigs were randomly assigned to 10 groups and provided with feed containing 3 ppm salbutamol for 14 days. The dosage of salbutamol was based on the findings by Warriss et al. (1990). Three untreated pigs were used as control tissue samples, slaughtered by electric stunning before the administration. Thirty treated pigs of 10 groups were killed at day 1, 2, 3, 4,

5, 8, 11, 16, 23 and 30 after the last feeding. Serum and urine samples were centrifuged at 1000xg for 30 minutes and stored at -20°C. Portion of heart, liver, spleen, lung, kidney, stomach, intestine, uterus, bladder, and longissimus muscle were collected from each carcass and stored at -20°C and the remaining tissues were cremated.

**Enzyme-linked immunosorbent assay (ELISA):** The concentrations of salbutamol in urine, serum and tissue samples were determined using a commercial  $\beta$ -agonist ELISA kit (Adimmune, Taichung, Taiwan). The ELISA was performed according to the manufacturer's instructions. Tissue samples were minced and diluted 10-fold in sample buffer provided by the ELISA kit. The swine tissues in the sample buffer were homogenized using a homogenizer (ESGE, Mettlen, Switzerland), centrifuged at 7000xg for 10 minutes and the supernatant was collected for ELISA analysis. Urine and serum samples were diluted 10-fold in the sample buffer for the analysis. To obtain the detection range of the ELISA, a standard curve was set up by adding a known amount of salbutamol (Sigma, St Louis, MO, USA) to blank diluted urine, sera or supernatant of the homogenized and centrifuged tissues. The optical density (OD) values of the samples were determined at 450 nm using an ELISA reader (Dynatech, Chantilly, VA, USA).

**Solid phase extraction and capillary electrophoresis:** Solid phase extraction and capillary electrophoresis (Beckman, New Bedford, CA, USA) were used to further confirm the presence of salbutamol in swine urine, sera and tissues. Salbutamol in urine, serum and tissues were extracted using C18 columns (Varian, Palo Alto, CA, USA). The columns were pre-treated with methanol and deionized water. After washed with diethyl ether and acetonitrile, the target drug in the columns was eluted by methanol and then identified by capillary electrophoresis (adapted from Chuang et al., 2010). Briefly, before the first run (or between runs), the capillary was rinsed with 20mM phosphate buffer (pH 9.0) for 3 minutes and the separation time for salbutamol was 15 minutes. The capillary was washed with 1M NaOH and water before the next run.

## Results



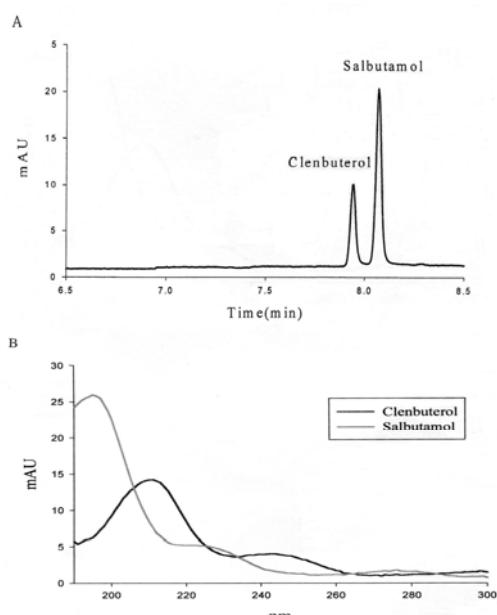
**Figure 1** The detection range of the salbutamol ELISA was 0.05-10 ng/ml.

**Analytical performance of the ELISA:** A standard curve with a linear relationship for the detection of salbutamol was established by preparing serial concentrations (0.01-1000 ng/ml) of the drug. The detection range (the linear part of standard curve) of the ELISA for salbutamol was approximately 0.05-10 ng/ml (Figure 1). The salbutamol ELISA kit can also detect clenbuterol while cannot detect terbutaline (Table 1).

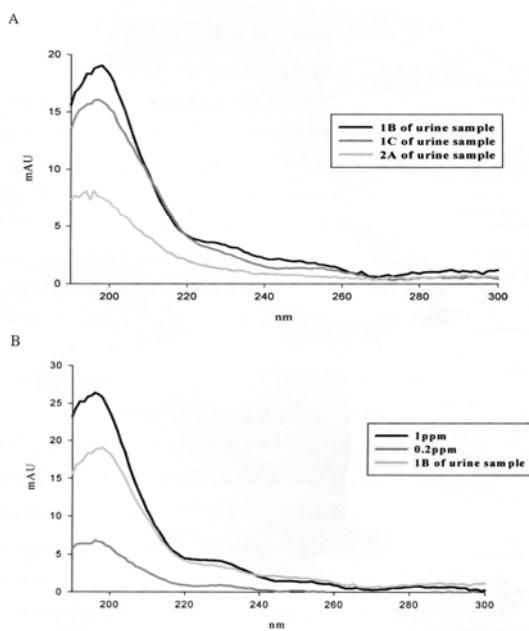
**Table 1** The crossreactivity of the ELISA with clenbuterol and terbutaline.

Compound	Crossreactivity (%)
Salbutamol	100
Clenbuterol	126.95
Terbutaline	<0.01

**Confirmation of salbutamol in swine tissues and body fluids:** Prior to capillary electrophoresis analysis, urinary, serum and tissue samples were extracted and the target drug was eluted as described above. When 5 ppm salbutamol and clenbuterol (Sigma, USA) standards were analyzed by capillary electrophoresis, the separation time of clenbuterol was approximately 0.2 minutes earlier than the counterpart of salbutamol (Figure 2A). The spectrum patterns of absorbance for salbutamol and clenbuterol standards were apparently different in the scanned range of wavelength between 190 and 300 nm (Figure 2B). The spectrum patterns of the suspected compounds in the samples were verified as the same drug (Figure 3A) and the patterns of the suspected drugs detected in the sample were identical to the standard of salbutamol (Figure 3B), confirming that the compound detected in the samples was salbutamol.



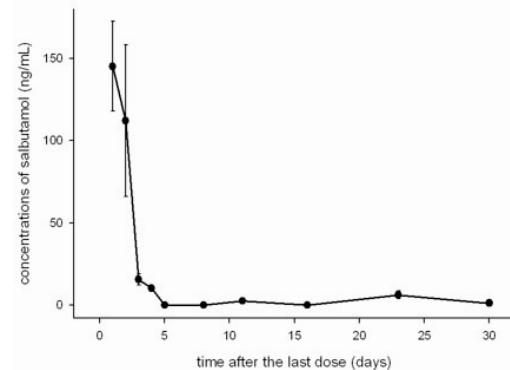
**Figure 2** (A) The separation time of clenbuterol was approximately 0.2 minutes earlier than the counterpart of salbutamol in the analysis of capillary electrophoresis and (B) the spectrum patterns of absorbance for salbutamol and clenbuterol were apparently different in the scanned range of wavelength between 190 and 300 nm.



**Figure 3** (A) The spectrum patterns of the suspected compounds were verified as the same drug by capillary electrophoresis and (B) the patterns of the suspected drugs detected in the sample were identical to the standard of salbutamol.

**Salbutamol concentrations in swine urine, sera and tissues:** In swine urine, the highest concentration was 145.12 ng/ml at day 1 after the last dose. The urinary concentrations of salbutamol remained detectable at day 30 after the last feeding (Table 2, Figure 4). The

highest levels of salbutamol in the liver, lung, kidney and longissimus muscle were approximately 70.42 ng/g, 26.06 ng/g, 31.88 ng/g and 2.97 ng/g, respectively (Table 2). The large intestine was the only tissue to have the highest residual level of salbutamol (43.58 ng/g) at day 2 after the feeding whereas the other tissues all showed the highest concentrations at day 1. The residues of salbutamol in the liver, lung and brain were detectable 4 days after the last feeding while salbutamol in the stomach and large intestine remained detectable up to day 11 (Table 2). The concentrations of salbutamol residues in the remaining tissues (small intestine, spleen, kidney, urinary bladder, heart, uterus and longissimus muscle) had detectable levels for up to 2 days (Table 2). Salbutamol was undetectable in serum samples.



**Figure 4.** The concentrations of salbutamol in swine urine following 14-day oral administration.

**Table 2** Salbutamol residues in swine tissues (ng/g) and urine (ng/mL) after 14-day feeding.

Sample Type	Days after the last dose									
	Day 1(18 hr)	Day 2	Day 3	Day 4	Day 5	Day 8	Day 11	Day 16	Day 23	Day 30
Urine	145.12±27.26	111.95±46.09	15.63±3.43	10.29±1.03	—	—	2.53±0.22	—	6.15±2.36	1.16±0.54
Liver	70.42±11.31	3.56±1.12	—	1.15±1.04	—	—	—	—	—	—
Lung	26.06±9.38	17.55±7.12	0.52±0.11	0.54±0.09	—	—	—	—	—	—
Kidney	31.88±25.29	6.87±4.81	—	—	—	—	—	—	—	—
Heart	6.76±3.54	1.06±0.38	—	—	—	—	—	—	—	—
Spleen	10.14±0.82	3.30±1.23	—	—	—	—	—	—	—	—
Brain	3.41±0.38	2.35±0.17	0.69±0.12	0.86±0.35	—	—	—	—	—	—
Stomach	18.33±12.96	1.92±1.78	0.43±0.11	0.41±0.17	0.11±0.06	0.12±0.05	0.19±0.09	—	—	—
Small intestine	51.46±12.29	7.77±1.08	—	—	—	—	—	—	—	—
Large intestine	25.09±9.30	43.58±3.55	1.32±0.65	0.84±0.13	0.60±0.20	0.67±0.31	0.73±0.21	—	—	—
Urinary bladder	17.56±7.08	4.27±1.13	—	—	—	—	—	—	—	—
Uterus	3.47±0.84	1.22±0.42	—	—	—	—	—	—	—	—
Muscle	2.97±0.83	1.20±0.17	—	—	—	—	—	—	—	—
Sera	—	—	—	—	—	—	—	—	—	—

## Discussion

Results of this study showed that the detection range of salbutamol by ELISA was between 0.05-10 ppb for swine tissues and spiked sera sample. These results are similar to results reported of 0.05-1 ppb (Lei et al., 2008). It is apparent that the concentration of salbutamol in swine serum was lower than the detection limit one day following the last feeding. It is likely that salbutamol had already cleared from the sera by this time. It has been reported that the half life of salbutamol is less than 4 hours in rabbits and human (Smith, 1998).

In cattle and humans, treatments with salbutamol were over 50% of the doses excreted via

urine (Montrade et al., 1995; Smith 1998). In dogs orally administered [<sup>3</sup>H] salbutamol, 70-80% of the dose was excreted unchanged in the urine (Brittain et al., 1968). Furthermore, salbutamol has been shown to undergo active renal excretion in humans (Boulton et al., 1996; Ward et al., 2000). These data may explain why in the current study the highest urinary concentration of salbutamol reached 145.12 ng/ml and the compound in urine had a long detectable period of 30 days. In our study the residues of salbutamol in the kidney were approximately 31.88 ng/g at day 1 and detectable until day 2 after the feeding. The residual level in the kidney is lower and the detectable period is also shorter than the counterparts reported in calves and chickens (Montrade et al., 1995; Malucelli et al., 1994).

On the other hand, the highest concentration of salbutamol in swine liver reached 70.42 ng/g and the residues could be detected until day 4 after feeding in the current study. These two data are lower or shorter than the results previously shown in bovine liver (Montrude et al., 1995). In chicken, salbutamol was also reported to have a long detectable period of 2 weeks in liver (Malucelli et al., 1994). Taken together, in pigs, cattle and chicken, high salbutamol residues were detected in liver after feeding whereas in cattle liver salbutamol seemed to have longer detectable periods, up to day 7 after oral administration, rationalizing that the source of the previous  $\beta$ 2-agonist poisoning cases was mainly from the consumption of cattle liver. In Asia, in some countries or areas such as China, Taiwan, Hong Kong and Korea, swine or poultry livers are considered as a nutritious food or nutritional supplementation for the ill, pregnant women and teenagers. The residual data of salbutamol in swine liver in our study showed another potential risk of public health for these consumers. It should also be noted that the residual levels of salbutamol in bovine muscle (5 ng/g) after 7-day withdrawal periods were still higher than those detected in lung (3 ng/g) and heart (2 ng/g) despite a low sample number (Montrude et al., 1995). However, in the current study, salbutamol concentrations in swine muscle at day 2 after feeding rapidly declined to 1.20 ng/g, which is much lower than the value detected in lung (17.55 ng/g) and similar to that in heart (1.06 ng/g) and salbutamol in swine muscle was undetectable after day 3, suggesting more potential risk of public hygiene caused by the illicit use of salbutamol in cattle than in pigs.

The use of  $\beta$ 2-agonists in livestock like pigs and cattle is forbidden for growth-promoting purposes, and the drugs are locally not permitted to be detected in all livestock products since 2006, according to the Announcement of Veterinary Drug Residue Limits in Foods. To date, residual or tissue distribution data of salbutamol in swine tissues and body fluids are still limited (Chalermchaikit et al., 1994). Therefore, the data on the concentrations and periods of salbutamol in tissues and urine in pigs after 14-day feeding shown in the current study should be essential for public health in countries feeding domestic pigs. These data may be helpful for the screening of salbutamol residues and regulations on its illegal use in pigs.

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