Effects of medicinal plants mixture on growth performance, nutrient digestibility, blood profiles, and fecal microbiota in growing pigs

Nguyen Cong Oanh1,2*, Truong Quang Lam3*, Nguyen Dinh Tien1*, Jean-Luc Hornick1,2* and Vu Dinh Ton1*

1. Vietnam National University of Agriculture, Faculty of Animal Science, Ngo Xuan Quang Street, Trauquy, Gia Lam, 100000 Hanoi, Vietnam; 2. University of Liège, Faculty of Veterinary Medicine, PARAH Center, Department of Veterinary Management of Animal Resources, Quartier vallée 2, Avenue de Cureghem 6, B43a, 4000 Liège, Belgium; 3. Vietnam National University of Agriculture, Faculty of Veterinary Medicine, Key Laboratory for Veterinary Biotechnology, Ngo Xuan Quang Street, Trauquy, Gia Lam, 100000 Hanoi, Vietnam.

Corresponding author: Nguyen Cong Oanh, e-mail: ncoanh@gmail.com
Co-authors: TQL: truongquanglam2806@gmail.com, NDT: ndtien.hd@gmail.com, HJ: jlhornick@uliege.be, VDT: vdtont@vnua.edu.vn
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Abstract

Background and Aim: Alternative natural materials to antibiotics for improving digestive health and growth performance are needed due to strengthening regulations related to the use of antibiotic growth promoters. The study aimed to evaluate the effects of medicinal plants mixture (60% Bidens pilosa L., 15% Urena lobata L., 15% Pseudernanthemum palatifurum, 5% Ramulus cinnamomi, and 5% Star anise) as alternative growth promotors on animal health, nutrient digestibility, blood parameters, and growth performance of growing pigs.

Materials and Methods: The study was conducted, from April 2020 to June 2020, at a private pig production farm located in Cam Giang district Hai Duong Province, Vietnam. Forty-eight 10-week-old crossbred (♂Duroc×♀[Landrace×Yorkshire]) pigs, average initial body weight 30.3±1.42 kg, were randomly allocated to four dietary groups, three replicate pens per experimental group, with 4 pigs/pen. For 7 weeks, the pigs were fed a basal diet supplemented with the mixture at levels of 0, 20, 40, and 60 g/kg of feed.

Results: Final body weight, average daily gain, average daily feed intake, and feed conversion ratio, as well as apparent total tract digestibility of dry matter, organic matter, crude protein, ether extract, and gross energy were not significantly influenced by the diets (p>0.05). Inclusion of the plant mixture decreased significantly red blood cell count, blood cholesterol, urea nitrogen, and low-density lipoprotein (LDL) concentrations (p<0.05) compared with the control diet. No diet effect was observed on fecal Escherichia coli, Salmonella spp., Clostridium spp., and total bacteria counts.

Conclusion: The incorporation of the plant mixture into the diet of growing pigs reduced serum cholesterol, LDL, and urea concentrations with no adverse effect on performance and nutrient digestibility.

Keywords: animal performance, blood profile, digestibility, growing pig, medicinal plants powder.

Introduction

Overuse of antibiotics for growth promotion and disease prevention in animals can contribute to the emergence of antibiotic resistance and increase human health risks [1]. Therefore, many countries have already taken action to reduce the use of antibiotics in food-producing animals. European Union has banned the use of antibiotics for growth promotion since 2006. Since then phylogenic compounds have been studied and used in animal feeds with the objectives to substitute antibiotics as growth promoters and disease prevention or treatment [2-5].

Several herbal extracts have been reported to promote feed intake, feed digestibility, and beneficial intestinal microbiome and also to improve immune system [6,7]. Medicinal plants Ramulus cinnamomi (Cinnamon twig), Star anise (Illicium verum Hook. f.), Bidens pilosa L., Urena lobata L., and Pseudernanthemum palatifurum are widely distributed in tropical and sub-tropical areas of Asia, especially Vietnam, and are considered as feed additives for animals [8]. The phytochemical components of these plants are tannins, saponins, phenols, alkaloids, and glycosides [9-13], which are known to be potential sources of useful drugs [14], especially exhibiting high antibacterial, immune, and antioxidant properties [12,15-18]. Flavonoids of B. pilosa protect the liver function by limiting an increase in hepatic levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) on the carbon tetra-chloride model in mice and no evidence of animal toxicity at 160 g/kg of animal live weight was observed [19]. A previous in vitro study [20] reported that water extract of B. pilosa had higher activity against Bacillus cereus and Escherichia coli than gentamycin sulfate. Dried and fresh leaves of *P. palatifurum* are used to treat diarrhea in piglets [21]. Recent in vitro studies [22,23] showed...
that *Star anise* and *R. cinnamomi* inhibited replication of influenza and flu virus. Moreover, weaning pigs fed a diet supplemented with 0.05% *S. anise* oil had a higher average daily gain (ADG) than those fed a control diet [24]. As far as we know, no studies reported the effects of a blend of more than 2 herbal plants of *B. pilosa*, *P. palatiferum*, *U. lobata*, *R. cinnamomi*, and *S. anise* on pig production.

The study aimed to evaluate the effects of medicinal plants mixture (60% *B. pilosa* L., 15% *U. lobata* L., 15% *P. palatiferum*, 5% *R. cinnamomi*, and 5% *S. anise*) as alternative growth promoters on animal health, nutrient digestibility, blood parameters, and growth performance of growing pigs.

**Materials and Methods**

**Ethical approval**

In the absence of proper regulation in Vietnam, all procedures included in the experiment were performed according to the best practices recommended by the ethical committee for experiments in animals of University of Liège, Belgium.

**Study period and location**

The study was conducted from April 2020 to June 2020 at a private pig production farm located in Cam Giang district, Hai Duong Province, between latitude 20° 57′ 0″ E and longitude 106° 13′ 0″ E.

**Preparation of the medicinal plants**

The plant species were chosen according to their availability around the farm and their constituents. Aerial parts of *P. palatiferum*, *U. lobata*, and *B. pilosa* were harvested during the vegetative growth phase at the medicinal garden of private agricultural farms located Cam Giang district, Hai Duong Province, Vietnam. *R. cinnamomi* and *S. anise* only were harvested during fructification from a forest garden at Huu Lung district, Lang Son Province, Vietnam.

The plants were dried separately in an oven at 60-75°C for 8 h. After drying, their materials were separately stored in air-tight bags. They were ground into fine powder and proportionally mixed as a powder (MP) containing 60% *B. pilosa*, 15% *U. lobata*, 15% *P. palatiferum*, 5% *R. cinnamomi*, and 5% *S. anise*. The weight contribution (60:15:15:5:5) of the medicinal plants in the mixture was determined by the availability of resources, their costs, and the recommendations of traditional medicine.

**Experimental design**

Forty-eight crossbred (*♂Duroc×♀[Landrace×Yorkshire]*) pigs, originating from eight sows, 3 to 4 litters order, initial body weight (IBW) 30.3±1.42 (SD), and age about 10 weeks old were used in this experiment. The pigs were individually ear tag numbered and randomly allocated to the four dietary treatments according to similar IBW, sex, and sow origin by treatment. There were three replicate pens per treatment, two barrows, and two gilts per pen (2 m×3 m) equipped with one stainless steel feeding and two automatic water drinking nipples. Animals were kept in a climate-controlled building with the temperature between 27 and 29°C, and the relative humidity between 70 and 85%.

Raw feed ingredients were purchased all at once from a local feed company. Feed was ground into flour through a 2 mm screen before the basal diet (T0) formulation. The experimental diets (T1, T2, and T3) were made of the basal diet mixed with MP at 20, 40, and 60 g/kg, respectively. Each was offered during a total experiment duration of 49 days. In the last week of the experiment, chromium oxide (Cr$_2$O$_3$) was added at a level of 5 g/kg of diet, as an inert marker to measure digestibility parameters.

The complete diets were collected for chemical analysis. The basic ingredients and nutrient levels of the diets are shown in Table-1 [25]. The nutrients composition of the diets met the recommended requirements for growing pigs [26]. Pigs were fed *ad libitum* during the whole experimental period and animals had free access to water by nipple drinkers.

**Measurements**

**Animal performance**

The animals were individually weighed at the start and the end of the experiment. Average feed intake, ADG, and feed conversion ratio (FCR) were calculated for each pen and diet treatment. In addition, disease symptoms such as diarrhea were recorded daily during the experiment.

**Digestibility**

For apparent total tract digestibility (ATTD), fresh fecal samples caught immediately after emission from all of the pigs were collected per pen 2 times/day (100 g/pig) for the past 5 days of the experiment and stored at 20°C. At the end of the collection, the fecal samples were thawed, mixed, and pooled per pen, after which they were dried and analyzed for ATTD determination. The ATTD of nutrients was calculated using the following equation [27]:

$$ \text{Nutrient digestibility (％ of intake)} = \left(1 - \frac{\text{Cr}_2\text{O}_3(\text{diet}) \times \text{nutrient (faces)}}{\text{Cr}_2\text{O}_3(\text{faces}) \times \text{nutrient (diet)}} \right) \times 100 $$

Where, nutrient digestibility is apparent digestibility of a nutrient or energy in the diet (％); nutrient (diet) is a nutrient (g) or gross energy (GE) (kcal) content per kg DM in diet and feces samples; and Cr$_2$O$_3$ (diet) and Cr$_2$O$_3$ (feces) are Cr$_2$O$_3$ content (g/kg DM) in diet and feces samples, respectively.

**Blood and serum parameters**

At the end of the experiment, 24 pigs (six pigs per treatment, one male and one female in each pen) were
Fecal microbiota

On day 28 of the experiment, fecal samples (about 50 g) of one male and one female per pen were collected directly from rectum, transferred to sterile Falcon, and immediately placed on ice in an insulating box for a maximum of 1 h transportation to the laboratory for further analysis. At the laboratory level, 0.01 g of fresh fecal sample of each animal was taken and placed into Eppendorf tubes supplemented with 990 µL 1× phosphate-buffered saline and 6-fold dilutions were prepared. The samples were plated on agar plates. Bacteria were quantified based on colony-forming units (cfu) counting on the culturing plates (log10 cfu g⁻¹). *E. coli* were enumerated on eosin methylene blue agar (Merck, Germany) after aerobic incubation at 37°C for 1 day. *Salmonella* species were enumerated on xylose lysine deoxycholate agar (Merck, Germany) after aerobic incubation at 37°C for 1 day. *Clostridium perfringens* were enumerated on Tryptose Sulfite Cycloserine agar (Merck, Germany) after anaerobic incubation at 37°C for 1 day. Total anaerobic bacteria were enumerated on nutrient agar (Merck, Germany) after anaerobic incubation at 37°C for 1-2 days. Before statistical analysis, fecal microbiota concentrations were transformed (log).

Chemical analysis

Diets and feces samples were pre-dried by oven drying at 65°C for 72 h and then milled separately through a 1 mm screen before analysis. Diets and feces were analyzed for crude protein (CP); method 954.01 [28], ether extract (EE) with petroleum ether solvent (method 920.39 [28]), ash (method 942.05 [28]), crude fiber (CF; method 962.09 [28]), neutral detergent fiber (NDF) with fiber filter bags of Ankom Technology F57 (method 973.18 [28]), GE through bomb calorimeter E2K (Germany), and chromium concentration was analyzed using ultraviolet absorption spectrophotometry (DR 3000, Germany) as previously described [29].

Statistical analysis

All data were analyzed using the PROC MIXED procedure of SAS (version 9.4, Institute Inc., Cary, NC, USA), and each pen was used as an experimental unit. The statistical model included the diets (n=4) as a fixed effect and the blocks (n=3) as random effects. Pen was used as an experimental unit for growth performance and digestibility data, and individual pig as an experimental unit for fecal microbiota, and blood profiles. For repeated measures performed on the same experimental unit, a similar model was used but including the effect of a compound symmetry structure of covariance. Orthogonal polynomials were calculated data according to the equation of [25] for ME estimation: $ME=4168-12.3\times Ash+1.4\times CP+4.1\times EE-6.1\times CF$ (g/kg DM). Calculated data.

| Table-1: Ingredients and nutrient composition of the experimental diets. |
|------------------|------------------|------------------|------------------|------------------|
| **Ingredients (%)** | **T0** | **T1** | **T2** | **T3** |
| Dry matter | 88.9 | 88.4 | 89.1 | 89.2 | 92.4 |
| Crude protein | 18.8 | 18.3 | 18.7 | 18.7 | 12.7 |
| Ether extract | 7.55 | 7.22 | 7.26 | 7.30 | 1.63 |
| Ash | 8.86 | 8.73 | 8.80 | 8.86 | 12.3 |
| Crude fiber | 5.24 | 5.89 | 5.97 | 6.95 | 28.4 |
| Calcium | 1.39 | 1.11 | 1.21 | 1.18 | 1.32 |
| Total phosphorus | 0.85 | 0.79 | 0.86 | 0.86 | 0.34 |
| Gross energy | 18.5 | 18.5 | 18.2 | 18.5 | 18.3 |
| Metabolizable energy | 13.9 | 13.8 | 13.7 | 13.5 | - |
| Lysine | 1.33 | 1.32 | 1.31 | 1.30 | - |
| Methionine | 0.54 | 0.53 | 0.53 | 0.53 | - |

MP=Medicinal plants power, ^1^T0=Control diet; T2, T4, and T6: T0 supplemented with 2, 4, and 6% of MP, respectively. ^2^Premix in 1 kg: ZnSO₄ (min-max): 250-300 mg; FeSO₄ (min-max): 150-200 mg; MnSO₄ (min-max): 150-200 mg; CuSO₄ (min-max): 250-300 mg; Biotin: 8 mg; activity enzyme: 100 g; coarse sand (max): 2%; sufficient enzyme in 1 kg: Saccharomyces boulardii: 10^5-10^7 CFU/g, Saccharomyces fibuliger: 10^5-10^7 CFU/g, Lactobacillus acidophilus: 10^5-10^7 CFU/g, Candida tropicalis: 10^5-10^7 CFU/g, moisture (max): 10%. ^A^blend powder of medicinal plants in 1 kg: 60% B. pilosa, 15% U. lobata, 15% P. palatiferum, 5% Ramulus cinnamomi, and 5% Star anise. ^3^Calculated data.
performed to determine linear and quadratic effects of increasing levels of MP in diets [30]. Data for pigs fed diets containing MP were compared with data for pigs fed control diet using orthogonal contrasts. The multiple comparisons of least-square means were performed according to Tukey adjustment. Significance was defined as \( p<0.05 \) and 0.05<\( p<0.10 \) were considered as a trend.

Results

Animal performance, health, and digestibility

The performances of the experimental pigs are shown in Table-2. There were no animal losses during the experimental period in the present study and no episodes of diarrhea or other symptoms were observed in the different groups. The IBW was not significantly different between dietary treatments and there were no significant effects between groups on FBW, ADG, and FCR (Table-2). Effects of increasing levels of MP on ATTD are presented in Table-3. No significant differences in the ATTD of DM, OM, CP, EE, and GE were observed between diet groups.

Blood characteristics

Significant linear decreases in RBC (\( p=0.003 \)), and plasma cholesterol, LDL, and urea nitrogen (\( p\leq0.04 \)) were observed in pigs fed MP diets compared with those fed the control diet. A trend for a linear decrease in lymphocytes proportions (\( p=0.05 \)) and a trend for open-up quadratic effect (\( p=0.08 \)) on creatinine and total protein concentrations were observed between diet groups. The other blood parameters including WBC, Hb, AST, ALT, bilirubin, or HDL were unaffected by the dietary treatments (Table-4). Besides, using orthogonal contrasts, decrease in RBC (\( p<0.05 \)), decrease trend in plasma cholesterol, and LDL (\( p\leq0.09 \)) were observed when animals fed MP diets were compared with the control animals.

Fecal microbiota

Fecal *E. coli*, *Salmonella*, *Clostridium*, and total bacteria concentrations were not significantly altered (\( p>0.05 \)) by the treatments (Table-5).

Discussion

In the present study, feeding increasing levels of MP to growing pigs had no significant effect on FBW, ADG, ADFI, and FCR. In agreement with our results, Hanczakowska et al. [31] reported that a diet containing herbal extract mixture of *Salvia officinalis*, *Urtica dioica*, *Magnolia officinalis*, and *Echinacea purpurea* unaffected the growth performance of pigs when compared to those fed a control diet. Ahmed et al. [32] reported that growing-finishing pigs fed a diet containing medicinal plants (pomegranate, *Ginkgo biloba*, and licorice) showed no changes in live body weight and ADG compared to a control group. Utiyama et al. [33] reported a similar ADG in weaning pigs fed a diet with medicinal extract (garlic, clove, cinnamon, pepper, thyme, cinnamaldehyde, and eugenol) compared to a control group. However, Yan et al. [34] demonstrated that growing pigs fed a diet supplemented with the inclusion of medicinal extract (buckwheat, thyme, curcuma, black pepper, and ginger) had improved ADG and ADFI but not FCR. Marcin et al. [35] reported that piglets fed a diet supplemented with extracts of sage and oregano also had significantly improved ADG. The world of phytochemistry being vast, the contradictory results regarding growth performance responses to natural, dried, or extracted herbs may be easily ascribed to different species, plant parts and their physical properties, age of plant, harvest time, processing method, and different dosage used [6,36,37]. Secondarily, different physiological periods of animal and housing conditions may respond differently to herb supplementation [6,31]. ATTD of nutrients was similar in pigs fed MP diets compared with those fed the control diet during 7 experimental weeks. Similar results were observed in the previous experiment [38] who reported that nutrient digestibility of growing pigs fed diet with herbal flavor supplementation for 10 experimental weeks was not affected. This suggests that the digestibility of moderate amounts of herbal plants could be similar to that of a control diet.

Dietary supplementation with medicinal plants may have a beneficial effect on hematological and biochemical characteristics of pigs. Supplementation with fermented medicinal plants (*Gynura procumbens*, *Rehmannia glutinosa*, and *Scutellaria baicalensis*) increased WBC concentration in growing pigs [39]. Increase in RBC counts and blood lymphocytes concentrations was found in weaning pigs fed a diet supplemented with fermented garlic powder at 0.5,

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**Table-2**: Feed intake, average daily gain, and feed conversion ratio (LSM) of growing pigs fed diets containing different levels of medicinal plants powder.

| Items      | Dietary treatment\(^a\) | SEM  | \( p\)-value |
|-----------|------------------------|------|-------------|
|           | T0         | T1   | T2   | T3   | Linear Quadratic |
| n         | 12         | 12   | 12   | 12   |                  |
| IBW (kg)  | 30.6       | 30.4 | 30.3 | 30.3 | 0.42             | 0.93 | 1.00 |
| FBW (kg)  | 65.6       | 65.1 | 64.6 | 64.2 | 1.49             | 0.48 | 0.98 |
| ADFI (kg/d)| 1.84      | 1.86 | 1.83 | 1.80 | 0.04             | 0.37 | 0.56 |
| ADG (g/d) | 750        | 737  | 731  | 720  | 31.8             | 0.50 | 0.98 |
| FCR (kg/kg)| 2.46      | 2.53 | 2.51 | 2.50 | 0.06             | 0.64 | 0.53 |

LSM=Least squares mean; SEM=Standard error of the mean; n=Number of animals; d=Day; g=Gram; IBW=Initial body weight; FBW=Final body weight; ADG=Average daily gain; ADFI=Average daily feed intake; FCR=Feed conversion ratio (kg feed/kg gain). \(^a\)T0=Control diet; T1, T2, and T3 supplemented with 2, 4, and 6% of blend powder of medicinal plants.
Dietary treatment: T0, control diet; T1, T2, and T3 supplemented with 2, 4, and 6% of blend powder of medicinal plants. DM=Dry matter; OM=Organic matter; CP=Crude protein; EE=Ether extract; LSM=Least squares mean; SEM=Standard error of the mean.

Table 3: Apparent total tract digestibility (LSM) of growing pigs fed diets containing different levels of medicinal plants powder (%).

| Items     | Dietary treatment | SEM | p-value |
|-----------|-------------------|-----|---------|
|           | T0 | T1 | T2 | T3 | Linear | Quadratic |
| DM        | 92.49 | 92.61 | 91.56 | 92.44 | 0.40 | 0.79 | 0.90 |
| OM        | 79.15 | 78.29 | 77.91 | 77.20 | 1.01 | 0.69 | 0.28 |
| CP        | 80.88 | 79.71 | 78.68 | 78.43 | 1.07 | 0.51 | 0.14 |
| EE        | 72.51 | 70.02 | 70.47 | 71.52 | 2.49 | 0.47 | 0.92 |
| GE        | 77.99 | 75.46 | 76.90 | 76.29 | 1.19 | 0.22 | 0.92 |

Table 4: Blood parameters (LSM) of growing pigs fed diets containing different levels of medicinal plant powders.

| Items     | Dietary treatment | SEM | p-value |
|-----------|-------------------|-----|---------|
|           | T0 | T1 | T2 | T3 | Linear | Quadratic |
| Number of animals | 6 | 6 | 6 | 6 |   |   |
| WBC (G/L) | 27.0 | 24.4 | 22.0 | 26.7 | 2.28 | 0.76 | 0.15 |
| Hb (g/dL) | 12.4 | 11.9 | 11.5 | 11.2 | 0.59 | 0.17 | 0.85 |
| LYM (%) | 69.5 | 58.8 | 60.6 | 52.0 | 4.97 | 0.05 | 0.84 |
| AST (U/L) | 37.7 | 57.9 | 37.9 | 43.1 | 8.63 | 0.92 | 0.40 |
| ALT (U/L) | 51.7 | 61.2 | 54.0 | 65.7 | 4.61 | 0.12 | 0.82 |
| Bilirubin (µmol/L) | 1.75 | 0.50 | 0.60 | 0.75 | 0.58 | 0.28 | 0.25 |
| Creatinine (µmol/L) | 137 | 117 | 118 | 134 | 9.63 | 0.88 | 0.08 |
| Total cholesterol (mmol/L) | 2.37 | 2.16 | 2.06 | 1.91 | 0.10 | 0.01 | 0.77 |
| HDL (mmol/L) | 0.97 | 1.04 | 0.99 | 0.98 | 0.03 | 0.97 | 0.22 |
| LDL (mmol/L) | 1.09 | 0.98 | 0.86 | 0.77 | 0.08 | 0.02 | 0.92 |
| Total protein (g/L) | 70.4 | 66.0 | 66.7 | 68.5 | 1.42 | 0.44 | 0.05 |
| Urea nitrogen (mmol/L) | 3.91 | 3.90 | 3.13 | 2.90 | 0.37 | 0.04 | 0.77 |

In our study, MP diets did not inhibit in pig feces the counts of the pathogenic bacteria studied compared with the control diet, which is consistent with a variety of medicinal plants as reported previously [46] who indicated that herbal plants did not inhibit the growth of E. coli and even enhance bacteria.
growth in gut intestinal of animals. Similarly, no significant differences in intestinal microbiology and diarrhea occurrence were observed in weanling pigs fed a diet with herbal extract mixture (garlic, clove, cinnamon, pepper, thyme, cinnamaldehyde, and eugenol) compared to those fed a control diet [33]. The present results indicate that the plant mixture did not show any bacteria modulating effect in gut on growing pigs.

Conclusion

From the present study, it can be concluded that growing pig diets containing up to 60 g/kg of a blended powder of medicinal plants (B. pilosa, U. lobata, P. palatiferum, R. cinnamomi, and Star anise) reduced blood cholesterol, LDL, and urea nitrogen concentrations without influencing performance, nutrient digestibility, and fecal microbiota of growing pigs. A limitation of this study is lacking interaction tests among medicinal plants. Further studies should be performed to obtain more comprehensive results.

Authors’ Contributions

NCO, VDT, and HJ: Conceived and designed research. NCO and NDT: Conducted the experiment and collected samples. NCO and TQL: Analyzed the samples. NCO and HJ: Analyzed the data. NCO: Wrote original draft. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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