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RESEARCH ARTICLE

Effect of Ultrafine Pulverization of *Senecio Scandens* on Growth, Immune System and Faecal Microorganisms in Piglets

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ABSTRACT

There is increased interest in using naturally occurring compounds subjected to new technologies for enhancing pig nutrition to replace antibiotic usage in swine production. The effects of ultrafine pulverization on the size distribution, morphology of *Senecio scandens* Buch.-Ham., and the growth performance, serum immunity parameters and faecal microorganisms of piglets fed this powder were investigated. The size distribution and morphology of *S. scandens* were characterized by using a laser diffraction analyser and scanning electron microscopy respectively. Ninety Duroc×Landrace×Yorkshire piglets (average body weight of 10.43kg) were randomly assigned to six treatments with three pens of five pigs per treatment. Group 1 (Control) piglets were fed the basal diet only. Groups 2 to 5 were fed with the basal diet supplemented with ultrafine powder (median diameter [$d_{0.5}$] of 8.89μm) of *S. scandens* at 0.3, 0.6, 0.9, and 1.2% of the basal diet, respectively, for 30 days. For group 6, 1.2% of ordinary *S. scandens* powder ($d_{0.5}$=88.59μm) was added to the basal diet. Both *S. scandens* ordinary and ultrafine powder increased piglet body weight and reduced the feed to gain ratio, but the performance of piglets fed the ultrafine powder was better. In groups 4 to 6, the number of *Escherechia coli* in faeces and the diarrhoeal incidence were significantly lower ($P<0.05$) and the serum IgA, IgG, IgM contents significantly higher ($P<0.05$). Feeding *S. scandens* ultrafine powder in the diet improved piglet performance and the diet supplemented with 0.9% of the ultrafine powder was the most effective.

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INTRODUCTION

The genus *Senecio* belongs to the Asteraceae plant family of more than 1000 species, of which 63 are found in China (Chen, 1999). *Senecio scandens* Buch. –Ham. is widely used, in single or formulated forms, in Chinese folk and herbal medicine. The main components in *S. scandens* are flavonoids, alkaloids and volatile oils (Li *et al*., 2008; Wang *et al.*, 2013). The herb has been shown to have anti-inflammatory, antioxidant, hepatoprotective, antibiotic and antiviral properties (Shi *et al*., 2007; Wang *et al*., 2013). A 78% ethanolic solution of *S. scandens* had an antibacterial effect against *Salmonella enteritidis* and *Escherichia coli* (*E. coli*) (Chen *et al.*, 2001).

Ultrafine pulverization is a new technology currently used in the processing of traditional Chinese medicines. Ultrafine pulverization can change the structure, surface area and functional properties of herbs, and thereby make them more easily digestible and absorbed (Chau *et al*., 2007; Zhao *et al.*, 2009). To our knowledge, there is no report on the effects of ultrafine pulverization on the size distribution and morphological characterization of *S. scandens* or of adding *S. scandens* powder to swine feed to improve piglet performance.

The aims of the present study were to determine the effect of pulverization of *S. scandens* on (1) the size distribution and morphological characteristics of the herb, (2) body weight including feed to gain ratio of piglets fed the powdered herb and (3) piglet serum IgA, IgG and IgM content, and (4) to evaluate overall piglet performance and the potential application of ultrafine *S. scandens* in swine diet to improve production.

MATERIALS AND METHODS

Preparation of *S. scandens* powder: Herbal samples of *S. scandens* were collected from Lipu County, Guangxi Province, and confirmed as *Senecio scandens* Buch.-Ham. by the Institute of Agricultural Biology of Guangdong Ocean University, Zhanjiang, Guangdong, China. The herbal samples were cut, air-dried, and ground in anRT-34 miniature grinder (Beijing Huanya Tianyuan Machinery Technology Co., Beijing, China) to prepare the *S. scandens* powder. Most of this was then pulverized using YSC-701 ultra-micro pulverizer (Beijing Yanshan Zhengde Instrument Equipment Co., Beijing, China) to obtain the ultrafine powder.

Size distribution of *S. scandens* powder: The *S. scandens* powder was dispersed in a container with ultrapure water and the size distribution of the ordinary and ultrafine powders was measured using a Mastersizer 2000 laser diffraction analyser (Malvern Instruments, Malvern, UK).

Morphological characterization of *S. scandens* powder: *S. scandens* powder and ultrafine samples were coated with gold using an ion sputter coater (Hitachi E-1045, Tokyo, Japan), examined by scanning electron microscopy (SEM) (Hitachi S-4800, Tokyo, Japan) and photomicrographs taken for morphological characterization.

Animal, treatment, diets and housing: The feeding experiment was conducted at the Guangxi Judong Animal Farming Group, Yulin, Guangxi, China. Ninety Duroc× Landrace×Yorkshire piglets with an average initial body weight of 10.43kg were randomly and equally assigned to six treatment groups, each with three pens(2.5×3m) of five pigs (three castrated male piglets and two female piglets). The piglets in group 1 were fed the basal balanced piglet diet, and those in groups 2, 3, 4 and 5 the basal diet supplemented with 0.3, 0.6, 0.9, and 1.2% of *S. scandens* ultrafine powder, respectively. For group 6, ordinary *S. scandens* powder (1.2%) was added to the basic diet. The composition and nutrient concentrations of the basal balanced diet are shown in Table 1.

Growth performance of piglets: Piglet body weight and supply of diet feed were measured at the beginning and end of the experiment. These data were used to calculate the average daily feed intake, average daily gain and feed to gain ratio (Qiao *et al*., 2013).

Diarrhoeal incidence: The piglets appeared normal except diarrhoea was observed daily in all piglets from the beginning of the experiment. The faecal consistency was characterized using a scale of 0 to 3, with 0=normal, solid faeces; 1=soft, loose faeces with slight diarrhoea; 2=moderate diarrhoea; 3=liquid, severe diarrhoeic faeces (Wang *et al*., 2009). When the score was 2 or above 3, the piglets were considered to have diarrhoea (Kong *et al*., 2007). The diarrhoeal incidence was determined according to the method of Huang *et al.* (2004).

Serum immunity parameters: At the end of experiment, blood was obtained from the piglets via the jugular vein. The serum was separated and stored at −20°C. A commercial swine enzyme-linked immunosorbent assay kit (ELISA, Jiancheng Institute of Biological Technology, Nanjing, China) was used to measure serum immunoglobulin G, A and M (IgG, IgA and IgM) in an MK3 microplate spectrophotometer (Thermo Electron Corp., Massachusetts, USA).

Faecal microorganisms: On day 1 and day 30 of the experiment, faeces were collected from all animals and temporarily stored at 4°C to determine the numbers of *E. coli* and *Lactobacilli*, using the plate colony counting method of Giang *et al*. (2012). The results are expressed as CFU/g faeces.

Statistical analyses: Data are expressed as the mean ±standard error and were analysed using ANOVA (SAS Institute 2001 software) as factorial experiments. Duncan's multiple range test was used to compare differences among the treatment groups. Probability values were considered significant when $P<0.05$.

Ethical considerations: All piglets were housed in an environmentally-controlled nursery with slatted cement flooring and a mechanical ventilation system. The piglets were given free access to feed and water during the 30-day experiment. The piglet research carried out in this study was approved by the Animal Ethics Committee of Guangdong Ocean University (Approval No: NXY2014-002). The ARRIVE guidelines reporting *in vivo* animal experiments were adhered to.

RESULTS

Size distributions of *S. scandens:* The size distributions of ordinary powder and ultrafine powder of *S. scandens* were shown in Figure 1. After ultrafine pulverization, the diameter distribution of ultrafine powder approached normal distribution. The ultrafine powder was smaller and the diameter narrower than the ordinary powder. The volume median diameter ($d_{0.5}$) values of the ordinary and ultrafine powders were 88.59μm and 8.88μm, respectively. In 90% of the ordinary powder sample, the diameter ($d_{0.9}$) was 15.56 times greater than in the ultrafine powder (Table 2).

Morphological characterization: The electron micrographs of the ordinary and ultrafine powders of *S. scandens* (Figure 2) show that the ultrafine powder had no unbroken structures and was narrower and more uniform in size than the ordinary powder.

Piglet growth performance: With increasing concentrations of *S. scandens* powder from groups 2 to 4, the final weight of piglets and average daily gain also showed an increasing trend, while the feed to gain ratios reduced (Table 3). Average daily feed intake values were not significantly different. Compared with group 1 (control), mean final piglet weights in groups 2, 3, 4, 5 and 6 increased by 0.96, 2.25, 10.81 ($P<0.05$), 3.37 and 9.09%, respectively and the feed to gain ratios were reduced by 1.53, 7.14, 17.35 ($P<0.05$), 9.69 and 15.31% ($P<0.05$) in the respective groups. Groups 4, 5 and 6 were not significantly different, although growth performance in group 4 appeared the best.

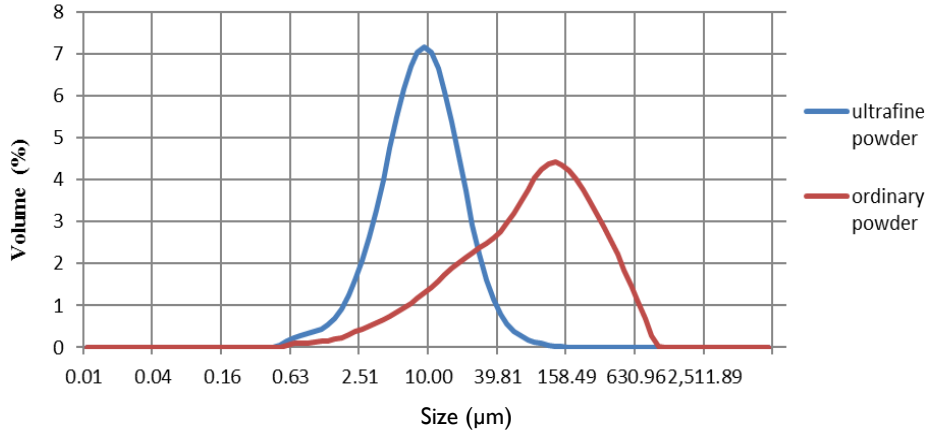

Fig. 1: Size distribution of ordinary powder and ultrafine powder of *S. scandens*.

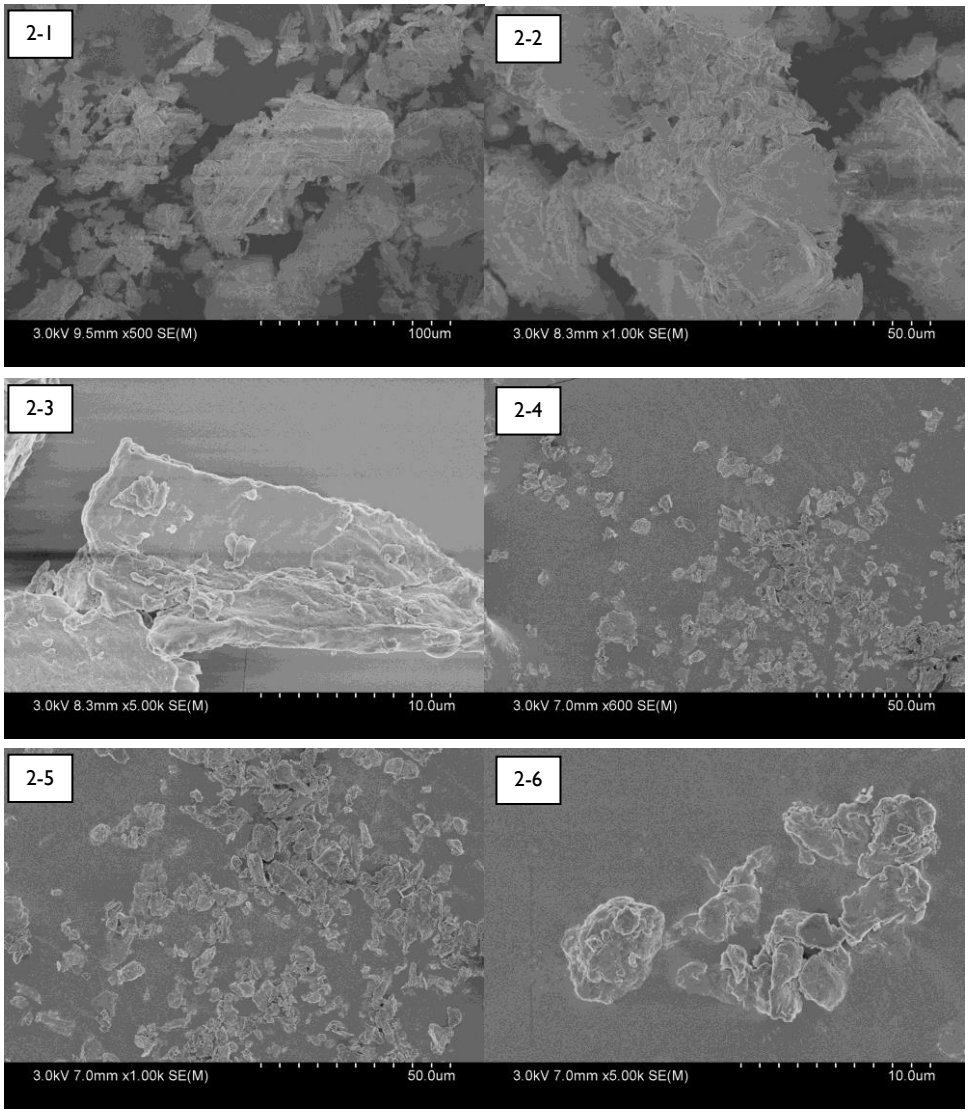

Fig. 2: Scanning electron microscopy images of ordinary powder and ultrafine powder of *S. scandens*. Micrographs 2-1 to 2-3 are of the ordinary powder and 2-4 to 2-6 the ultrafine powder.

Diarrhoeal incidence: Adding *S. scandens* powder reduced the incidence of diarrhoea in the piglets (Table 4). Initially (0 to 15 days), the diarrhoeal incidence of groups 3 to 6 was significantly lower ($P<0.05$) than that of the control group 1. However, during the second fortnight (15 to 30 days), a significant reduction ($P<0.05$) in diarrhoeal incidence was observed only in group 5>4>6, compared with group 1.

Serum immunity parameters: The serum IgG, IgA, IgM values were shown in Table 5. Compared with the control values for group 1, the IgG of groups 2 to 6 increased by *1.55, 4.07, 10.86, 12.50, and 8.89%, respectively. IgA increased by *1.27, 5.15, 13.69, 13.40 and 8.65%, and IgM by *3.55, *9.43, 18.85, 27.56 and 26.41%, respectively. All increases were significant ($P<0.05$) except the four marked with an asterisk. However, there were no significant differences between groups 4, 5 and 6. Collectively, the effect on group 4 was the best.

Faecal microorganisms: Table 6 showed the effects of adding *S. scandens* powder on faecal microorganism count.

On day 1, no significant difference was observed between *E. coli* and *Lactobacillus* in the different piglet groups. At the end of the experiment, compared with group 1, a significant reduction in the number of *E. coli* ($P<0.05$) was observed in groups 4, 5 and 6. However, there was no significant difference among these three groups. For *Lactobacillus*, a non-significant increasing trend was observed.

DISCUSSION

According to British Standards, the width of particle size distribution is measured by span. A smaller span value means a powder has a more uniform size and a narrower size distribution (Zhang *et al*., 2012). Particle size distributions are often characterized by $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ values (Giry *et al*., 2006). In our study, following ultrafine pulverization, the span value changed from 1000μm in the ordinary to 100μm in the ultrafine powder, and the values of $d_{0.5}$ and $d_{0.9}$ also decreased. All these parameters indicated that the ultrafine powder had a more uniform and smaller size.

Table 1: Composition and nutrient levels of the basal piglet diet

Ingredients	Content (%)	Nutrient Levels[1)]	Content
Corn	63.65	DE (MJ/kg)	14.12
Soybean meal	22.70	CP (%)	18.00
Whey powder	6.00	Lys (%)	1.16
Fish meal	4.00	Ca (%)	0.74
Soybean oil	0.90	AP (%)	0.46
$CaHPO_4$	0.83		
Limestone	0.67		
NaCl	0.25		
Premix[2)]	1.00		
Total	100.00		

[1)] Calculated value. [2)] The premix provided the following per kilogram of diet mix: vitamin A 10,000 IU; vitamin D_3 1600 IU; vitaminE 80 mg; vitamin B_2 8 mg; choline chloride 540 mg; Fe 100 mg; Cu 120 mg; Zn 90 mg; Mn 60 mg.

Table 2: Size of ordinary and ultrafine powder of *S. scandens*

Sample	$d_{0.1}$ (μm)	$d_{0.5}$ (μm)	$d_{0.9}$ (μm)
Ordinary powder	9.85	88.59	365.68
Ultrafine powder	3.06	8.88	23.37

Note: $d_{0.1}$, $d_{0.5}$, $d_{0.9}$ respectively mean equivalent volume diameters at 10, 50, and 90% cumulative volumes.

Scanning electron microscopy is used in both the biological and material sciences to examine the topology and elemental composition of specimen surfaces (Paredes, 2014). In more recent years, SEM has been increasingly used for research in Chinese traditional herbal medicine. Mechanical damage transforms the structure of powders from ordered to disordered or amorphous because of breakage of intermolecular bonds (Zhao *et al*., 2010). Use of different grinders can cause marked changes to the structure and diameter of *S. scandens*. SEM confirmed that the structure of the ultrafine powder used in this study was smaller and also more uniform than the ordinary powder.

In our study, adding *S. scandens* powder to the diet enhanced the growth performance of piglets. The main components of flavonoids, alkaloids and volatile oils from *S. scandens* (Tan *et al*., 2010; Wang *et al*., 2013) probably work synergistically to improve growth performance. In this study, the effect of 0.9% ultrafine powder of *S. scandens* in piglet feed performed better than other concentrations. The dosage of 1.2% of the ordinary *S. scandens* powder also had an effect on the piglets' average daily feed intake. It appears that the ultrafine pulverization of *S. scandens* changed its cellular structure and allowed the more active constituents of the ultrafine powder to be more easily assimilated and thus improved digestibility and absorption of dietary nutrients within the piglet gastrointestinal system. Therefore, from an economic point of view, addition of the ultrafine powder can reduce the dosage and indirectly reduce feed costs.

Diarrhoea is a pathological state with an increase in faecal water content and increased frequency of defecation (Wang *et al*., 2014). This is an economic loss because this results in less time being available for absorption of important nutrients by the small intestine. The aetiology of diarrhoea is multifactorial, with nutrition, viruses, and bacteria being mostly implicated (Petri *et al*., 2008; Sørensen *et al*., 2009). Post-weaning diarrhoea is common in piglets and is in most instances caused by proliferation of enterotoxigenic *E. coli* and toxins produced by these bacteria (Wang *et al*., 2009; Zhang *et al.*, 2010). *S. scandens* has been used for antibiosis and as an anti-inflammatory agent in Chinese traditional medicine. It has broad-spectrum antibacterial activity, mostly against *E. coli*, *Staphylococcus aureus* and *Dysenteric bacilli* (Wang *et al*., 2013). In this study, adding the *S. scandens* powder to the feed offered some protection against diarrhoea in piglets. At weaning, usually the gut population of *Lactobacilli* decreases and the *E. coli* population increases (Jensen, 1998). *E. coli* is the main pathogen responsible for piglet diarrhoea after weaning (Fairbrother *et al*., 2005). Chen *et al.* (2001) found that 78% ethanolic solution of *S. scandens* produced a marked antibacterial effect against *E. coli in vivo* and *in vitro*. In this current study, adding 0.9% or 1.2% ultrafine powder of *S. scandens* (groups 4 and 5) and 1.2% ordinary powder (group 6) significantly reduced the number of *E. coli* and decreased diarrhoeal incidence in piglets. In addition, there was a trend of *Lactobacilli* increasing in numbers in those fed *S. scandens.* Flavonoids and alkaloids of *S. scandens* play an important role in broad-spectrum antibacterial activity. A possible mechanism is via inhibition of *E. coli* proliferation by flavones and alkaloids in *S. scandens*, thereby improving the microbial community balance for *Lactobacilli* to thrive in the gut and improve the intestinal environment of piglets. A decrease in diarrhoeal incidence improves nutrient absorption thereby enhancing the growth performance of piglets and consequently reducing economic losses associated with the disease.

All mammals have IgG, IgA and IgM in their blood. IgG plays an important role in humoral immunity, and functions as an antiviral and antibacterial agent and also to neutralize toxins. The functions of IgA are similar to IgG, but it especially has an anti-infection effect on membrane infection by pathogenic microorganisms. On first contact with an antigen, IgM is the earliest antibody to act (Zhao *et al*., 2012). In this study, compared with group 1, adding *S. scandens* powder increased the IgG, IgA, IgM concentrations, with significant increases ($P<0.05$) in piglets in groups 4 to 6. It appears that appropriate addition of *S. scandens* powder can markedly improve the humoral immunity of piglets. This will no doubt enhance immune resistance and, coupled with a significant decrease in gut *E. coli*, reduce the pathogenic bacterial load to piglets as shown by a reduction in diarrhoeal incidence and an improved growth performance.

Table 3: Effect on piglet growth performance of adding increasing amounts of *S. scandens* powder to the basic diet

Group[1]	Initial weight (kg)	Final Weight (kg)	ADG[2] (g)	ADFI[3] (g)	Feed to Gain Ratio[4]
1	10.43±0.17	20.91±0.66^{b}	349.33±17.48^{c}	683.29±20.29	1.96±0.04^{a}
2	10.43±0.15	21.11±0.67^{b}	356.11±26.74^{bc}	682.51±19.81	1.93±0.09^{a}
3	10.43±0.15	21.38±0.83^{ab}	365.00±24.96^{abc}	662.47±32.66	1.82±0.04^{ab}
4	10.43±0.12	23.17±0.53^{a}	424.56±19.37^{a}	686.91±17.33	1.62±0.04^{c}
5	10.44±0.14	21.69±0.45^{ab}	375.00±10.41^{abc}	662.69±0.79	1.77±0.05^{abc}
6	10.43±0.02	22.81±0.17^{ab}	413.33±5.36^{ab}	685.91±28.35	1.66±0.07^{bc}

[1] Groups 1 to 5 supplemented with 0, 0.3, 0.6, 0.9, and 1.2% ultrafine powder, respectively, for 30 d. Group 6, 1.2% of ordinary powder added. [2] ADG: average daily gain; [3] ADFI: average daily feed intake. [4] In the same column, values with the same superscript letters or no superscript indicate no significant difference (P>0.05), and different superscript letters indicate a significant difference (P<0.05).

Table 4: Effect on piglet diarrheal incidence (%) of adding *S. scandens* powder. Groups 1 to 5 supplemented with 0, 0.3, 0.6, 0.9, and 1.2% ultrafine powder, respectively, for 30 d. Group 6, 1.2% of ordinary powder added

	1	2	3	4	5	6
0 to 15 d	14.22±0.45^{a}	12.00±0.77^{ab}	10.67±0.77^{b}	4.89±0.89^{cd}	3.56±0.89^{d}	6.22±0.89^{c}
15 to 30 d	7.11±1.60^{a}	6.67±0.77^{a}	5.33±0.77^{ab}	2.22±0.45^{c}	1.33±0^{c}	2.67±0.77^{bc}

In the same line, values with the same superscript letters indicate no significant difference (P>0.05), and different superscript letters indicate a significant difference (P<0.05).

Table 5: Effect on piglet serum immunity parameters of adding *S. scandens* powder. Groups 1 to 5 supplemented with 0, 0.3, 0.6, 0.9, and 1.2% ultrafine powder, respectively, for 30 d. Group 6, 1.2% of ordinary powder added

Group	IgG (μg/mL)	IgA (μg/mL)	IgM (μg/L)
1	2108.71±30.05^{d}	553.93±3.36^{d}	163.65±2.65^{c}
2	2141.30±19.62^{cd}	560.99±2.25^{d}	169.46±5.11^{c}
3	2194.54±19.86^{c}	582.45±3.64^{c}	179.08±4.46^{bc}
4	2337.71±24.93^{ab}	629.79±2.88^{a}	194.49±5.39^{ab}
5	2372.38±21.55^{a}	628.14±4.00^{a}	208.75±9.27^{a}
6	2296.09±12.64^{b}	601.84±4.13^{b}	206.87±3.64^{a}

In the same column, values with the same superscript letters or no superscript mean no significant difference (P>0.05), and different superscript letters indicate a significant difference (P<0.05).

Table 6: Effects on fecal microorganisms (log_{10}CFU/g) of adding *S. scandens.* Groups 1 to 5 supplemented with 0, 0.3, 0.6, 0.9, and 1.2% ultrafine powder, respectively, for 30 d. Group 6, 1.2% of ordinary powder added

Group	*E. coli*		*Lactobacillus*	
	Day 1	Day 30	Day 1	Day 30
1	7.14±0.15	6.39±0.16^{a}	7.43±0.12	8.18±0.16
2	7.06±0.13	6.38±0.12^{a}	7.43±0.12	8.24±0.14
3	7.28±0.17	6.25±0.11^{ab}	7.43±0.09	8.35±0.15
4	7.09±0.18	5.94±0.02^{b}	7.40±0.13	8.36±0.17
5	7.04±0.20	5.92±0.06^{b}	7.35±0.10	8.38±0.17
6	7.05±0.18	5.90±0.01^{b}	7.37±0.11	8.38±0.18

In the same column, values with the same superscript letters or no superscript indicate no significant difference (P>0.05), and different superscript letters indicate a significant difference (P<0.05).

Conclusions: Dietary supplementation with *S. scandens* ordinary and ultrafine powders reduced the number of faecal *E. coli* but increased the number of *Lactobacilli*, and improved the serum IgA, IgG, IgM content in piglets. Collectively, this resulted in a reduced diarrhoeal incidence, and an enhanced growth performance in piglets. Adding *S. scandens* ultrafine powder (which has a narrower and a more uniform size than the 'normal' powder) at 0.9% concentration is an effective method to improve piglet production performance.

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