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Tolerance and Residue Study for Standardized *Macleaya cordata* Extract Added to Chicken Feed

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Abstract: Feed palatability is necessary for optimum feed intake and utilization in all animal species. The effects of consumption of a standardized preparation of an extract of *Macleaya cordata* (*M. cordata*), a herbaceous flowering perennial of the Papaveraceae family that contains isoquinoline alkaloids, has been evaluated when fed to chickens for 35 consecutive days from birth. The chicks were divided into 4 treatment groups (8 replicates of 10 birds/pen) and fed corn/soybean meal-based diets supplemented with a standardized *M. cordata* extract preparation at 0,100, 500 and 1000 mg/kg feed. After the treatment period, eight animals/treatment were randomly selected for blood collection and necropsied after slaughtering. Routine plasma biochemistry and a gross pathology examination were performed. Tissue and organ samples were analyzed for the isoquinoline alkaloids sanguinarine and chelerythrine. No statistical differences were found between treatment groups for live weight (LW), feed intake (FI) or average daily gain (ADG). Blood biochemical analyses showed significant changes in plasma creatinine and alkaline phosphatase levels, but these changes were not dose-dependent and not considered treatment-related. No treatment-related changes were found after necropsy of the selected organs and tissues. No chelerythrine was found in any tissues, while low levels of sanguinarine were found in two fat+skin samples in the 500 mg/kg feed and three fat+skin samples and one kidney sample in the 1000 mg/kg feed. The results of this study show that consumption of a standardized *M. cordata* extract preparation, at up to 1000 mg/kg feed, was well tolerated by chickens for 35 consecutive days.

Key words: *Macleaya cordata*, chicken, isoquinoline, sanguinarine, tolerability, residues

INTRODUCTION

Ingredients to be added to animal feed must undergo an assessment of the safe consumption by the target species prior to inclusion of the ingredient into commercial production, as well as the safe consumption by humans of the animal-derived products. Sangrovit® is a herbal preparation composed of an extract from *Macleaya cordata* (*M. cordata*) which is added to a carrier prior to inclusion into feed. *M. cordata* is a herbaceous flowering perennial of the Papaveraceae family, which contains isoquinoline alkaloids, mainly sanguinarine and chelerythrine, that are bittering substances that are considered flavoring components that influence the feed palatability. The palatability of feed may play a significant role in overall feed consumption and ultimately, in animal health and growth. Previous research has shown that a variety of commercial animal species perceive taste and respond by altering feed consumption (negatively or positively) (Ginane *et al*., 2011; Figueroa *et al*., 2012). Approximately 300 taste buds have been identified in the chicken and the neuronal processes of chickens have been analyzed utilizing taste aversion methodology, indicating a strong ability of taste to influence feed consumption by chickens (Kuenzel, 1989; Gibbs *et al*., 2008).

M. cordata extracts have also been studied for a variety of other effects when consumed by poultry. Research in chickens has found that a *M. cordata* alkaloid extract fed to male chickens for fattening for five weeks at 15 mg/kg feed decreased beta-glucuronidase and beta-glucosidase ceacal activities, while increasing select ceacal short chain fatty acids (Juskiewicz *et al*., 2011), similar to other studies in which chickens for fattening were fed *M. cordata* extract-containing product (MCEP; Sangrovit®) at 30 mg/kg feed (Juskiewicz *et al*., 2013). Additional studies have concluded that addition of MCEP to feed for chickens for fattening at 20 mg/kg feed may reduce excessive ceacal fermentation pathways without increasing ceacal pH or diminishing glycolytic activity, potentially optimizing nutrient absorption (Jankowski *et al*., 2009). However, the tolerance of chickens consuming the *M. cordata* extract at levels greater than what would typically be added to chicken feed, in case of feed formulation errors, has not previously been evaluated. The public

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literature does not provide sufficient detailed analysis of the hematology and pathology of tissues and organs of chickens for fattening that consume the *M. cordata* extract. Therefore, the aim of the present study was to determine the tolerance of chickens for fattening to the consumption of *M. cordata* extract when provided to the chicken by the standardized *M. cordata* extract market preparation (Sangrovit®) for 35 days at levels of 100, 500 and 1000 mg/kg feed.

MATERIALS AND METHODS

Test substance: The test substance was a standardized *Macleaya cordata* extract preparation (MCEP; Sangrovit®), consisting of the *M. cordata* extract combined with a carrier, in this case the dried, ground plant material from the Papaveraceae family, standardized to provide at least 1.5% sanguinarine. The MCEP was provided by Phytobiotics Futterzusatzstoffe (Eltville, Germany).

Animals and diets: Three-hundred-twenty male Hubbard chickens for fattening (Hatchery: Vallespluga, Strada per Veleia, 101 Carpaneto Piacentino (PC)-Italy) with an initial live weight of 42.6±2.3 were obtained for this study at one day of age. Upon arrival at the test facility (Cerzoo, Italy) the animals were examined for general health conditions and unfit animals excluded, then randomly placed in 32 floor pens with a stocking density of 0.12 sqm/bird. All chicks were vaccinated in the hatchery at the day of hatching against Mareck disease, bronchitis and New-Castle disease. The animals were also vaccinated during the study against Gumboro disease at day 14 of age (with Nobilis Gumboro D78 produced by Intervet Italia srl, Italy-batch n. A029AJ01) and against New-Castle disease at day 16 of age (with Nobilis ND Hitchner produced by Intervet Italia srl, Italy-batch n. A003DJ02). Ten birds were reared in each pen, with eight replicates/treatment group. The trial facility was equipped with a dynamic ventilation system and the ventilation rate varied from 0 m^3/h to the maximum ventilation rate required, according to the desired temperature and the age of the chickens. The temperature and relative humidity were recorded every 20 minutes during each day of the trial. For the first two weeks of the study the temperature ranged from approximately 25-30°C and 50-70% relative humidity. For the remainder of the study the temperature and relative humidity ranged from 18-25°C and 55-80%, respectively. The lighting period was 23:1 h (light: dark) during the 35-day study.

The chickens for fattening were fed typical corn-soybean meal-based basal diets in dry powder form (Table 1). The basal diets (one for each of the three growing periods: Day 0-Day 10 (D0-D10), Day 10-Day 28 (D10-D28) and Day 28-Day 35 (D28-D35)), were formulated to meet or exceed nutrient requirements defined in NRC (1994). The basal diets were free from any zootechnical feed additive as defined in European Commission No. 1831/2003. The water quality was analytically evaluated annually. Feed and water were provided *ad libitum*.

Study design: The chicks were assigned to receive one of the four dietary treatments: Control basal diet (T1); Basal diet supplemented with the MCEP at 100 mg/kg feed (T2); Basal diet supplemented with the MCEP at 500 mg/kg feed (T3); and Basal diet supplemented with the MCEP at 1000 mg/kg feed (T4). The test substance was added to the experimental diet over the 100% of the volume of the basal diet at Cerzoo feed mill.

The general health status of the chickens for fattening and the correct functioning of the equipment were evaluated twice daily. Individual body weights were obtained on Days 0, 10, 28 and 35. Any animals found dead were weighed on the date of death and recorded. Average feed intake (FI) by pen was defined as the difference between the feed offered and the feed refused and measured back for the growing periods (i.e., D0-D10, D10-D28 and D28-D35) and for the complete study period (i.e., D0-D35). Average feed intake and average daily weight gain (ADG) *per* pen were used to calculate the average feed:gain ratio (F:G). Blood collection was conducted on Day 35 from one randomly selected animal from each replicate (pen) (32 chickens in total, eight *per* treatment group). The blood samples were drawn by wing vein puncture using 10 cc heparinized (lithium heparin) disposable syringes by Becton Dickinson Acute Care (U.S.A.), centrifuged for 10 min. at 3000 rpm and then the plasma was transferred with Pasteur pipette in a plastic test tube and frozen for later routine biochemistry analysis of the parameters: glucose, calcium, inorganic phosphorus, cholesterol, triglycerides, phospholipids, uric acid, urea, creatinine, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and total bilirubin. The same animals utilized for blood analysis were necropsied and evaluated by a veterinary surgeon for gross pathology of: external skin, eyes and any injuries, 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. Tissue and organ samples (liver, both kidneys and breast muscular tissue, fat+skin tissue around the breast) were also retained (frozen at -20°C) for sanguinarine and chelerythrine analysis.

Statistical analysis: Data were analyzed by the General Linear Model (GLM) procedure of SAS (release 9.1) (Cary, NC, USA). The Student "t" test was used to compare the means of each group. The level of significance stated in the ANOVA model was $p = 0.05$ when the difference was statistically significant and $0.05<p = 0.10$ when the difference was a near-significant trend. The raw data was also analyzed for outliers. No data was excluded from the statistical analysis as all animals were in good health and none required removal from the trial.

Feed, chicken for fattening organ and tissue analysis for sanguinarine and chelerythrine

Sample extraction: Sanguinarine and chelerythrine were extracted from feed samples using acidified methanol, under reflux for 45 min at 60°C. After centrifugation and dilution, the extracts were analyzed by HPLC-MS/MS using a Phenomenex Gemini column (Phenomenex, Torrance, CA, USA). Organ and tissue samples were homogenized and sanguinarine/ chelerythrine was extracted using acidified methanol. After purification through an SPE column, the purified extract was concentrated, diluted, filtered and analyzed by HPLC-MS/MS using a Phenomenex Hilic-Kinetex column (Phenomenex, Torrance, CA, USA). Standard solutions were prepared by solubilizing a reference standard of sanguinarine and chelerythrine (Sigma-Aldrich, St. Louis, MO, USA) in untreated (blank) purified extract.

Analytical method validation: Prior to sample analysis, the method was validated for each matrix, with a known volume of standard solution (three different levels of standards) added to untreated matrix and the extraction for sanguinarine/chelerythrine conducted as stated above. Three replicates were carried out for each level. Untreated matrix (control) for each organ/tissue/feed was also extracted and analyzed. Validation was conducted for: specificity, accuracy, precision, detection limit, quantification limit, linearity and range. The limit of detection (LOD) was found to be 10 µg/kg in muscle, liver and fat and 15 µg/kg in kidney tissue, for both sanguinarine and chelerythrine. The LOD in feed was 0.05 mg/kg. The limit of quantification (LOQ) was 45 µg/kg (ppb) in muscle, liver and fat and 60 µg/kg (ppb) in kidney tissue for both sanguinarine and chelerythrine. The LOQ was 0.1 mg/kg (ppm) in feed. Three replicates were carried out for each matrix and precision was evaluated calculating the relative standard deviation (RSD) between the replicates, which were lower than 10%.

Recovery analysis was calculated from the difference between the amount of standard added and the amount detected in the validation assay. The standard solution was added at three different concentrations and three replicates were carried out for each level. Sanguinarine recovery in the feed for test item levels of 1.5, 7.5 and 15.0 mg sanguinarine/kg feed ranged from 93.1-106.7%, while recovery of chelerythrine for the same addition levels ranged from 87.6-96.7%. Recovery of sanguinarine/chelerythrine from muscle, liver, fat and kidney samples at three concentrations (80, 200 and 400 µg/kg tissue) were also analyzed for the evaluation of sanguinarine levels in biological matrices. Recovery of chelerythrine for the same concentrations ranged from 81.1-108.8% for all tissues.

RESULTS AND DISCUSSION

Daily inspection of the animals did not indicate any adverse effects on the animals; there was no difference in fecal consistency between groups and low mortality (0.63% as mean of all treatments) with no test substance-related increases in mortality. No statistical differences were found among feeding treatments for the parameters of live weight, ADG, FI and F:G (Table 2). One study published by Kozlowski *et al.* (2008) found that consumption of rations supplemented with 20 mg/kg feed MCEP for five weeks had no significant effect on feed intake, live weight gain or feed conversion ratio of fattening chickens, while work by Vieira *et al.* (2008) reported transient increases in live weight and cumulative effects of MCEP (50 mg/kg feed from D1 to D21 and 25 mg/kg feed from D22 to D42) containing 1.5% sanguinarine, on feed conversion during a 42-day feeding study in male Cobb broiler chicks. Vieira *et al.* (2008) also reported a dose-dependent increase in feed intake with MCEP, which was not observed in the current study.

The results of plasma analysis are summarized in Table 3. Statistical differences were only found for two of the blood plasma parameters among the treatment groups. The creatinine level was significantly lower in the T3 group (500 mg/kg feed) than in the T1 (control) and T4 (1000 mg/kg feed) groups (39.76 *vs.* 45.53 and 47.17 µmol/L, respectively, $p = 0.0463$). Also the alkaline phosphatase level was significantly higher in the T2 (100 mg/kg feed) group, compared to the T1 (control) and T3 (500 mg/kg feed) groups (2072.25 vs. 1831.25 and 1809.00 U/L, respectively, $p = 0.0267$). However, these responses were not dose-dependent and therefore were not considered treatment-related. No statistical differences were found among feeding treatments for all other parameters (Table 3).

Statistical evaluation of the necropsy results showed no statistically significant differences between feeding treatments for all parameters (Table 4). Minor variations among the treatment groups occurred in group mean weights for the leg ($p = 0.1653$), stomach

Table 1: Composition (%) and analytical characteristics (as feed) of the basal diets in the 3 growing periods

Ingredient (%)	First experimental period (D0-D10)	Second experimental period (D10-D28)	Third experimental period (D28-D35)
Corn meal	50.00	56.00	62.90
Soybean meal 44%	42.25	36.18	30.00
Soybean oil	2.00	2.40	2.00
Hydrogenated palm fat	2.00	2.00	2.00
DL Methionine (95%)	0.18	0.17	0.10
Calcium carbonate	0.25	0.25	0.22
Dicalcium phosphate	2.50	2.20	2.00
Salt	0.30	0.30	0.20
Sodium bicarbonate	0.27	0.25	0.33
Vitamins and minerals[1]	0.25	0.25	0.25
Analytical characteristics (%)			
Dry matter	89.82	88.91	87.69
Crude protein	22.20	20.45	18.54
Ether extract	6.52	6.69	6.32
Crude fibre	3.34	3.29	3.47
Ash	6.42	6.36	4.85
Starch	37.97	40.28	40.02
Carbohydrate	4.41	3.88	3.91
Metabolizable energy[2]	12.59	12.69	12.33
Sanguinarine in the diets			
T1	<0.10	<0.10	<0.10
T2	2.11	2.08	1.95
T3	7.75	8.12	8.10
T4	15.47	15.70	15.31
Chelerythrine in the diets			
T1	<0.10	<0.10	<0.10
T2	1.03	1.04	0.99
T3	3.74	3.97	3.86
T4	7.27	7.38	6.52

[1]Vitamin/mineral premix employed at 5 kg/ton feed. Each kg premix contains: Vit. A: 2,500,000 IU; Vit D3: 600,000 IU; Vit. E: 15,000 IU; Vit. K: 1,200 mg; Vit. B1: 400 mg; Vit. V2: 1,600 mg; Pantothenic acid: 2,500 mg; Vit. B6: 1,200 mg; Biotin: 30 mg
Folic acid: 250 mg; Vit. C: 20,000 mg; Niacin: 8,000 mg; Vit. B12: 6 mg; Cu: 1,000 mg; Fe: 10,000 mg; Mn: 30,000 mg; Se: 40 mg; Zn: 15,000 mg; I: 200 mg; Co: 40 mg;
[2]According to the equation proposed by the EU legislation (G.U.CE L54 of February 26,2009)

(p = 0.5796), gizzard (p = 0.8637), small intestine (p = 0.5796), caecum (p = 0.8637), colon (p = 0.5496), liver (p = 0.5496), lungs (p = 0.8637), muscle (p = 0.5496) and fat (p = 0.5796), but none were statistically significant effects. No dose-dependent differences were noted for any of the parameters analyzed and no statistically significant effects occurred. Therefore, it was concluded that there were no test-article related effects on the tissues.

Analyses for residual levels of sanguinarine or chelerythrine were conducted on organs and tissues from eight chickens/treatment. No residual levels of sanguinarine or chelerythrine were found in the organs and tissues of T1 (Control) and T2 (100 mg/kg feed) animals. Residual levels of sanguinarine were found in two fat+skin tissue samples, one containing 89.79 µg/kg and the other 56.68 µg/kg sanguinarine in the T3 (500 mg/kg feed) group. In the T4 (1000 mg/kg feed) group three fat+skin tissue samples contained residual levels of sanguinarine (55.36, 117.64 and 62.32 µg/kg) and one kidney sample contained a residual level of sanguinarine at 96.61µg/kg (Table 5). All other samples were either below the LOQ or LOD (data not shown). No chelerythrine was found in any of the tissues (Table 5). The absence of sanguinarine or chelerythrine in the muscle tissue is in agreement with work conducted by Kosina *et al.* (2004), who did not find sanguinarine or chelerythrine residues in muscle tissues of swine fed a *M. cordata* extract at 2 and 100 mg/kg feed for 90 days, but did find sanguinarine in plasma, liver gingiva, tongue, stomach and intestine (4-79 µg/kg range) when *M. cordata* extract was consumed at the 2 mg/kg feed level. The *M. cordata* extract used by Kosina *et al.* (2004) contained approximately 64% sanguinarine, this and the higher dosage may explain the higher levels of sanguinarine found in the tissues than what was found in the current study. In swine consuming *M. cordata* extract at 100 mg/kg feed, sanguinarine was found in the liver (113±33 µg/kg), plasma (108±4 µg/kg), gingiva (514±29 µg/kg), tongue (32±8µg/kg), stomach (52±22 µg/kg) and intestine (124±41 µg/kg) (Kosina *et al.*, 2004). No residues where found in the muscle tissue, consistent with the current study, although the pigs received a much higher dosage then the chickens were provided in the current study.

Table 2: Live weight and feed intake results

Experimental period	T1	T2	T3	T4	p-value	SEM
Live weight (g)						
D0	43	43	42	43	0.7817	0.1955
D10	249	247	244	250	0.8572	5.2489
D28	1270	1253	1240	1288	0.4609	21.9797
D35	2131	2144	2096	2159	0.6362	35.6704
Average daily gain (g)						
D0-D10	20.6	20.4	20.2	20.8	0.8687	0.5256
D10-D28	36.5	35.7	35.6	37.1	0.4651	0.7337
D28-D35	123.1	127.2	122.2	124.4	0.8074	3.8313
D0-D35	46.4	46.3	45.6	47.0	0.6689	0.7944
Daily feed intake (g)						
D0-D10	30.5	29.7	29.8	31.0	0.3849	0.6256
D10-D28	60.0	59.4	59.2	62.1	0.3375	1.2050
D28- D35	249.0	247.8	245.4	247.3	0.9726	5.4614
D0-D35	82.9	81.7	81.6	84.0	0.4203	1.1428
Feed:gain ratio						
D0-D10	1.49	1.46	1.48	1.50	0.9542	0.0478
D10-D28	1.65	1.67	1.67	1.68	0.8834	0.0289
D28-D35	2.03	1.95	2.01	2.00	0.6935	0.0490
D0-D35	1.79	1.77	1.79	1.79	0.8916	0.0256

All data mean values±SEM = standard error of the mean. T1: Control group; T2: MCEP (100 mg/kg feed); T3: MCEP (500 mg/kg feed); T4: MCEP (1000 mg/kg feed)

Table 3: Biochemical analysis of plasma

Experimental period	T1	T2	T3	T4	p-value	SEM
Glucose (mmol/L)	13.93	13.51	12.38	13.83	0.3739	0.6833
Calcium (mmol/L)	2.58	2.19	2.46	2.59	0.4959	0.2065
Inorganic phosphorus (mmol/L)	2.24	2.30	2.22	1.91	0.1320	0.1234
Cholesterol (mmol/L)	3.50	3.95	3.83	3.85	0.3318	0.1774
Triglycerides (mmol/L)	1.12	1.07	1.22	1.05	0.8248	13.1116
Phospholipids (mg/100 mL)	234.50	253.63	257.75	247.75	0.6217	13.1116
Uric acid (mg/dL)	3.87	4.07	3.55	3.55	0.5740	0.3136
Urea (mmol/L)	0.38	0.40	0.43	0.52	0.2972	0.0568
Creatinine (µmol/L)	45.53^{b}	42.98	39.76^{a}	47.17^{b}	0.0463	1.8588
Alkaline phosphatase (U/L)	1831.25^{a}	2072.25^{b}	1809.00^{a}	1948.88	0.0267	64.2694
Aspartate aminotransferase (U/L)	246.75	211.63	280.88	242.25	0.2461	23.4423
Alanine aminotransferase (U/L)	3.50	3.25	3.13	4.00	0.8436	0.7391
Bilirubin (µmol/L)	3.04	2.74	2.78	3.72	0.4976	0.5045
Lactate dehydrogenase (U/L)	1158.88	987.13	1260.50	1259.25	0.4262	131.4661

All data mean values±SEM = standard error of the mean; a, b in the same row = statistically significant difference at $p<0.05$
T1: Control group; T2: MCEP (100 mg/kg feed); T3: MCEP (500 mg/kg feed); T4: MCEP (1000 mg/kg feed)

Table 4: Necropsy results

Experimental period	T1	T2	T3	T4	p-value	SEM
External skin	0.000	0.000	0.000	0.000	-	0
Leg	0.000	0.125	0.500	0.125	0.1653	0.1602
Eyes	0.000	0.000	0.000	0.000	-	0
Anus	0.000	0.000	0.000	0.000	-	0
Oral cavity	0.000	0.000	0.000	0.000	-	0
Esophagus	0.000	0.000	0.000	0.000	-	0
Stomach	0.000	0.000	0.125	0.125	0.5796	0.0884
Gizzard	0.125	0.250	0.250	0.125	0.8637	0.1456
Small Intestine	0.000	0.000	0.125	0.125	0.5796	0.0884
Caecum	0.250	0.125	0.250	0.125	0.8637	0.1456
Colon	0.250	0.125	0.125	0.000	0.5496	0.1205
Pancreas	0.000	0.000	0.000	0.000	-	0
Liver	0.250	0.125	0.125	0.000	0.5496	0.1205
Lungs	0.250	0.125	0.250	0.125	0.8637	0.1456
Kidneys	0.000	0.000	0.000	0.000	-	0
Heart	0.000	0.000	0.000	0.000	-	0
Muscle	0.250	0.125	0.125	0.000	0.5496	0.1205
Fat	0.125	0.000	0.125	0.000	0.5796	0.0884

All data mean values±SEM = standard error of the mean, (1) Scored according to the following:
0 = no alteration found; 1.000 = Slight alterations; 2.000 = Alteration of medium intensity; 3.000 = Serious alteration
T1: Control group; T2: MCEP (100 mg/kg feed); T3: MCEP (500 mg/kg feed); T4: MCEP (1000 mg/kg feed)

A comprehensive analysis of the health of swine consuming a *M. cordata* extract, including clinical pathology, hematology, histology and clinical chemistry parameters has been previously published

Table 5: Quantifiable levels of sanguinarine and chelerythrine in tissues

Treatment group*	Matrix	Sanguinarine (µg/kg)	Chelerythrine (µg/kg)
T1 (Control)	All matrices	<LOD	<LOD
T2 (100 mg/kg)	All matrices	<LOD/< LOQ	<LOD
T3 (500 mg/kg)	Fat	89.79	<LOQ
	Fat	56.68	<LOQ
	All other matrices	<LOD/<LOQ	<LOD/<LOQ
T4 (1000 mg/kg)	Fat	55.36	<LOQ
	Fat	117.64	<LOQ
	Fat	62.32	<LOQ
	Kidney	96.61	<LOQ
	All other matrices	<LOD/<LOQ	<LOD/<LOQ

LOQ: limit of quantification; *Tissue samples from eight animals/treatment were analyzed for sanguinarine and chelerythrine; only those samples with quantifiable levels are shown. All other samples were below limit of detection (LOD and below the LOQ)

indicating the safety of the *M. cordata* extract, but the current work is the first such comprehensive tolerance study conducted in chickens up to date. The current work confirms that consumption of MCEP at up to 1000 mg/kg feed was well tolerated by chickens for fattening for 35 days.

Conclusion: In conclusion, the results of the study showed no adverse effects of consumption of the MCEP provided to chickens for fattening and incorporated into the diet at 100, 500 or 1000 mg MCEP/kg feed. No residual levels of sanguinarine or chelerythrine were found in organs or tissues of chickens fed 100 mg MCEP/kg feed, while sanguinarine was found in two fat+skin tissue samples at 89.79 and 56.68 µg/kg when MCEP was fed at 500 mg/kg feed. Three fat+skin tissue samples (55.36, 117.64 and 62.32 µg/kg) and one kidney sample (96.61 µg/kg) contained residual levels of sanguinarine at 1000 mg/kg feed. MCEP was well tolerated by the animals, with no adverse effects noted at levels up to 20 times the recommended consumption level.

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