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Residue Study for a Standardized *Macleaya cordata* Extract in Growing-Finishing Swine

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Abstract

The aim of this study was to assess the effects of Sangrovit®, a standardized preparation of *Macleaya cordata* extract (MCE), on the health status and retained residues in growing-finishing swine. A total of twelve growing-finishing swine (n = 6 for each group) were randomly divided into two groups and fed either a control feed or the control feed supplemented with 100 mg/kg Sangrovit® (3.5 mg/kg MCE) for 28 days. The parameters for growth and health status were evaluated during the trial and after which the animals were slaughtered. Residual levels of MCE in swine organs and tissues were determined by measuring sanguinarine and chelerythrine levels by LC-MS/MS. The results showed no statistically significant differences in live weight, feed intake and average daily gain between the treatment and control groups. The feed supplemented with 100 mg/kg Sangrovit® was well tolerated by the swine, with no adverse effects noted during the feeding period or in the necropsy results. Residue analysis indicated that levels of sanguinarine or chelerythrine were under the limit of detection in all the examined tissues and organs from the treated swine. This study demonstrates that Sangrovit®, a standardized preparation of MCE when fed to growing-finishing swine for 28 days, at the level of 100 mg/kg in feed, does not result in sanguinarine or chelerythrine residues in the organs or tissues.

Keywords

Macleaya cordata Extract, Swine, Sanguinarine, Tolerability, Residues

1. Introduction

Macleaya cordata is a plant native to eastern Asian countries such as China and Japan, but has found a wide spread throughout Europe as ornamental plant and

can also be found growing wild east of the Mississippi river in North America. As a deciduous perennial plant, *M. cordata* is a hollow-stemmed, leafy, fast growing plant that can grow to 2.0 m high by 1.0 m wide. *M. cordata* has been reported to contain considerable amounts of isoquinoline alkaloids, mainly sanguinarine and chelerythrine [1]. Sanguinarine and chelerythrine are bitter in taste and have the potential use as flavoring components to increase the feed intake in farm animals [2]. Swine is one of the most important livestock that provides more than one third of the meat production in the world [3]. Previous research has found that swine favor the bitter taste [4] and therefore, a bitter tasting substance can be used as flavor modifier for addition to swine feed [5].

In recent years, plant derived (phytogenic) compounds have been increasingly used in livestock feeding practices. *M. cordata* extract (MCE)-containing products, with a bitter flavor, have been used as a naturally-derived appetizing agent for addition to the feed of production animals including bovine [6] [7], poultry [8] [9], fish [10] and swine [2] [11] Sangrovit® is a standardized preparation of the botanically-derived feed additive that contains 3.5% MCE (providing at least 1.5% sanguinarine). Dietary supplementation with Sangrovit® has been demonstrated to increase the feed intake and optimize nutrient absorption in chickens and other species [12]. Research in swine has shown that Sangrovit® exerts a positive effect to increase the body weight gain and feed conversion rate in growing swine [13]. Supplementation of 50 mg/kg Sangrovit® in the feed for four weeks greatly promotes the growth in weaning piglets [2]. However, few studies have been performed to examine the safety of MCE consumption for swine and MCE residues remaining in swine organs and tissues, which are the major edible parts for humans. In this study, the objective was to determine the tolerance of growing-finishing swine to the consumption of a standardized preparation of MCE, containing sanguinarine and chelerythrine as major active components, for 28 days at the level of 100 mg/kg feed, and evaluate the residue levels of sanguinary and chelerythrine in the organs and tissues of the swine.

2. Materials and Methods

2.1. Test Substance

The experiments were conducted by using a manufactured preparation of MCE, (trade name knownas Sangrovit®) as test substance. Sangrovit® preparation was standardized to contain at least 1.5% sanguinarine. The level of sanguinarine and chelerythrine in Sangrovit® was measured before adding to the feed. The standardized preparation of MCE used in this study was analyzed and found to contain 1.67% of Sanguinarine and 0.65% of chelerythrine. The test product was provided by Phytobiotics Futterzusatzstoffe GmbH (Eltville, Germany).

2.2. Experimental Animals and Diets

The trial was carried out on twelve growing-finishing Hybrid swine at the age of six months old (six females and six castrated males) with an initial live weight (LW) of 102.5 (±2.2) kg (Breeding farm code: 007 LO 011, located in Comezza-

no-Cizzago (BS), Italy). The animals were weighed, selected out of groups on the farm breeding normal without disease problems, and assigned to the control and treatment groups to achieve maximum possible homogeneity within each group and minimize differences between the groups. The trial took place at the CERZOO S.r.l. facility (Piacenza, Italy) and the conditions were regarded as representative for a modern commercial operation in Europe. The animals were examined for health status upon arrival at the test facility, and fed with basal diet for 31 days (Table 1). After this period, the animals were weighed and assigned to control or the treated groups, housed individually and fed with control or experimental diets (*i.e.*, Day 0). The test facility was equipped with a dynamic ventilation system that enabled the facility to control the ventilation rate according to the temperature and age of the pigs. The temperature and relative humidity were recorded every 20 min and temperature was automatically adjusted as necessary. Natural daylight was utilized as a light source during the whole trial period.

Table 1. Composition (%) and nutrient characteristics (g/kg) of the diets.

Ingredient (%)	Basal feed (for pre-experimental period and control group)	Treatment feed (for treatment group)
Diet composition (%)		
Corn meal	60.00	60.00
Barley meal	15.00	15.00
Wheat bran	10.30	10.30
Soybean meal 44%	12.40	12.40
Dicalcium phosphate	0.45	0.45
Limestone	1.20	1.20
Sodium bicarbonate	0.10	0.10
Salt	0.40	0.40
L-Lysine HCl	0.11	0.11
Vitamins and minerals[1]	0.04	0.04
Nutrient characteristics		
Dry Matter (%)	90.20	90.44
Crude Protein (%)	13.55	14.02
Ether extract (%)	3.20	3.36
Crude fiber (%)	3.65	3.80
Ash (%)	5.85	6.21
Starch (%)	51.30	51.42
Neutral detergent fiber (%)	22.70	22.64
Acid detergent fiber (%)	6.80	6.94
Digestible Energy[1] (MJ/kg)	14.48	14.51
Net energy[2] (MJ/kg)	10.68	10.70
Sanguinarine in the diet (mg/kg)	<LOD[3]	1.53 ± 0.27[3]
Chelerythrine in the diet (mg/kg)	<LOD[3]	0.53 ± 0.06[3]

Note: [1]Content of vitamins and Oligo minerals/kg of pre-mixture (manufactured by Unione Veneto Lombarda – Roé-Volciano (BS) – I): vit. A: 6,000,000 UI; vit. D_3: 600,000 UI; vit. E: 8000 mg; vit. K3: 800 mg; vit. B_1: 800 mg; vit. B_2: 150 mg; vit. B_6: 800 mg; vit. B_{12}: 6 mg; vit. H: 40 mg; vit. PP: 10,000 mg; D-pantothenic acid: 4000 mg; choline chlorure 150,000 mg; Mn: 24,000 mg; Fe: 80,000 mg; Cu: 8000 mg; Zn: 30,000 mg; Co: 300 mg; I: 960 mg; Se: 120 mg. Excipient to 1000 g: Ca carbonate; [2]According to the equation proposed by EU legislation (G. U.CE L54 published on February 26, 2009); [3]Limit of detection (LOD) for sanguinarine/chelerythrine are 0.05 mg/kg feed (as shown in Table 4), the values were obtained from 3 replicates ($n = 3$) and expressed as mean ± standard deviation.

The swine were fed a basal diet (for control group) or treatment diet (for treatment group) formulated without antibiotics, antibiotic growth promoters (AGP), or AGP alternatives. The basal diet was prepared by Rossana feed mill (Revere, MN, Italy) and provided sufficient nutrients to meet the NRC Swine Requirement (NRC 1998, diet composition was shown as Table 1), which was free from any zootechnical feed additive as defined in European Commission No. 1831/2003. The treatment diet was prepared by mixing 100 mg/kg Sangrovit® with the basal diet at CERZOO feed mill, and homogenous distribution was ensured. The treatment diet was similar with the basal diet in composition except for the addition of 100 mg Sangrovit® per kg of test diet. After mixing, both control and test diets were analyzed for moisture, ash, starch, crude protein, crude fat and fiber, as well as the content of sanguinarine and chelerythrine. The sanguinarine and chelerythrine content in the basal diet was under detection limit and the treatment diet was found to contain 1.53 mg sanguinarine and 0.53 mg chelerythrine per kg diet (Table 1). During the trial period, the animals were fed with a diet approximate to 3% of their LW in one steel feeder per cage per day. The drinking water was provided *ad libitum* by an internal water system in which the water quality was analyzed annually. Feed intakes were measured and recorded per swine.

2.3. Study Design

The swine were divided into two groups with equal sex distribution in each group (n = 6, three castrated males and three females). The control group was given a basal diet without MCE treatment; the treatment group was given a diet containing 100 mg Sangrovit® per kg feed. The treatment period was 28 consecutive days with a pre-experimental period of 31 days for adaptation. The general health status of the swine and the housing was inspected twice a day. Individual live weights (LW) of the swine were recorded at the beginning and the end of the study (Day 0 and Day 28). The feed intake, health/illness, and adverse events were analyzed daily and carefully recorded during the whole period of experiment (from Day 0 to Day 28). The feed conversion ratio (feed: gain ratio) in each period was calculated by dividing the total amount of feed consumed by the corresponding live weight gain. This study was conducted in full compliance with Good Laboratory Practice (GLP) guidelines under Italian Legislation and, an additional measure was taken to make the study also compliant with the U.S. GLP Guidelines (21 CFR Part 58).

2.4. Post-Mortem Analysis

At the end of the study (Day 29), the experimental animals were slaughtered and necropsied by a veterinary surgeon at CERZOO. A gross post-mortem examination was carried out by an animal health welfare manager to assess the general health and fitness of the animals for human consumption, which included a gross check on: external skin, eyes, feet, ears, head and tail, mouth and anus, gut (oral cavity, esophagus, stomach, upper, mid and lower small intestine, caecum,

and colon), pancreas, spleen, liver/gall bladder, kidneys, genitals, abdominal fat, omentum, heart and lungs, skeletal muscle, and fat.

2.5. Organ and Tissue Sampling

On Day 29, the animals were fed with control or treatment diet twelve hours before slaughter (the last feeding). The animals were slaughtered according to the international standard set by World Organization of Animal Health (OIE). The organs (*i.e.*, liver and kidney) and tissues (*i.e.*, muscular tissue (thigh), adipose tissue with skin in natural proportion (around the thigh) and fat (around kidney)) were sampled, prepared and frozen according to the Standard Operating Procedure (SOP) at CERZOO. The frozen samples were immediately transferred to the laboratory for further analysis.

2.6. Measurement of Sanguinarine and Chelerythrine

2.6.1. Sample Preparation and Purification

Samples of the control and treatment feeds were obtained and represented the beginning, middle and end of the diet production. The samples were ground with a 1 mm sieve mill, dissolved in 100 ml of acidified methanol (1% HCl), and refluxed for 45 min at 60˚C. After cooling to 20˚C, a 5 ml aliquot was centrifuged at 3000 rpm for 10 min and diluted prior to analysis. The organ and tissue samples (5 g for liver, muscle, adipose tissue with skin and fat; 3 g for kidney) were homogenized for 2 min with 20 ml (for liver, muscle, and adipose tissue) or with 15 ml (for kidney) of acidified methanol (1% HCl) by using an Ultra Turrax T25-IKA homogenizer (Staufen, Germany). The homogenized mixture was centrifuged at 5500 rpm for 10 min and the supernatant was obtained. The precipitated residue was re-extracted with 20 or 15 ml acidified methanol and centrifuged. The re-extracted supernatant was pooled with the one from previous extraction and the final volume was recorded. The extract was further purified by loading a 3 ml aliquot through an OASIS HLB column (pre-conditioned with 3 ml methanol and 2 ml distilled water) at a flow of 0.5 ml/min. The purified extract was eluted into a graduated vial, and followed by washing with 3 ml acidified acetonitrile (0.2% formic acid). The eluate was concentrated under nitrogen flow, brought to 1 ml using acidified (0.2% formic acid) acetonitrile, mixed and filtered (0.22 μm) prior to analysis.

2.6.2. Sample Analysis

The level of sanguinarine and chelerythrine in the samples were analyzed by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS) equipped with Phenomenex Hilic-Kinetex column (2.6 μm, and 150 × 2.1 mm). The mobile phase consisted of 1) 25 mM ammonium for mate buffer (pH 3.2) and 2) acetonitrile diluted with distilled water (19:81, v/v). The signal was detected by an ESI positive ionization detector with MS/MS. For each matrix, a calibration curve was obtained by using a matrix-matched standard of sanguinarine and chelerythrine (Sigma-Aldrich, St. Louis, MO, USA), prepared in a blank matrix (without MCE), for each feed/organ/tissue.

2.6.3. Validation of Analytical Methods

In conformance with GLP guidelines, all measuring/recording instruments used in this study were checked and calibrated before use. The methods to detect and quantify the sanguinarine and chelerythrine levels were validated in each blank matrix before the sample analysis was performed. Known amounts of standard solution were added to the blank matrix (untreated), and extraction of sanguinarine/chelerythrine was performed as described above. Three different levels of standard solution were added to each matrix, and three replicates (n = 3) were measured for each level. The blank matrix was also extracted and analyzed as a control. The parameters involved in the analysis were validated, including specificity, accuracy, precision, detection limit, quantification limit, linearity, and range. Recovery (%) of sanguinarine and chelerythrine in each matrix was calculated as the percentage of the detected level to the level that the standard was initially added.

2.7. Statistical Analysis

Data obtained in this experiment were analyzed by using the software program of SAS 2002-2008, Release 9.2 (Cary, NC, USA) under the General Linear Model (GLM) procedure. Analysis of Variance (ANOVA) was used as the main statistical test and the Student-t test was used to compare the means from each group. $P \leq 0.05$ in the ANOVA model was considered as statistically significant, while $0.05 < P \leq 0.10$ was described as a near-significant trend. The LW was analyzed for outliers, and no data were excluded. For each determination, including LW and feed intake, there were six replicates available for each treatment group.

3. Results and Discussion

The LW and feed intake for the growing-finishing swine during the 28-daytrial period are shown in Table 2. After 28-day feeding, there were no significant differences LW between the control and treatment groups (P = 1.00 and 0.92,

Table 2. Performance parameters (live weight, average daily gain, feed intake, and feed to gain ratio) in the growing-finishing swine.

Experimental period	Control Group	Treatment Group (Sangrovit® 100 mg/kg feed)	Treatment effect P=
Live weight (kg)			
D0	102.5 ± 2.1	102.5 ± 2.4	1.0000
D28	113.7 ± 2.6	113.8 ± 2.8	0.9166
Average daily gain (g)			
D0 - D28	398.8 ± 27.01	404.8 ± 29.4	0.7208
Feed intake (g/d)			
D0 - D28	1540 ± 31	1540 ± 35	1.000
Feed to gain ratio			
D0 - D28	3.87 ± 0.35	3.82 ± 0.09	0.7173

The values were obtained from 6 replicates (n = 6) and expressed as mean ± standard deviation.

respectively). Similarly, no differences were observed among the different feeding groups in the average daily gain ($P = 0.72$) and average daily feed intake ($P = 1.00$). The feed to gain ratio (feed conversion ratio) over the 28-day period was 3.82 in the treatment group, showing no statistical difference from that of the control group (3.87, $P = 0.72$). The results are in agreement with previous findings in which 21-day-old piglets fed a basal feed supplemented with 2 mg MCE (containing 1.28 mg sanguinarine and 0.44 mg chelerythrine)/kg feed and 100 mg MCE (containing 64.03 mg sanguinarine and 21.99 mg chelerythrine)/kg feed for 90 days displayed growth characteristics (e.g., feed conversion efficiency) very similar to the corresponding control piglets [14]. However, Kantas *et al.* found that supplementation of 50 mg/kg Sangrovit® (containing 0.75 mg/kg sanguinarine) in the feed significantly increased feed consumption and feed conversion ratio in six-week-old piglets [2]. The performance discrepancy between different studies may be attributed to different sample size, difficulty in showing significant growth changes in short periods or growth variation between different swine breeds, as [13] reported that Sangrovit® exerted a significant effect on body weight gain and feed conversion ratio in two out of four experiment conducted on swine of different breeds and ages, but there were no effects on growth performance in the other two experiments.

During the 28 days of this experiment, all animals remained apparently healthy and manifested no signs of toxicity. After the swine were slaughtered, the general health of the swine and their fitness for human consumption were evaluated. As shown in **Table 3**, the gross examination of the external skin, gastrointestinal tract (mouth, anus, oral cavity, esophagus, stomach, intestine, caecum and colon), major organs (pancreas, spleen, liver/gall bladder, kidney, genitals, heart and lungs), and tissues (abdominal fat, omentum, skeletal muscle and fat) did not reveal any pathological alterations in the control and MCE-fed animals (all the scores less than or equal to 1, all p values greater than 0.05 between the groups). All the parameters, including general performance and necropsy results, of the treated animals were similar to those given control feed under commercial growing conditions, indicating that there were no testarticle-related adverse effects from the consumption of MCE via the diet on the health status of growing-finishing swine.

To address the safety concerns for human consumption, the evaluation for potential residues of MCE in the major edible parts of the swine was conducted utilizing validated assays (LC-MS/MS). As the main active flavoring components in MCE, sanguinarine and chelerythrine were used as phytochemical makers for detection of MCE residues in the feed, organ and tissue samples obtained in this study. The limit of detection (LOD) for sanguinarine and chelerythrine was found to be 10 μg/kg in muscle, liver, and adipose tissue (with skin and fat), and 15 μg/kg in the kidney. The limit of quantification (LOQ) for both test substances was 45 μg/kg in the muscle, liver and adipose tissue (with skin and fat), and 60 μg/kg in the kidney (**Table 4**). The LOD for sanguinarine and chelerythrine in feed was 50 μg/kg, and the LOQ for both test substances in feed was 100

Table 3. Necropsy results[1].

Tissue or organs	Control Group						Treatment Group (Sangrovit® 100 mg/kg feed)						Treatment effect *P* =
	F1	F2	F3	CM1	CM2	CM3	F1	F2	F3	CM1	CM2	CM3	
External skin	0	0	0	0	0	0	0	0	0	0	0	0	-
Eyes	0	0	0	0	0	0	0	1	0	0	0	0	0.3409
Anus	0	0	0	0	0	1	0	0	0	0	0	0	0.3409
Oral cavity	0	0	0	0	0	0	0	0	0	0	0	0	-
Esophagus	0	0	0	0	0	0	0	0	0	0	0	0	-
Stomach	0	1	0	0	0	0	0	0	0	0	0	0	0.3409
Small intestine	0	0	0	0	0	1	0	0	0	0	0	0	0.3409
Caecum	0	0	0	0	0	0	0	0	0	0	0	0	-
Colon	0	0	0	0	0	0	0	0	0	0	1	0	0.3409
Pancreas	0	0	0	1	0	0	0	0	0	0	0	0	0.3409
Liver	1	0	0	1	0	0	1	0	1	0	1	0	0.5995
Kidneys	0	0	0	0	0	0	0	0	0	0	1	0	0.3409
Heart	0	0	0	0	0	0	0	0	0	0	0	0	-
Lungs	0	0	0	0	0	0	0	0	1	0	0	0	0.3409
Skeletal muscle	0	0	0	0	0	0	0	0	0	0	0	0	-
Fat	0	0	0	0	0	0	0	0	0	0	0	0	-

F = female swine, CM = Castrated male swine, n = 6 for each group; [1]Scored according to the following: 0 = no alteration found; 1.000 = Slight alterations; 2.000 = Alteration of medium intensity; 3.000 = Serious alteration; In the final report, it also stated that the mean scores for feet, ears, head, tail, mouth, spleen, gall bladder, abdominal fat, and omentum were below 1 and no statically significant differences were observed between control and treatment group in these tissue or organs.

Table 4. Limit of detection and quantification of sanguinarine and chelerythrine in matrix (feed/organ/tissue).

Matrix	Limit of detection (LOD)		Limit of quantification (LOQ)	
	Sanguinarine (μg/kg)	Chelerythrine (μg/kg)	Sanguinarine (μg/kg)	Chelerythrine (μg/kg)
Feed	50	50	100	100
Liver	10	10	45	45
Kidney	15	15	60	60
Muscular tissue	10	10	45	45
Adipose tissue with skin	10	10	45	45
Fat	10	10	45	45

μg/kg (Table 1). To validate the analytical method, the recovery of the test items (sanguinarine and chelerythrine) was measured in feed and tissue/organ samples. Recovery of sanguinarine in the feed for three addition levels (1.5, 7.5, and 15.0 mg/kg feed) was 94% ± 17%, and the recovery of chelerythrine in the feed for the same three addition levels was 91% ± 11%. Recovery of sanguinarine and chelerythrine in the biological matrices including muscle, liver, fat/skin, and kidney samples at three levels (80, 200 and 400 μg/kg tissue) were also analyzed. Recovery of sanguinarine ranged from 71% - 107% for all tissues, and recovery

of chelerythrine for the same three levels ranged from 73% - 109% for all tissues. The results from the recovery tests demonstrated that the accuracy (as indicated by mean recovery) and precision (as determined by the standard deviation of the recoveries) were adequate to determine the contents of sanguinarine and chelerythrine in the feed, organs and tissues.

The residual levels of sanguinarine and chelerythrine in the organs and tissues were analyzed in all the swine fed with either control or treatment feed ($n = 6$ for each group). The levels of sanguinarine and chelerythrine in the liver and kidney of all the animals were below LOQ and LOD (Table 5). Likewise, no residue of sanguinarine and chelerythrine were detected in the muscular tissue, adipose tissue with skin, and fat of control and Sangrovit® groups. The findings in this study are consistent with previous results, which showed that the sanguinarine and chelerythrine levels were below LOD in fattening chickens that were given 100 mg/kg Sangrovit® in feed (the same dose as the current study) for 35 days [8]. The absence of sanguinarine and chelerythrine in the organs/tissues of fattening swine is also in agreement with other studies performed in piglets. Kosina *et al.* did not find sanguinarine or chelerythrine residues in the muscle of piglets fed 2 or 100 mg/kg feed MCE (extracted by the authors) for 90 days, but minimal amounts of sanguinarine and chelerythrine were present in the liver and gastrointestinal (GI) tract (*i.e.*, gingiva, tongue, stomach, and intestine), and ranged from 4 - 79 μg/kg sanguinarine and 5 - 36 μg/kg chelerythrine for the 2 mg/kg group and 52 - 514 μg/kg sanguinarine and 40 - 50 μg/kg chelerythrine for the 100 mg/kg group [14]. It is noted that the 100 mg/kg dose of MCE used in Kosina's study is much higher than what we used in the present study. MCE (extracted by the authors) at 100 mg/kg contained 64.03 mg/kg sanguinarine and 21.99 mg/kg chelerythrine, which is about 40 times higher than the dosage used in this study (1.5 mg/kg sanguinarineand 0.5 mg/kg chelerythrine). Kosina *et al.* also found that the sanguinarine and chelerythrine levels retained in the piglets was very small (13 μg/kg sanguinarine and 5 μg/kg chelerythrine in the liver and 15 μg/kg sanguinarine and chelerythrine (not found) in the intestine) when fed with a lower dose of MCE (2 mg/kg, containing 1.28 mg/kg sanguinarine) [14], which are under the LOQ in current study.

Table 5. Sanguinarine and chelerythrine levels in organs and tissues.

Matrix	Control Group		Treatment Group (Sangrovit® 100 mg/kg feed)	
	Sanguinarine (μg/kg)	Chelerythrine (μg/kg)	Sanguinarine (μg/kg)	Chelerythrine (μg/kg)
Liver	<LOD	<LOD	<LOD	<LOD
Kidney	<LOD	<LOD	<LOD	<LOD
Muscular tissue	<LOD	<LOD	<LOD	<LOD
Adipose tissue with skin	<LOD	<LOD	<LOD	<LOD
Fat	<LOD	<LOD	<LOD	<LOD

LOD = Limit of detection, for kidney is 15 μg/kg and 10 μg/kg for other organs/tissue as shown in Table 4.

MCE, is listed in the European Feed Additive Register and therefore authorized for use in animal feed. Previous studies in swine and other animal species have shown that sanguinarine and chelerythrine, are largely retained in the GI tract; the small portion absorbed from the GI tract is metabolized in the liver and eliminated in feces and urine [14] [15] [16]. MCE consumption has not been shown to alter the hematology, histology or clinical chemistry parameters in chickens and weanling swine [2] [8] [14]. However, to our knowledge, this is the first study to comprehensively evaluate the tolerance and residues of MCE in growing-finishing swine at ready-to-market weights (>100 kg). The current work is consistent with previous studies that a dosage of 3.5 mg/kg (or lower) MCE does not have any adverse effects on the health status of swine and no residues of sanguinarine or chelerythrine were retained in the organs and tissues in the swine [2] [14]. Moreover, it was demonstrated in this study that the addition of Sangrovit® to swine feed at up to 100 mg/kg does not result in sanguinarine or chelerythrine in the swine-derived meat products and it is thus there is no safety concern for sanguinarine or chelerythrine exposure for people that consume the swine-derived meat products.

4. Conclusion

In conclusion, the results of this study showed no adverse effects after28 days of consumption of Sangrovit®, a standardized MCE at a level of 100 mg/kg feed in growing-finishing swine. No residual levels of sanguinarine or chelerythrine were detected in the tissues and organs of the swine.

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Conflict of Interests

The authors declare there is no conflict of interests.

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