

# STUDIES ON HERBAL EXTRACT PRODUCTION WITH DIARRHEA PREVENTION AND GROWTH PROMOTION PROPERTIES TO REPLACE ANTIBIOTICS IN PIG AND CHICKEN FEEDS

La Van Kinh

## INTRODUCTION

One of the future research strategies in animal husbandry for safe animal products is the application of herbal extract products to replace antibiotics and artificial growth promotants in animal feed. The natural growth promoters (herbal extracts) with meaning properties in nutrient digestion and absorption, strengthening gut flora health and immune system bring a promising way for the replacement of artificial growth promotants (may poison for animal products consumers). Many companies from USA and Denmark such as Alltech, Biomin have researched, produced and commercialized their herbal extract products. In Vietnam, the studies on herbal extract products have got preliminary results. The objective of this research aims to produce some herbal extract products for replacing antibiotics in pig and chicken feeds in order to prevent diarrhea disease and stimulate animal growth.

## MATERIALS AND METHODS

- Extracting and determining the active compounds from the *Tinospra cordifolia*
- Qualitative analysis of active compounds by chemical method and HPLC.
- Analysis of dry matter, total ash and acid insoluble ash by drying and burning methods.
- Quantitative analysis of Flavonoid by weighing method after extraction with methanol and ethyl acetate.
- Diterpen lacton was determined by the optical absorption measurements with Thermo Spectromic HELIOS.
- Building up the selection, extraction and checking procedures of medicinal herbs and herbal extract products.
- Medical properties tests including acute toxicity, growth, immunity and hematology tests carrying out in mouse.
- Studies on using herbal products (the mixture of ground herbs) in chicken, weaning piglets and growing pigs.
- Studies on using herbal extract products (the mixture of herb extracts) in chicken, weaning piglets and growing pigs.

## RESULTS

1. A total of 11 chemical compounds from the *Tinospra cordifolia* were extracted and determined their chemical structures. Among them, there are 4 new compounds such as: TC2C1: 2-hydroxy-borapetoside F; 2. TC3C4: N-acetylasimilobine 2-O- $\beta$ -D-glucopyranosyl-(1->2)- $\beta$ -D-glucopyranoside; 3. TC3C5: N-acetylasimilobine 2-O- $\beta$ -D-glucopyranosyl-(1->2)- $\beta$ -D-glucopyranoside; 4. TC3A1: Sinapyl 4-O- $\beta$ -D-ribofuranosyl (1->6)-O- $\beta$ -D-glucopyranoside.

2. The specific extraction procedures were established for *Pouzolzia zeylanica* Benn, *Andrographis paniculata* Nees., *Mahonia nepalensis* DC., *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe, *Coscinium usitatum* (Gaertn.) Colebr, *Wedelia chinensis* (Osbek.) Merr, *Glycyrrhiza glabra* L., *Curcuma longa* L., *Astragalus membranaceus* (Fish.) Bunge.
3. Six herbal products were prepared:
  - a) Product 1: a mixture of extracts from *Mahonia nepalensis* DC. (2.7%); *Pouzolzia zeylanica* (L.) Benn (13.6%); *Zingiber officinale* Roscoe (17.0%), adjuvant 66.7%. Total flavonoid content in product 1 was 6.64%. In addition, there were alkaloid compounds.
  - b) Product 2: a mixture of extracts from *Andrographis paniculata* (Burm.f.) Nees (19.9%); *Mahonia nepalensis* DC. (1.9%), *Zingiber officinale* Roscoe (11.6%), adjuvant 66.6%. Total flavonoid content in product 2 was 8.65%. In addition, there were alkaloid and diterpen lacton compounds.
  - c) Product 3: a mixture of extracts from *Andrographis paniculata* (Burm.f.) Nees (9.3%); *Tinospora crispa* (L.) Miers (10.2%), *Zingiber officinale* Roscoe (5.5%), adjuvant 75.0%. Total flavonoid content in product 3 was 4.59%. Alkaloid and diterpen lacton compounds were also detected.
  - d) Product 4: a mixture of extracts from *Pouzolzia zeylanica* (L.) Benn (5.4%); *Tinospora crispa* (L.) Miers (12.8%); *Zingiber officinale* Roscoe (6.8%), adjuvant 75.0%. Total flavonoid content in product 4 was 1.83%. Alkaloid and diterpen lacton compounds were also detected.
  - e) Product 5: a mixture of extracts from *Coscinium usitatum* (Gaertn.) Colebr 1595 mg; *Wedelia chinensis* (Osbek.) Merr 1905 mg; *Glycyrrhiza glabra* L 6820 mg; adjuvant to 1000 g. Berberin clorid content in product 5 was from 0,015 to 0,02%.
  - f) Product 6: a mixture of extracts from *Coscinium usitatum* (Gaertn.) Colebr 1595 mg; *Zingiber officinale* Roscoe 2096 mg; *Astragalus membranaceus* (Fish.) Bunge 2324 mg; adjuvant to 1000 g. Berberin clorid content in product 6 was from 0.015 to 0.02%.
4. Base on above 6 mentioned products, we selected 4 products for *in vitro* experiments in mouse to asses their effect. The experiment results are as follow:
  - a) Product 1 (*Mahonia nepalensis* DC., *Pouzolzia zeylanica* (L.) Benn, *Zingiber officinale* Roscoe): Dose 0.705 g/kg body weight had a good effect on weight gain. However, the impact on immunity was unclear.
  - b) Product 2 (*Mahonia nepalensis* DC., *Andrographis paniculata* (Burm.f.) Nees., *Zingiber officinale* Roscoe): Dose 0.825 g/kg body weight improved weight gain of mouse. Doses 1.65-3.3 g/kg body weight had good effects on hematology indexes. However, the effect on immunity was unclear.
  - c) Product 3 (*Andrographis paniculata* (Burm.f.) Nees., *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe): Dose 2.46 g/kg body weight was efficient on weight gain improvement. Dose 1.23 g/kg body weight improved the immunity of mouse. Dose 1.23 and 2.46 g/kg body weight increased

hematology indexes. So that, doses from 1.23 to 2.46 g/kg body weight should be used in further experiments in animal.

- d) Product 4 (*Pouzolzia zeylanica* (L.) Benn, *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe): Doses from 0.295 to 1.179g g/kg body weight had good effects on weight gain. However, the effects on immunity were unclear. Doses from 0.589 to 1.179 g/kg bodyweight enhanced hematology index. In sum, doses from 0.295 to 1.179 g/kg body weight should be used in further experiment in animal.

5. Experiment results on using herbal products (the mixture of ground herbs) in chicken, weanling and growing pigs feeding.

Basing on the mouse experiment results, two herbal products [**Product 3:** *Andrographis paniculata* (Burm.f.) Nees., *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe and **Product 4:** *Pouzolzia zeylanica* (L.) Benn, *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe] were selected for trials in pig and chicken. The results of trial results showed that:

**On chicken:** The addition of a mixture of herbal products (ground powders) extracted from *Andrographis paniculata* (Burm.f.) Nees, *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe (Product 3) or *Pouzolzia zeylanica* (L.) Benn, *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe (Product 4) into the feed for COBB-308 chicken had good effects on growth stimulation and economic efficiency improvement. Supplementating 1.1% product 3 improved 4.7–15.9% weight gain and decreased 7.9–11.2% FCR. Whereas, 0.5% product 3 improved 3.5–4.2% weight gain and decreased 5.2–7.5% FCR. Supplementing both products 3 (1.1%) and 4 (0.5%) improved 4.8–5.7% weight gain and decreased 7.9 – 10.5% FCR.

**On weaning piglet:** The supplementation of product 3 (0.425-1.275%) as well as using Berberin and *Andrographis paniculata* (Burm.f.) Nees to replace Colistin in weaning pig diet had not effected to the weight gain and diarrhea disease prevention, however, the FCR was improved. Supplementing 0.5%-1.5% product 4 improved pig weight gain but had negative effects on FCR and diarrhea prevention compared with Colistin.

**On growing pig:** The supplementation of 2% product 4 in growing pig diet improved weight gain, FCR, however, the effect on diarrhea was limited in comparison with Colistin.

6. Experiment results on using herbal extract products (the mixture of dry and ground herbal extracts) in chicken, weanling and growing pigs feeding.

The active compounds from *Andrographis paniculata* (Burm.f.) Nees, *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe (Product 3) and *Pouzolzia zeylanica* (L.) Benn, *Tinospora crispa* (L.) Miers, *Zingiber officinale* Roscoe (Product 4) were extracted, dried and ground to make two herbal extract products named IAS-1 and IAS-2, respectively. The supplementation of IAS-1 or IAS-2 into pig and chicken diets improved their weight gain and FCR, decreased the diarrhea level, feed cost and saved 4-10% of feed expense without effecting on feed intake, survival rate and meat quality.

**On Chicken:** At the rate of 0.375% IAS-1 product in chicken diet improved 9.8% weight gain, decreased 7.1% FCR, 43% diarrhea ratio (from 14.3% down to 9.7%) and 2% death ratio while maintained the meat quality. The optimum supplementation level of IAS-2 product is 0.12%. This addition level increased 9.4% weight gain, decreased 8.1% FCR, 2% death rate and 26.7% diarrhea rate. The supplementation of both products IAS-1

(0.375%) and IAS-2 (0.12%) into chicken diet did not enhance the weight gain and FCR. However, it reduced 66% diarrhea rate.

**On weanling piglet:** At the rate of 0.375% IAS-1 product in weaning piglet diet improved 8% weight gain, decreased 9.5% FCR, 72% diarrhea rate. At the rate of 0.12% IAS-2 product improved 3% weight gain, decreased 10% FCR, 284% diarrhea and 240% cough rates.

**On growing pig:** Supplementing 0.375% or 0.5% IAS-1 product in growing pig diet improved 2-3% weight gain, decreased 2 % FCR in comparison with negative control. However, the effect on diarrhea disease was limited in comparison with positive control (Colistin supplement). Adding 0.12% or 0.18% IAS-2 product improved 3% weight gain, decreased 3% FCR, however, it did not reduce the diarrhea compared to supplementation of Colistin, Berberin or *Andrographis paniculata* (Burm.f.) Nees. The optimum addition rate of IAS-2 in growing pig diet is 0.12%.

## **CONCLUSION**

Herbal products IAS-1 and IAS-2 can replace antibiotics in feeds for diarrhea prevention and growth stimulation on pigs and chicken.