Effects of dietary Macleaya cordata extract on growth performance, immune responses, antioxidant capacity, and intestinal development in weaned piglets

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1. Introduction

Weaning is probably the most stressful time in a pig’s life. From the physiological point of view, they are physiologically not fully competent to deal with the multiple social, nutritional, environmental and immunological changes associated with weaning (Pluske et al. 1997). Weaning is associated with changes to the architecture of the small intestine, disturbed intestinal microbiota and diminished immune responses (Spreeuwemberg et al. 2001; Boudry et al. 2004; Pieper et al. 2008), as well as accompanied by a decreased nutrient intake and digestibility, weight loss and growth performance depression (Kim et al. 2012). Antibiotics have been widely used to improve the health and growth performance in weaned piglets (Frydenhahl 2002; Pluske 2013). However, the misuse of antibiotics has led to bacterial resistance in livestock animal and humans, as well as antibiotics-residue in animal products, which may affect for human health (Schwarz et al. 2001; Kolpin et al. 2002). Therefore, alternative feed additives should be investigated in livestock.

Macleaya cordata extract (MCE) is a rich source of sanguinarine. Many studies reported that sanguinarine is an antimicrobial (Newton et al. 2002; Kosina et al. 2010), anti-oxidant (Chaturvedi et al. 1997), anti-inflammatory (Tanaka et al. 1993; Yiu and Wei 1993; Niu et al. 2012), and immuno-modulatory products (Chaturvedi et al. 1997). Dietary supplementation with MCE has beneficial effects for pigs (Jeroch et al. 2009; Kantas et al. 2015), broiler chickens (Vieira et al. 2008), and fish (Zhang et al. 2013). Supplementation of MCE into the broilers’ diet at 20 or 50 ppm improved growth performance, increased relative jejunal or ileal length, and altered gut microbiota in broiler chickens (Lee et al. 2015). A recent study showed that supplementation of post-weaning piglet diets with 120 mg MCE per kg diet improved growth performance and nutrient digestibility (Goodarzi et al. 2018). Although MCE appears to increase the production performance of multiple species including swine, little data are available on its effects on weanling stage pigs. We hypothesized that MCE affects gastrointestinal tract integrity, immune response, and microbial populations in weanling pigs. Thus, we envision that growth performance and gastrointestinal tract health and function of MCE supplemented weanlings will be similar to antibiotic-treated animals. Therefore, the objective of this study was to investigate the effect of dietary MCE on growth performance, immune
status, antioxidant capacity, intestinal microbiota and morphology in weaned piglets.

2. Materials and methods

All procedures were approved by the Committee of Animal Care at the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Sangrovit® is a standardized pre-mixture of MCE, combined with a carrier of dried and ground plant material from the Papaveraceae family, standardized to provide at least 12.5 g/kg mixture of quaternary-benzo(c)phenanthridine alkaloids (e.g. sanguinarine and chelerythrine) and protopine alkaloids (e.g. protopine and allocryptopine), among which 1.5% w/w sanguinarine was used as a main marker, and is manufactured from extracts of M. cordata (Micolta Bioresource Co. Ltd., Changsha 410128, China).

2.1. Experimental design, animals, diets, and husbandry

A total of 36 crossed healthy weaned piglets [Duroc × (Large White × Landrace)] with an average body weight (BW) of 6.55 ± 0.32 kg (21 d of age) were used in this experiment. The piglets were randomly classified into 3 groups including basal diet (Control), basal diet + 50 mg/kg MCE (MCE group), and basal diet + 20 mg/kg flavomycin + 100 mg/kg aureomycin (ABO group). The feeding protocol was carried out for 22 days until 42 days of age. A corn-soybean meal basal diet containing no antibiotics was present in Table 1 according to the nutritional requirements (NRC 2012). Individual animals were housed in stainless steel metabolic cages (150 cm long × 105 cm wide × 120 cm high) in a temperature-controlled nursery house (25°C to 27°C). Relative humidity was controlled at 60–70%. Each animal was housed in a stainless steel metabolic cage (150 cm long × 105 cm wide × 120 cm high). All the pigs had ad libitum access to the diets and clean drinking water.

Table 1. Ingredients and chemical composition of experimental diets (as-fed basis).

| Ingredients                  | Content (%) |
|------------------------------|-------------|
| Corn                         | 35.00       |
| Soybean meal                 | 19.00       |
| Full-fat soybean powder      | 10.00       |
| Fish meal                    | 5.00        |
| Whey powder                  | 6.15        |
| Soybean oil                  | 1.50        |
| Dicalcium phosphate          | 0.90        |
| L-Lysine-HCl                 | 0.48        |
| L-Threonine                  | 0.05        |
| DL-Methionine                | 0.10        |
| L-Tryptophan                 | 0.02        |
| Salt                         | 0.30        |
| Limestone                    | 0.50        |
| Premix®                      | 1.00        |
| Total                        | 100.00      |

Calculated nutrients

| Nutrients       | Content (MJ/kg) |
|-----------------|-----------------|
| Digestible energy | 14.64          |
| Crude protein   | 20.15           |
| Lysine          | 1.38            |
| Methionine      | 0.82            |
| Methionine + cysteine | 1.01        |
| Tryptophan      | 0.97            |
| Calcium         | 0.80            |
| Total phosphorus| 0.73            |

*The premix provided the following (per kilogram of compound feed): Vitamin A, 12,000 IU; Vitamin D, 2500 IU; Vitamin E, 30 IU; Vitamin B12, 12 μg; Vitamin K, 3 mg; d-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline chloride, 400 mg; Mn, 40 mg; Zn, 100 mg; Fe, 90 mg; Cu, 8.8 mg; I, 0.35 mg; Se, 0.3 mg.

2.2. Growth performance and diarrhea incidence

The individual BW and feed consumption were measured at the beginning and the end of the trial. Those data were used for calculating the average daily gain (ADG), average daily feed intake (ADFI), and the ratio of feed to gain (F/G). The clinical signs of diarrhea were visually assessed every day by observers blinded to treatments, and a scoring system was applied to indicate the presence and severity of diarrhea as follows: 1 = hard feces; 2 = slightly soft feces; 3 = soft, partially formed feces; 4 = loose, semiliquid feces; and 5 = watery, mucous-like feces. When the average score was over 3, pigs were identified as having diarrhea. Diarrhea rate was calculated according to the formula (Sun et al. 2008):

Diarrhea rate (%) = \( \frac{\text{the number of diarrhea pigs × diarrhea days}}{\text{the total number of pigs × experiment days}} \)

2.3. Sample collection and preparation

At the end of experiment (21 days of experiment), 5 mL blood samples were collected via anterior vena cava puncture from all of pigs and centrifuged at 3000 × g and 4°C for 15 min to separate out the serum. The serum samples were then stored at −20°C for immunoglobulin (Ig) and antioxidative index analysis. Then all of the animals were humanely killed by lethal intraperitoneal injections of sodium pentobarbital, as described by Yao et al. (2011). Segments of the mid-duodenum, mid-jejunum and mid-ileum in each animal were collected for morphological examination. The luminal digesta of the cecum was collected and stored at −80°C until the gut microbial composition analysis.

2.4. Bacterial quantification by real-time PCR

Total bacterial DNA was extracted from the contents of each intestinal sample (0.2 g) according to a previously described protocol (Kraler et al. 2016), using a QIAamp DNA Stool Mini Kit (Qiagen, Germany). Those extracts were stored at −80°C. They were then quantified on a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Courtaboeuf, France) before the results were adjusted to a concentration of 10 ng/μL.

Methods based on 16S rRNA were used to assess the abundances of Bifidobacterium spp., Escherichia coli, Lactobacillus spp., and Salmonella spp. as previously described (Chen et al. 2018). All PCR primers are listed in Table 2. Duplicate sample analyses were performed in mixtures (final volume, 10 μL) that contained 1 μL of diluted DNA sample and 0.2 μL of each primer, using a 1× of SYBR® Premix Ex Taq™ II Kit (TaKaRa Bio Inc., Shiga, Japan). The amplification programme included 95°C for 30 s; followed by 40 cycles of 95°C for 5 s and 60°C for 30 s; and then a final melting curve for SYBR Green tests. The melting curve analysis and size-
determination of amplificates on agarose gels verified that the target fragments had been amplified. Standard curves were generated as described by Qi et al. (2011). Results were expressed in log_{10} copies of 16S rRNA genes per gram of intestinal material in each sample and bacterial group (Metzler-Zebeli et al. 2015).

2.5. Serum parameters assay

Serum antioxidant-related indices, including total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT) and the concentration of malondialdehyde (MDA), were determined with commercially available reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Moreover, the levels of immunoglobulin (IgM, IgG and IgA) in serum was detected by an enzyme-linked immunosorbent assay (ELISA) kit (Bogoo Biotechnology Co. Ltd., Shanghai, China). All measurements were done at least in triplicate and according to the manufacturer’s instructions.

2.6. Morphological measurements

Histological samples were rapidly fixed in neutral-buffered formalin. Sections of the small intestine were excised, dehydrated, and embedded in paraffin wax before four transverse sections were cut, placed on glass slides, and stained with haematoxylin and eosin. The villus height and crypt depth of 10 well-oriented villi per segment were measured using a Nikon ECLIPSE 80i light microscope equipped with a computer-assisted morpho-metric system (Nikon Corporation, Tokyo, Japan) (Chen et al. 2016).

Table 2. Sequences of primers and probes used for group-specific quantitative PCRs.

| Bacterial group/species | Forward | Reverse | Probe |
|-------------------------|---------|---------|-------|
| *Bifidobacterium* spp.  | CGG GTG AGT AAT GCG TGA CC | TGA TAG GAC GCG ACC CCA | (6FAM)CTCTGAAAAGCGTGT(BHQ1) |
| *Salmonella* spp.       | CGGCGGTGGAGGAGGATA | AGCAATGAAAACGAGGATG | FAM-CATTCTAAACCCGCGGTCTTCCCT-MGB |
| *E. coli*               | CGG GTA AGC TCA ATG AGC AAA | CAT GCC GCG TGT ATG AAG AA | (6FAM)TATTAACCTTTATCTTCCCTCCCGGTGA(BHQ1) |
| *Lactobacillus* spp.    | AGC AGT AGG GAA TCT TCCA | CGC CAC TGG TGT TCY TCC ATA |

Table 3. Effects of dietary MCE supplementation on growth performance of weaned piglets.

| Items*                | Control | MCE | ABO | SEM† | P-value |
|-----------------------|---------|-----|-----|------|---------|
| Initial weight, kg    | 6.56    | 6.54| 6.55| 0.05 | 0.910   |
| Final weight, kg      | 12.36   | 13.29| 13.06| 0.31 | 0.235   |
| ADG, g                | 276 b   | 321 a| 310 a| 7.34 | 0.021   |
| ADFI, g               | 496 b   | 522 a| 522 a| 12.52| 0.048   |
| F/G                   | 1.80 a  | 1.64 b| 1.68 b| 0.07 | 0.036   |

*ADFI, average daily feed intake; ADG, average daily gain; F/G, Feed/gain.
†Treatments consisted of (1) Control; basal diet, (2) MCE; basal diet + 50 mg/kg MCE and (3) ABO; basal diet + 20 mg/kg flavomycin + 100 mg/kg aureomycin.
‡SEM, pooled standard error of mean.
§Means within each row with different superscripts differ significantly (P < 0.05).

2.7. Statistical analysis

All data were analyzed by analysis of variance (ANOVA) as a randomized complete block design using the General Linear Model (GLM) procedure of the IBM SPSS v 20.0 (IBP SPSS Institute Inc., 2011). Individual pig served as the experimental unit for the analysis of all data. Significant differences between means were determined using Tukey’s multiple comparison test. The results were shown as mean and standard error of the mean (SEM). A value of P ≤ 0.05 was considered statistically significant, whereas 0.05 < P < 0.10 was considered as trend.

3. Results

3.1. Growth performance and diarrhea ratio

As shown in Table 3, piglets fed with MCE increased the ADG by 16.38% (P < 0.05) and ADFI by 6.35% (P < 0.05) compared with the piglets fed the control diet, decreased the F/G by 8.89% (P < 0.05). Supplementation with ABO also significantly increased ADG and ADFI (P < 0.05) in comparison to the control group. However, there was no significant effects between MCE and ABO groups.

As shown in Figure 1, supplemental MCE and ABO decreased the diarrhea ratio by 83.8% (P < 0.05) and 64.0% (P < 0.05), respectively.
respectively, as compared with control group. There was no significant difference between MCE and ABO groups.

### 3.2. Serum immunity and antioxidant capacity

As shown in Table 4, the amount of IgG in MCE group was higher than control group ($P < 0.05$). However, there was no difference in serum IgA and IgM levels among the groups ($P > 0.05$).

The effects of experimental diets on the activities of antioxidant enzymes in the serum of piglets are presented in Table 5. A higher ($P < 0.05$) serum T-AOC, GSH-Px and SOD was observed in MCE-supplemented piglets than those in the control and ABO groups, while piglets fed with MCE decreased the serum MDA content ($P < 0.05$), and tended to increase the activities of serum CAT ($P < 0.10$) compared with the control and ABO groups. Antibi-otics supplementation had no significant effect ($P > 0.05$) on the serum antioxidant indices compared to the control group.

### 3.3. Intestinal microflora

After a 21-d feeding, piglets fed the MCE diet had higher ($P < 0.05$) cecal Lactobacillus spp. population than piglets fed the control and ABO diet, and had lower ($P < 0.05$) Salmonella spp. than piglets fed the control diet (Figure 2). Piglets fed the ABO diet had lower ($P < 0.05$) cecal E. coli population than piglets fed the control diet. Piglets fed the MCE diet had tended to decrease ($P < 0.10$) the E. coli than piglets fed the control diet. No treatment effect was found for Bifidobacterium spp. ($P > 0.05$).

### 3.4. Small intestinal morphology

Piglets fed the diet supplemented with MCE had greater ($P < 0.05$) villus height and the villus height to crypt depth ratio respectively, as compared with control group. There was no significant difference between MCE and ABO groups.

### 4. Discussion

Natural compounds extracted from *M. cordata*, such as the quaternary benzo[c]phenanthridine alkaloids (QBAs) sanguinarine has been used as feed additive in both swine and poultry (Vieira et al. 2008; Pellikaan et al. 2010). Gradually, they have evoked attention as a substitute to antibiotic growth promoters (Kim et al. 2012). Kosina et al. (2004) conducted an in vivo safety assessment of sanguinarin (a mixture of sanguinarine and a

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**Table 4.** Effects of dietary MCE supplementation on immune response of weaned piglets.

| Items* | Control | MCE | ABO | SEM$^1$ | $P$-value |
|--------|---------|-----|-----|---------|-----------|
| IgA, g/L | 1.77    | 1.53 | 1.66 | 0.28    | 0.743     |
| IgG, g/L | 6.77$^b$ | 11.53 | 6.86$^b$ | 1.01    | 0.017     |
| IgM, g/L | 0.63    | 0.78 | 0.65 | 0.05    | 0.275     |

$^*$IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

$^1$SEM, pooled standard error of mean.

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**Table 5.** Effects of dietary MCE supplementation on serum antioxidant activity of weaned piglets.

| Items* | Control | MCE | ABO | SEM$^1$ | $P$-value |
|--------|---------|-----|-----|---------|-----------|
| T-AOC, U/mL | 8.22$^b$ | 9.49$^a$ | 8.15$^b$ | 1.27    | 0.013     |
| GSH-Px, U/mL | 659$^a$ | 751$^b$ | 647$^a$ | 13.77   | 0.018     |
| SOD, U/mL | 111$^b$ | 142$^a$ | 118$^b$ | 5.72    | 0.026     |
| CAT, μM  | 7.23    | 8.14 | 7.09 | 1.03    | 0.075     |
| MDA, nmol/mL | 5.49$^a$ | 3.18$^b$ | 5.36$^a$ | 0.11    | 0.003     |

$^*$T-AOC: Total antioxidant capacity; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde.

$^1$SEM, pooled standard error of mean.

**Table 6.** Effects of dietary MCE supplementation on intestinal morphology of weaned piglets.

| Items* | Control | MCE | ABO | SEM$^1$ | $P$-value |
|--------|---------|-----|-----|---------|-----------|
| Villus height, μm | 437$^b$ | 474$^a$ | 472$^a$ | 36.78   | 0.015     |
| Jejunum | 353$^b$ | 398$^a$ | 380$^a$ | 42.19   | 0.027     |
| Ileum   | 413$^b$ | 444$^a$ | 432$^a$ | 38.82   | 0.038     |
| Crypt depth, μm | 306    | 303 | 298 | 27.91   | 0.619     |
| Duodenum | 244    | 219 | 232 | 24.65   | 0.034     |
| Jejunum | 224    | 201 | 216 | 31.42   | 0.174     |
| Ileum   | 1.43$^b$ | 1.56$^a$ | 1.58$^a$ | 0.23    | 0.047     |
| Jejunum | 1.44$^b$ | 1.82$^a$ | 1.64$^a$ | 0.31    | 0.009     |
| Ileum   | 1.85$^b$ | 2.20$^a$ | 2.00$^a$ | 0.40    | 0.018     |

**Figure 2.** Occurrence of bacterial groups in cecal samples of weaned piglets fed dietary treatments, determined by qPCR (log$_{10}$) 16S rRNA gene copy number/g fresh matter. Treatments consisted of (1) Control; basal diet, (2) MCE; basal diet + 50 mg/kg MCE and (3) ABO; basal diet + 20 mg/kg flavomycin + 100 mg/kg aureomycin. Bars represent mean ± SD of 12 pigs per group. The letters a, b, c indicate the difference between treatment groups ($P < 0.05$).
structurally similar alkaloid, chelerythrine) (5 mg/kg body weight) in pigs following its oral administration for 90 days. Similar to the findings with sanguinarin, administration of MCE for 90 days at doses higher than the daily recommended dosage were well tolerated in rats (Zdarilova et al., 2008). They demonstrated no signs of toxicity and impairment in the health status of the animals, and the remaining alkaloid was unabsorbed and excreted in the feces (Psotova et al. 2006). Furthermore, *M. cordata* was showed up in the European Food Safety Authority (EFSA) database (Ni et al. 2016). Previous study demonstrated that dietary supplementation with MCE improved growth performance, gut health, immune systemand digestive function and reduced the diarrhea in nonruminant mammals (Gudev et al. 2004; Jankowski et al. 2009; Zhang and Coultas 2013; Liu et al. 2016). In the present study, piglets fed with MCE increased the ADG and ADFI and decreased F/G. In agreement with previous studies, improvement of growth performance (increase of BW, ADG and feed intake, reduction of feed conversion rate) in weaning pigs was found (Kantas et al. 2015). Similarly, supplementation of post-weaning piglet diets with 120 mg MCE per kg diet also caused improvement in body weight gain and feed conversion rate (Goodarzi et al. 2018). Moreover, the growing broilers fed MCE at 50 and 25 ppm (1–21 d and 22–42 d, respectively) had improved BW and feed conversion rate at 21 d of age (Vieira et al. 2008). It has been reported that sanguinarine can regulate the serotonin synthesis by employing tryptophan and has been reported that sanguinarine can regulate the serotonin synthesis by employing tryptophan and could be used as an anti-parasitic and anti-diarrheal agent in piglets fed with MCE-containing diets increased the levels of serum IgG. Liu et al. (2016) reported that supplementing pig diet with MCE increased the concentration of IgG which is in consistent with the present findings. In addition, dietary supplementation with MCE increased the levels of serum IgG and IgM in broilers (Yakhkeshi et al. 2011). The improvement on anti-inflammatory results in better immunomodulatory properties (Gessner et al. 2017). Chaturvedi et al. (1997) have demonstrated that sanguinarine is a potent inhibitor of NF-κB activation via inhibiting the phosphorylation and degradation of IkBα, an inhibitory subunit of NF-κB. As the activity of the transcription factor NF-κB influences the immune system. These findings suggest that dietary MCE can improve humoral immunity of pigs.

Weaning has been demonstrated to cause oxidative stress reflected by oxidative imbalance in piglets (Zhu et al. 2012; Yin et al. 2014). Previous studies have shown that such oxidative stress problems can be alleviated by supplementation with herbal plant (e.g. *alfalfa saponin* and *piper sarmentosum*) (Shi et al. 2014; Wang et al. 2016). Sanguinarine may have exerted its anti-oxidative function by impairing the activity of the nitroso-cysteine adenine dinucleotide phosphate (NADPH) enzyme, which is supported by a study by Qin et al. (2006), which demonstrated that sanguinarine is an enzyme inhibitor rather than a reactive oxygen species (ROS) scavenger. Liu et al. (2015) confirmed that sanguinarine can inhibit NADPH oxidase 2 (NOX2) NADPH oxidase activity and ROS generation of H9c2 cardiac cells induced by Angiotensin II. Antioxidant capacities of MCE were shown in this study by measuring some antioxidant parameters in serum, such as T-AOC, SOD, GSH-Px, CAT and MDA. The SOD, GSH-Px and CAT are the main parameters used to assess oxidative status in the enzymatic system. The SOD is a group of metalloenzymes that protect cells from superoxide radicals by degrading the superoxide radicals into hydrogen peroxide (Hao et al. 2015), and the CAT decompose hydrogen peroxide into water (Desagher et al. 1996). The GSH-Px can catalyze the reduction reaction of lipid peroxides caused by reduced glutathione, which plays a role in the cell membrane protection (Johnson et al. 2003). The T-AOC level indicates total antioxidative capacity and reflects the nonenzymatic antioxidant defense system (Tao et al. 2014). The MDA is one of the most frequently used indicators of lipid peroxidation. Increased MDA is the consequence of cellular membrane damage initially caused by increased formation of radicals (Niedernhofer et al. 2003). Supplementation of laying hens diet with sanguinarine decreased serum MDA concentration (Bavarsadi et al. 2016). Our results indicated that MCE supplementation can contribute to the improvement of antioxidant capacity for counteracting the oxidative stress caused by weaning.

*Macleaya cordata* extract is known to have anti-inflammatory effects and immunomodulatory properties (Lenfeld et al. 1981; Yiu and Wei 1993). It has been reported that MCE stimulates phagocyte activity and promotes host protective responses (Gudev et al. 2004). The immunoglobulin (IgG, IgA and IgM) protect the extravascular compartment against pathogenic bacteria and microorganisms (Kong et al. 2007; Lauridsen 2010). Furthermore, IgG also has antibacterial and antitoxin effects (Li et al. 2009). In the current study, piglets fed with MCE-containing diets increased the levels of serum IgG. Liu et al. (2016) reported that supplementing pig diet with MCE increased the concentration of IgG which is in consistent with the present findings. In addition, dietary supplementation with MCE increased the levels of serum IgG and IgM in broilers (Yakhkeshi et al. 2011). The improvement on anti-inflammatory results in better immunomodulatory properties (Gessner et al. 2017). Chaturvedi et al. (1997) have demonstrated that sanguinarine is a potent inhibitor of NF-κB activation via inhibiting the phosphorylation and degradation of IkBα, an inhibitory subunit of NF-κB. As the activity of the transcription factor NF-κB influences the immune system. These findings suggest that dietary MCE can improve humoral immunity of pigs.
Generally, microflora will maintain a dynamic equilibrium with each other in order to maintain normal physiological function in the host (Wang et al. 2009). It has been shown that sanguinarine was a potent inhibitor of E. coli, Aeromonas hydrophila, and Salmonella aureus infection (Miao et al. 2011). Dietary supplementation with MCE increased the amount of lactic-acid bacteria in the ileal and cecal contents in broiler chickens (Juskiewicz et al. 2011). Administering sanguinarine in drinking water reduced the Salmonella enteritidis count in the cecum of broilers (Pickler et al. 2013). In addition, the administration of sanguinarine suppressed ileal counts of E. coli and Salmonella spp. in laying hens (Bavarsadi et al. 2016). In the present study, we found that piglets fed diets supplemented with MCE increased the amount of the beneficial microorganisms Lactobacillus spp., and decreased those of the harmful microorganism E. coli and Salmonella spp. Other studies on medicinal plant additives have shown a reduction of Strep tococcus spp. and Clostridium Cluster XIVa in pigs fed polyphenol-rich plant products (Fiesiel et al. 2014). This suggested that MCE has the potential to resist harmful intestinal microflora and enhanced beneficial microflora, thus maintaining intestinal microfloral homeostasis (Zhao et al. 2018). Therefore, supplementation with MCE may improve the intestinal microflora and decreased diarrhea rates, leading to enhance growth performance and health status.

It has been reported that dietary sanguinarine is not metabolized into potentially harmful benz[c]acridine and passes along the small intestine with almost no absorption (Psotova et al. 2006). In this study, an increase in villus height and the ratio of villus height to crypt depth in the duodenum, jejenum and ileum was observed in piglets fed 50 mg/kg MCE after weaning compared with control diet. Similar results were also reported by Bavarsadi et al. (2016), supplementation of laying hens diets with sanguinarine (3.75 and 7.5 mg/kg) increased the ratio of villus height to crypt depth and decreased crypt depth in the jejunum. Moreover, MCE supplementation at 60 mg/kg post-weaning piglets diets increased villus length (Goodarzi et al. 2018). Furthermore, broiler chickens fed diets supplemented with 20 mg/kg MCE had increased relative jejunal and ileal lengths (Lee et al. 2015). In addition, supplementation with isoquinoline alkaloids also reduced the occurrence of lesions in the duodenum, jejenum, and ileum of broiler chickens that were challenged with Eimeria spp. (Xue et al. 2017). Neither sinus dystrophy nor inflammation of the mucosal epithelium was observed when pigs were given a diet supplemented with either 2 ppm (0.0002%) pure sanguinarine or 100 ppm (0.01%) of a sanguinarine preparation (Kosina et al. 2004). Commercial products that contain QBAs (primarily sanguinarine) exhibit the antimicrobial properties that are inherent to those QBAs (Lenfeld et al. 1981). Sanguinarine improved the time of recovery of the gut walls from infections (e.g. clostridia and E. coli) by 60% within hours of the first treatment (Mellor 2001), indicating that sanguinarine inhibited the action of harmful bacteria in the intestinal wall and reduced the production of toxic compounds and damage to intestinal epithelial cells, thereby protecting the intestinal mucosa (Yakhkeshi et al. 2011). Therefore, supplemented with MCE had higher villus height and lower crypt depth at the small intestinal mucosa could result in the low diarrhea and good growth performance.

5. Conclusions

In conclusion, the present data demonstrated that supplementation of weaning piglet diets with MCE improved growth performance, reduced rate of diarrhea among supplemented weanlings. These MCE effects were associated with increased serum IgG and changes in intestinal morphology and function including increased villus height, greater intestinal antioxidant activity, and a higher proportion of beneficial bacteria. Together findings support that MCE supplementation may be a good alternative to adding antibiotics to weaning piglet diet.

Disclosure statement

No potential conflict of interest was reported by the authors.

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