Effects of Gelsemium elegans Extract on Growth Performance, Intestinal Morphology, and Cecal Microbiota in Growing Pigs: Postprint

Authors: Chen Xiaojun, Wang Yu, Wang Shuaishuai, Wu Yong, Li Yuanyi, Sun Zhiliang

Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of Gelsemium elegans extract on growth performance, intestinal morphology, and cecal microbiota in growing pigs. Seventy-five 56-day-old growing pigs were selected and randomly divided into 5 groups with 3 replicates per group and 5 pigs per replicate. The control group was fed a basal diet, the Gelsemium elegans ethanol extract groups were fed experimental diets supplemented with 100, 50, and 25 mg/kg Gelsemium elegans ethanol extract in the basal diet, respectively, and the Gelsemium elegans acid extract group was fed an experimental diet supplemented with 150 mg/kg Gelsemium elegans acid extract in the basal diet, with an experimental period of 49 days. The results showed that, compared with the control group, dietary supplementation with Gelsemium elegans extract had no significant effect on average daily feed intake of growing pigs ($P>0.05$), significantly increased average daily gain ($P<0.05$), and significantly decreased feed-to-gain ratio ($P<0.05$), with the 100 mg/kg Gelsemium elegans ethanol extract demonstrating the optimal effect; compared with the control group, dietary supplementation with 100 mg/kg Gelsemium elegans ethanol extract significantly increased villus height in the jejunum and ileum ($P<0.05$) and significantly decreased crypt depth in the duodenum ($P<0.05$); transmission electron microscopy observation revealed that dietary supplementation with Gelsemium elegans extract resulted in more uniform, orderly, and dense microvilli in jejunal tissue, with tight junctions, intermediate junctions, and desmosomes between adjacent cells all being clearly defined and intact; compared with the control group, dietary supplementation with 50 and 100 mg/kg Gelsemium elegans ethanol extract significantly reduced Escherichia coli populations and significantly increased Lactobacillus populations in the cecum ($P<0.05$). Thus, both Gelsemium elegans ethanol extract and Gelsemium elegans acid extract can improve intestinal morphology, modulate cecal microbiota composition, increase average daily gain, and decrease

feed-to-gain ratio in growing pigs, with 100 mg/kg Gelsemium elegans ethanol extract demonstrating the optimal effect.

Full Text

Effects of Gelsemium elegans Extracts on Growth Performance, Intestinal Morphology and Caecal Microflora in Growing Pigs

CHEN Xiaojun[1], WANG Yu[1], WANG Shuaishuai[1], WU Yong[2], LI Yuanyi[3], SUN Zhiliang[1]*

[1]College of Veterinary Medicine, Hunan Agricultural University, Changsha 410128, China

[2]Hunan Engineering Research Center of Veterinary Drug, Changsha 410128, China

[3]McGill University Health Centre, Montreal H3G 1A4, Canada

Abstract: This experiment was conducted to investigate the effects of Gelsemium elegans extracts on growth performance, intestinal morphology and caecal microflora in growing pigs. A total of seventy-five 56-day-old growing pigs were randomly assigned into 5 groups with 3 replicates per group and 5 pigs per replicate. Pigs in the control group were fed a basal diet, while pigs in the Gelsemium elegans alcohol extract groups were fed the basal diet supplemented with 100, 50 or 25 mg/kg Gelsemium elegans alcohol extract, and pigs in the Gelsemium elegans acidic extract group were fed the basal diet supplemented with 150 mg/kg Gelsemium elegans acidic extract. The experiment lasted for 49 days. The results showed that compared with the control group, dietary supplementation with Gelsemium elegans extracts had no significant effect on average daily feed intake (ADFI) ($P>0.05$), but significantly increased average daily gain (ADG) ($P<0.05$) and significantly reduced feed/gain ratio (F/G) ($P<0.05$), with the 100 mg/kg Gelsemium elegans alcohol extract showing the best effect. Compared with the control group, dietary supplementation with 100 mg/kg Gelsemium elegans alcohol extract significantly increased villus height in the jejunum and ileum ($P<0.05$) and significantly decreased crypt depth in the duodenum ($P<0.05$). Transmission electron microscopy observations revealed that dietary supplementation with Gelsemium elegans extracts resulted in more uniform, orderly and dense microvilli in jejunal tissue, with clearly visible and intact tight junctions, intermediate junctions and desmosomes between adjacent cells. Compared with the control group, dietary supplementation with 50 and 100 mg/kg Gelsemium elegans alcohol extracts significantly reduced the number of E. coli and significantly increased the number of Lactobacillus in the caecum ($P<0.05$). It is concluded that Gelsemium elegans alcohol and acidic extracts can improve intestinal morphology, modulate caecal microflora, increase ADG and reduce F/G in growing pigs, with 100 mg/kg Gelsemium elegans alcohol extract being the most effective.

Keywords: Gelsemium elegans extracts; growing pigs; growth performance; intestinal morphology; caecal microflora

Gelsemium elegans, also known as heartbreak grass, large tea medicine, or Humenteng, is the whole plant of the Loganiaceae family. It is widely distributed in Zhejiang, Fujian, Guangdong, Guangxi, Yunnan, Guizhou and other provinces in China. Gelsemium elegans is a notoriously toxic plant that is highly poisonous to humans; ingestion of 10 g of its stems and leaves or 2–8 g of its roots can cause poisoning and even death, with respiratory failure being the primary cause of mortality. Indole alkaloids are its main chemical components and also its primary toxic and active constituents, among which koumine has the highest content and gelsemine has the strongest toxicity. As understanding of its toxicity has deepened, research on the applications of Gelsemium elegans has become increasingly extensive, expanding from external use only to applications in anti-inflammatory, analgesic, sedative, antitumor and immunomodulatory functions. Although highly toxic to humans, Gelsemium elegans is not overtly toxic to cattle, pigs and sheep, and appropriate doses can actually promote weight gain and prevent plague. According to *Guangdong Chinese Veterinary Herbal Medicine*, "Gelsemium elegans, when regularly administered to pigs and sheep, can strengthen the stomach, kill parasites and promote fattening." Thus, Gelsemium elegans is a promising Chinese herbal feed additive, and studying its growth-promoting effects and mechanisms is important for its development and application. China has a long history of using Gelsemium elegans to promote animal growth; in Guangdong and Fujian, it is known as "pig ginseng" and is often dried, pulverized and mixed into feed to fatten pigs. Studies have shown that compound pig ginseng can increase piglet daily weight gain by 16.6% and improve feed conversion rate by 18.2%. Research on Gelsemium elegans powder, total alkaloids and injection for promoting piglet growth found that all three significantly increased weight gain, with total alkaloids showing better effects than powder and injection at equivalent doses due to smaller volume and better palatability. Due to differences in structure types and physicochemical properties of various alkaloids, the total alkaloid yield and activity of Gelsemium elegans extracts obtained by different methods also differ, resulting in varying growth-promoting effects. Currently, research on Gelsemium elegans for promoting pig growth is limited and mainly focuses on the efficacy of Gelsemium elegans powder or its compounds, with no comparative studies on the growth-promoting effects and mechanisms of different Gelsemium elegans extracts in pigs. This experiment compared the growth-promoting effects of Gelsemium elegans alcohol and acidic extracts by adding them to growing pig diets and investigated their effects on growth performance, intestinal morphology and caecal microflora to provide basic data for the application of Gelsemium elegans extracts as feed additives.

1.1 Experimental Materials

Gelsemium elegans alcohol extract and acidic extract were provided by Hunan Taigu Biotechnology Co., Ltd. The alcohol extract was prepared by hot extraction with 95% ethanol, with a total alkaloid content of 6.44%. The acidic extract was prepared by hot extraction with 0.5% sulfuric acid, followed by pH adjustment to neutral with 8 mol/L sodium hydroxide, with a total alkaloid content of 2.15%. Both extracts were dried under reduced pressure, pulverized and stored in aliquots. The experimental growing pigs, basal diet and experimental site were provided by Yong' an Branch of Hunan New Wellful Co., Ltd.

1.2 Experimental Design

Seventy-five healthy 56-day-old "Landrace × Large White" crossbred growing pigs with similar body weight [(18.63±0.36) kg] were selected and randomly divided into 5 groups using a single-factor experimental design, with 3 replicates per group and 5 pigs per replicate, with each replicate housed in one pen. Based on preliminary trial results, three Gelsemium elegans alcohol extract groups were supplemented with 25 (low dose), 50 (medium dose) and 100 mg/kg (high dose) of the alcohol extract in the basal diet, while one Gelsemium elegans acidic extract group was supplemented with 150 mg/kg of the acidic extract in the basal diet. The control group was fed the basal diet, whose composition and nutrient levels are shown in Table 1 . The experimental period lasted 49 days.

1.3 Feeding Management

All experimental pigs were fed at regular times daily with ad libitum access to feed and water. Routine management practices including immunization, disinfection and sanitation were conducted according to standard protocols.

1.4 Growth Performance Measurement

Body weight was measured at 08:00 on an empty stomach on days 1, 15, 31 and 49 of the experiment. Daily feed consumption was accurately recorded to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed/gain ratio (F/G). Fecal conditions were observed daily, with diarrhea incidence and frequency recorded for each group to calculate diarrhea rate. Sleep conditions of the pigs were also observed daily.

1.5 Intestinal Morphology Observation

1.5.1 Light Microscopy Observation of Intestinal Morphology On day 49 of the experiment, 2 pigs with body weight close to the average were randomly selected from each replicate of each group. After 12 h of fasting and weighing, the pigs were completely anesthetized with sodium pentobarbital. The abdominal cavity was opened, and the small intestine was quickly removed and emptied of all chyme. Approximately 10 cm segments from the middle of the duodenum, jejunum and ileum were excised, rinsed with pre-cooled physiological saline, and 2 cm samples were fixed in 10% formalin. After dehydration, clearing, embedding, sectioning and hematoxylin-eosin staining, samples were observed under an optical microscope. Image analysis software was used to measure villus height and crypt depth, and to calculate the villus height/crypt depth ratio. For each sample, 3 non-consecutive sections were observed, 3 complete and typical villus fields were selected from each section, and 10 villus lengths and crypt depths were measured in each field, with the average value used as one measurement.

1.5.2 Electron Microscopy Observation of Intestinal Ultrastructure

Jejunal tissue samples were repeatedly rinsed with ice-cold phosphate buffer, fixed with 2.5% glutaraldehyde, washed with phosphate buffer, fixed with 1% osmium tetroxide, and then processed through dehydration, substitution, embedding, sectioning, staining and washing before being observed under a Hitachi H-7700 transmission electron microscope.

1.6 Caecal Microflora Count

The caecal segment was isolated and ligated, the ligation site was disinfected with alcohol cotton, and it was placed in a sterile plastic bag. In a super-clean workbench, caecal contents were aseptically collected into sterile centrifuge tubes, and contents from pigs in the same group were uniformly mixed. Ten-fold serial dilutions were prepared. Based on preliminary results, 10 , 10 and 10 dilutions were inoculated onto EMB (E. coli) and LBS (Lactobacillus) culture media. EMB plates were incubated at 37 °C for 24 h, while LBS plates were incubated in an anaerobic incubator for 72 h. Each concentration had 5 replicates, and colony numbers were counted and averaged. Results were expressed as log colony-forming units per gram of caecal content [lg(CFU/g)].

1.7 Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS 21.0 software, with multiple comparisons performed using the LSD method.

2.1 Effects of Gelsemium elegans Extracts on Growth Performance of Growing Pigs

As shown in Table 2 , dietary supplementation with Gelsemium elegans extracts significantly increased ADFI during days 1-15 ($P<0.05$), but there were no significant differences in ADFI among groups during the entire experimental period (days 1-49) ($P>0.05$). During the entire experimental period, ADG in all Gelsemium elegans extract groups was significantly higher than that in the control group ($P<0.05$), and F/G was significantly lower ($P<0.05$). There were no significant differences in ADG among the three Gelsemium elegans alcohol extract dose groups and the acidic extract group ($P>0.05$). ADG in the high-dose Gelsemium elegans alcohol extract group was numerically higher than that in the medium- and low-dose groups (P>0.05), showing a clear dose-dependent effect. The F/G in the Gelsemium elegans acidic extract group was not significantly different from that in the low-dose Gelsemium elegans alcohol extract group (P>0.05), but both were significantly lower than those in the medium- and high-dose Gelsemium elegans alcohol extract groups (P<0.05). No mortality, diarrhea or other adverse conditions occurred in the control or Gelsemium elegans extract groups during the experiment, and all pigs remained in good health.

2.2 Effects of Gelsemium elegans Extracts on Intestinal Morphology of Growing Pigs

2.2.1 Light Microscopy Observation Results As shown in Table 3 , dietary supplementation with Gelsemium elegans extracts increased villus height in the duodenum, jejunum and ileum to varying degrees. There were no significant changes in duodenal villus height in the Gelsemium elegans extract groups compared with the control group (P>0.05). Jejunal villus height in the high-dose Gelsemium elegans alcohol extract group and the acidic extract group was significantly higher than that in the control group and the medium- and low-dose Gelsemium elegans alcohol extract groups (P<0.05). Ileal villus height in the high-dose Gelsemium elegans alcohol extract group was significantly higher than that in the control group and all other Gelsemium elegans extract groups (P<0.05). Dietary supplementation with Gelsemium elegans extracts decreased crypt depth in the duodenum, jejunum and ileum to varying degrees, but compared with the control group, only the decrease in duodenal crypt depth in the high-dose Gelsemium elegans alcohol extract group reached statistical significance (P<0.05). There were no significant differences in villus height/crypt depth ratio in the duodenum, jejunum or ileum among all groups (P>0.05).

2.2.2 Transmission Electron Microscopy Observation Results Transmission electron microscopy observation revealed that compared with the control group, the jejunal tissue microvilli in all Gelsemium elegans extract groups were more uniform, orderly and dense, with clearly visible and intact tight junctions, intermediate junctions and desmosomes between adjacent cells. The jejunal mucosal structures of the control group and high-dose Gelsemium elegans alcohol extract group under transmission electron microscopy are shown in Figure 1 [Figure 1: see original paper].

2.3 Effects of Gelsemium elegans Extracts on E. coli and Lactobacillus Numbers in Caecum of Growing Pigs

As shown in Table 4 , compared with the control group, the medium- and low-dose Gelsemium elegans alcohol extract groups showed significantly reduced E. coli numbers and significantly increased Lactobacillus numbers in the caecum (P<0.05), with a clear dose-dependent effect on Lactobacillus proliferation. Dietary supplementation with Gelsemium elegans acidic extract decreased E. coli numbers and increased Lactobacillus numbers in the caecum, but these differences were not significant compared with the control group ($P>0.05$).

3.1 Effects of Gelsemium elegans Extracts on Growth Performance of Growing Pigs

Liu et al. found that adding Gelsemium elegans powder (20, 40 g/kg) to piglet diets increased ADG and decreased F/G, with effects intensifying as dosage increased. Compared with Gelsemium elegans powder, total alkaloids (0.22 g/kg, ethanol-extracted) or Gelsemium elegans injection [2 mL/(head · d)], there was no significant difference in ADG between the powder and injection groups, while the total alkaloids group showed significantly higher ADG than both powder and injection groups, indicating that alkaloids are the main active components for growth promotion. Consistent with these findings, our results showed that both Gelsemium elegans alcohol and acidic extracts significantly increased ADG and significantly reduced F/G in growing pigs, with the growth-promoting effect of Gelsemium elegans alcohol extract positively correlated with dosage. The F/G in the Gelsemium elegans acidic extract group (150 mg/kg) was not significantly different from that in the low-dose Gelsemium elegans alcohol extract group (25 mg/kg), but both were significantly lower than those in the medium- and high-dose Gelsemium elegans alcohol extract groups (50, 100 mg/kg), which may be related to total alkaloid content. Zhang et al. reported that adding 1.5% and 3.0% Gelsemium elegans powder to basal diets significantly promoted broiler growth, but when dosage reached 6.0%, broilers grew slowly and showed obvious poisoning symptoms. In our study, dietary supplementation with 100 mg/kg Gelsemium elegans alcohol extract and 150 mg/kg Gelsemium elegans acidic extract corresponded to approximately 0.16% and 0.08% Gelsemium elegans powder, respectively, and growing pigs showed no poisoning symptoms, though the toxic dosage of Gelsemium elegans extracts for growing pigs requires further investigation.

Gelsemium elegans is highly toxic to humans, and its alkaloid poisoning mechanisms include inhibition of cholinesterase activity, causing peripheral autonomic nervous system M-like effects, suppressing the respiratory center, inhibiting motor centers in the brain and spinal cord, causing respiratory muscle paralysis and leading to respiratory failure. In recent years, research on Gelsemium elegans has gradually expanded and deepened, revealing its antitumor, anti-inflammatory, immunomodulatory, sedative-analgesic and hematopoietic-promoting effects, particularly significant in prolonging survival of liver cancer patients and providing analgesia for cancer patients. Clinically, gelsemine has been used to treat neuralgia, indicating deepening understanding of its toxicity. Most clinical poisoning cases result from accidental ingestion or unauthorized use based on folk remedies. Although using Gelsemium elegans mixed in feed for fattening is quite common in Guangdong and Fujian, no reports of poisoning from consumption of animal products have been documented, possibly due to rapid elimination of Gelsemium elegans alkaloids in pigs, chickens and other animals. This speculation has been confirmed by residue and metabolism studies. Reportedly, no Gelsemium elegans alkaloids were detected in broiler liver and muscle (fed basal diet containing 2% Gelsemium elegans powder for 60 consecutive days) or pig muscle (fed basal diet containing 0.2% Gelsemium elegans powder for 40 consecutive days). Metabolism studies further confirmed that after oral administration of 10 mg/kg Gelsemium elegans alkaloids to rats, peak plasma concentrations of gelsemine and koumine occurred at 0.43 and 0.28 h, respectively, with half-lives (t /) of 1.59 and 1.60 h, indicating rapid absorption and elimination. In vitro studies found that koumine undergoes oxidation, reduction, dehydrogenation and demethylation in pig liver S9, while gelsemine undergoes oxidation and demethylation, suggesting rich metabolic pathways that may contribute to rapid elimination. These results indicate that Gelsemium elegans is a safe and reliable Chinese herbal feed additive, though more systematic studies are needed to clarify its safety as a feed additive, which will be the focus of our next research.

In this experiment, dietary supplementation with Gelsemium elegans extracts had no significant effect on ADFI in growing pigs compared with the control group. Han et al. reported that adding different doses (0.4%, 0.8%, 1.2%, 1.6%) of Gelsemium elegans powder to diets increased ADG but decreased ADFI in Gushi chickens, with ADFI reduction positively correlated with dosage, possibly due to poor palatability. Since our experiment used Gelsemium elegans extracts at relatively low doses, it is difficult to speculate whether palatability was affected. Some studies suggest that the growth-promoting effect of Gelsemium elegans is related to increased appetite, with experimental pigs showing "obvious voracious appetite, hunger, roaring, food competition and licking the trough clean," though in our experiment, no obvious differences in appetite were observed between Gelsemium elegans extract groups and the control group. According to *Guangxi Traditional Chinese Medicine Gazetteer*, "When pigs have poor appetite, feeding them Gelsemium elegans can increase appetite." Whether Gelsemium elegans can increase appetite in both healthy pigs and those with poor appetite requires further experimental observation.

Animals have lower basal metabolism in quiet states, which facilitates nutrient deposition in the body and promotes growth. Studies have shown that Gelsemium elegans total alkaloids significantly enhance the sedative-hypnotic effect of pentobarbital sodium in mice. Oral administration of higher doses (10.0 mg/kg) of koumine enhances the hypnotic effect of subthreshold doses of pentobarbital sodium, while 2.5 mg/kg koumine has no such effect, though intravenous injection of 2.36 mg/kg koumine has sedative effects in mice. Based on this, researchers have speculated that the growth-promoting effect of Gelsemium elegans is related to sedation and hypnosis. However, in our experiment, no obvious differences in sleep conditions were observed between Gelsemium elegans extract groups and the control group, possibly because the dosages used were far below sedative-hypnotic doses.

3.2 Effects of Gelsemium elegans Extracts on Small Intestinal Morphology of Growing Pigs

The small intestine is the primary site for nutrient absorption, and villus height and crypt depth are important indicators of small intestinal digestive and absorptive function. Crypt depth reflects cell generation rate; shallower crypts indicate increased cell maturation rate and enhanced secretory function. Villus height/crypt depth ratio comprehensively reflects small intestinal functional status, and an increased ratio indicates enhanced digestive and absorptive function. Our experiment found that 100 mg/kg Gelsemium elegans alcohol extract significantly increased jejunal and ileal villus height and significantly decreased duodenal crypt depth in growing pigs, while 150 mg/kg Gelsemium elegans acidic extract significantly increased jejunal villus height, indicating that Gelsemium elegans extracts can promote small intestinal villus growth and development, improve small intestinal mucosal structure and promote nutrient absorption. These results support the speculation of Han et al. that the growth-promoting effect of Gelsemium elegans may be mainly related to promoting nutrient digestion and absorption.

3.3 Effects of Gelsemium elegans Extracts on Caecal Microflora of Growing Pigs

The caecum is a site for extensive microbial growth and reproduction, and intestinal microflora is a major component of the intestinal mucosal barrier, closely related to nutrition, immunity, disease prevention and physiological functions. Our results showed that dietary supplementation with 50 and 100 mg/kg Gelsemium elegans alcohol extract significantly promoted Lactobacillus growth and inhibited E. coli growth in the caecum. Liu also confirmed that Gelsemium elegans total alkaloids dose-dependently reduced E. coli numbers and increased Lactobacillus numbers in broiler ileum. However, in vitro antibacterial test results are inconsistent with these findings; ethanol extract of Humenteng (Gelsemium elegans, 0.4 g/mL) showed no significant effects on numbers of Salmonella, non-pathogenic E. coli, fecal Streptococcus or Bacillus subtilis. Gelsemium elegans alcohol extract contains higher levels of alkaloids, carbohydrates and proteins than the acidic extract, providing a richer nutritional environment for intestinal microorganisms and effectively promoting caecal Lactobacillus growth. Lactobacillus inhibits proliferation of pathogenic bacteria such as E. coli by producing organic acids (lactic acid, acetic acid, propionic acid) and hydrogen peroxide. Therefore, although Gelsemium elegans alcohol extract has no antibacterial activity in vitro, it can enhance competitiveness against harmful bacteria by promoting beneficial bacteria proliferation in vivo, thereby improving intestinal microecological balance and intestinal morphology, with superior intestinal microecological regulation ability compared to Gelsemium elegans acidic extract.

4 Conclusion

Gelsemium elegans alcohol and acidic extracts can improve intestinal morphology, increase Lactobacillus numbers and reduce E. coli numbers in the caecum of growing pigs, thereby increasing ADG and reducing F/G, with 100 mg/kg Gelsemium elegans alcohol extract showing the best effect.

References

[1] HONG Xijun, LU Wenguang, SONG Cijiao, et al. Study on toxicity of different parts of Gelsemium elegans [J]. Pharmaceutical Bulletin, 1983, 18(12): 62.
[2] CHEN Yehe. Rescue and nursing of Gelsemium elegans poisoning [J]. Journal of Clinical Nursing, 2005, 4(1): 8-10.
[3] ZHANG Qiuping, ZHANG Binfeng, CHOU Guixin, et al. Chemical constituents from the aerial parts of Gelsemium elegans [J]. China Journal of Chinese Materia Medica, 2011, 36(10): 1305-1310.
[4] QU J, FANG L, REN X D, et al. Bisindole alkaloids with neural anti-inflammatory activity from Gelsemium elegans [J]. Journal of Natural Products, 2013, 76(12): 2203-2209.
[5] ZHANG J Y, GONG N, HUANG J L, et al. Gelsemine, a principal alkaloid from Gelsemium sempervirens Ait., exhibits potent and specific antinociception in chronic pain by acting at spinal 3 glycine receptors [J]. Pain, 2013, 154(11): 2452-2462.
[6] MARZOTTO M, OLIOSO D, BRIZZI M, et al. Extreme sensitivity of gene expression in human SH-SY5Y neurocytes to ultra-low doses of Gelsemium sempervirens [J]. BMC Complementary and Alternative Medicine, 2014, 14: 104.
[7] WANG Kun, XIAO Yanfen, YU Xiaoling, et al. Protective effect of Gelsemium elegans on acute radiation injury in mice [J]. Chinese Journal of Radiological Medicine and Protection, 2002, 22(2): 111-112.
[8] LIN Qiyuan. Fattening trial report of compound pig ginseng feed additive [J]. Animal Husbandry and Veterinary Medicine, 1993, 25(5): 207-208.
[9] LIU Daorong, HUANG Taikang, WANG Youshun, et al. Experimental study on feeding pigs with pig ginseng [J]. Animal Husbandry and Veterinary Medicine, 1992, 26(2): 67-68.
[10] ZHANG Dongying, YUAN Hui, LIU Yalin. Effect of Gelsemium elegans on immune function of broilers [J]. Journal of Hunan Agricultural University (Natural Sciences), 2004, 30(6): 538-541.
[11] XIE Lijing, HAN Xuefeng. Mechanism, clinical characteristics and management of Gelsemium elegans poisoning [J]. Adverse Drug Reactions Journal, 2006, 8(3): 202-204.
[12] LIU Xiaoyan. Study on growth-promoting mechanism and residue of Gelsemium elegans [D]. Master' s thesis. Changsha: Hunan Agricultural University, 2003.
[13] CHEN Bin. Study on efficacy and safety of Humenteng as feed additive

[D]. Master' s thesis. Wuhan: Huazhong Agricultural University, 2013.
[14] WANG L, WEN Y Q, MENG F H. Simultaneous determination of gelsemine and koumine in rat plasma by UPLC-MS/MS and application to pharmacokinetic study after oral administration of Gelsemium elegans Benth extract [J]. Biomedical Chromatography, 2018, doi:10.1002/bmc.4201.
[15] XIAO Sa. Content determination of Gelsemium elegans crude extract and in vitro metabolism of main alkaloids [D]. Changsha: Hunan Agricultural University, 2016.
[16] HAN Ruili, KANG Xiangtao, TIAN Yadong, et al. Effects of Gelsemium elegans on growth performance and immune performance of Gushi chickens [J]. Chinese Journal of Veterinary Drug, 2006, 40(6): 4–7.
[17] YUAN Hui, WEN Lixin. Research progress on Gelsemium elegans toxin and its recuperative effect on livestock and poultry [J]. Guangdong Feed, 2002, 11(2): 30–32.
[18] ZHOU Minglu, HUANG Cong, YANG Xiaoping. Study on analgesic, sedative and safety effects of total alkaloids of Gelsemium elegans [J]. Chinese Traditional Patent Medicine, 1998, 20(1): 35–36.
[19] CHEN Chaojie, XIN Zhiming, LIN Jing, et al. Effects of koumine on spontaneous behavior, coordination balance and subthreshold hypnotic effect of barbital in mice [J]. Journal of Fujian Medical University, 2014, 48(2): 83–85.
[20] CHI Debiao, LEI Linsheng, YANG Hongxuan, et al. General pharmacological study of koumine injection [J]. Journal of First Military Medical University, 2004, 24(1): 32–34.
[21] HU Xiaofei, WEI Fengxian, LIN Dongkang, et al. Application prospect of Gelsemium elegans toxin in animal husbandry [C]//Proceedings of the 8th National Feed Additives Academic and New Technology & New Products Exchange Conference. Yantai: China Feed Industry Association, 2004: 57–59.
[22] RIEGER J, JANCZYK P, HÜNIGEN H, et al. Intraepithelial lymphocyte numbers and histomorphological parameters in the porcine gut after Enterococcus faecium NCIMB 10415 feeding and Salmonella typhimurium challenge [J]. Veterinary Immunology and Immunopathology, 2015, 164(1/2): 40–50.
[23] XIAO Sa, WU Yong, HUANG Yajun, et al. Determination of component contents in Gelsemium elegans crude extracts extracted with different solvents [J]. Progress in Veterinary Medicine, 2016, 37(6): 72–76.

Note: Figure translations are in progress. See original paper for figures.