



Effect of Fermented *Chlorella* Supplementation on Growth Performance, Nutrient Digestibility, Blood Characteristics, Fecal Microbial and Fecal Noxious Gas Content in Growing Pigs

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ABSTRACT: A total of 96 growing pigs (Landrace×Yorkshire)×Duroc; BW = 26.58±1.41 kg) were used in a 6-wk feeding trail to evaluate the effects of fermented chlorella (FC) supplementation on growth performance, nutrient digestibility, blood characteristics, fecal microbial and fecal noxious gas content in growing pigs. Pigs were randomly allotted into 1 of 4 dietary treatments with 6 replicate pens (2 barrows and 2 gilts) per treatment. Dietary treatments were: i) negative control (NC), basal diet (without antibiotics); ii) positive control (PC), NC+0.05% tylosin; iii) (fermented *chlorella* 01) FC01, NC+0.1% FC, and iv) fermented *chlorella* 02 (FC02), NC+0.2% FC. In this study, feeding pigs PC or FC01 diets led to a higher average daily gain (ADG) and dry matter (DM) digestibility than those fed NC diet ($p < 0.05$), whereas the inclusion of FC02 diet did not affect the ADG and DM compared with the NC group. No difference ($p > 0.05$) was observed on the body weight, average daily feed intake (ADFI), gain:feed (G:F) ratio, the apparent total tract digestibility of N and energy throughout the experiment. The inclusion of PC or FC did not affect the blood characteristics ($p > 0.05$). Moreover, dietary FC treatment led to a higher ($p < 0.05$) *lactobacillus* concentration and lower *E. coli* concentration than the NC treatment, whereas the antibiotic supplementation only decreased the *E. coli* concentration. Pigs fed FC or PC diet had reduced ($p < 0.05$) fecal NH₃ and H₂S content compared with those fed NC diet. In conclusion, our results indicated that the inclusion of FC01 treatment could improve the growth performance, nutrient digestibility, fecal microbial shedding (lower *E. coli* and higher *lactobacillus*), and decrease the fecal noxious gas emission in growing pigs when compared with the group fed the basal diet. In conclusion, dietary FC could be considered as a good source of supplementation in growing pigs because of its growth promoting effect. (**Key Words:** Fermented *chlorella*, Growing Pig)

INTRODUCTION

Chlorella vulgaris (*C. vulgaris*) is a genus of unicellular green algae containing high level of protein (50 to 70% of dry matter), lipid, vitamins and minerals (Phang, 1992). Rania and Hala (2008) had previously suggested that *C. vulgaris* have a higher antimicrobial activity due to the presence of cyclic peptides, alkaloids, and lipopolysaccharides *in vitro*. Janczyk et al. (2009) also suggested that the inclusion of *C. vulgaris* could benefit the intestinal *lactobacillus* diversity in laying hens because of the unknown compounds such as polysaccharides, phenolic substances and aromatic compound (Halama, 1990). Therefore, *C. vulgaris* appears to have the highest potential as a function protein source for the livestock.

Buckenhuskes et al. (1990) suggested that a fermentation step could give several advantages (improved flavor, enrichment with desirable metabolites produced by the microorganisms, and enhanced safety) to the vegetable and crops. Our previous studies also confirmed that fermentation process could improve the quality of the soybean meal, fish meal and garlic powder, and subsequently improve the growth performance of the animal (Cho and Kim., 2011; Yan et al., 2011a; Yan et al., 2012a, b). Therefore, it is suitable to hypothesize that fermentation process could also benefit the chlorella utilization in livestock. Indeed, Keijiro (2011) suggested that the inclusion of fermented *C. vulgaris* significantly improved the protein digestibility compared with non-fermented chlorella in rat. Zheng et al. (2012) also demonstrated that dietary fermented *C. vulgaris* could improve the production performance of the laying hens.

However, there is no study concerning the effect of fermented *C. vulgaris* supplementation in pigs. So, the

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objective of the current study is to determine the effect of fermented *C. vulgaris* supplementation on growth performance, nutrient digestibility, blood characteristics, fecal microbial concentration and fecal noxious gas content in growing pigs.

MATERIALS AND METHODS

The experimental protocols were approved by the Animal Care and Use Committee of Dankook University.

Preparation of fermented *C. vulgaris*

A commercial product, *C. vulgaris* microalgae produced by fermentation (CBT[®]) was obtained from Celltech, Co. Ltd, Korea. *C. vulgaris* was inoculated with baker's yeast and lactic acid bacterium. After 72 h of dry fermentation with cereal broth, fermented *C. vulgaris* then dried at low temperature and ground into powder (Zhang et al., 2012).

Experimental design, animals, and housing

A total of 96 growing pigs ((Landrace×Yorkshire)×Duroc, BW = 26.58±1.41 kg) were randomly allotted into 4 dietary treatments with 6 replicate pens (2 barrows and 2 gilts per pen) in a complete block design model. Dietary treatments were: i) negative control (NC), basal diet (without antibiotics); ii) positive control (PC), NC+0.05% tylosin; iii) fermented *Chlorella* 01 (FC01), NC+0.1% FC, and iv) fermented *Chlorella* 02 (FC02), NC+0.2% FC. Diets (Table 1) were formulated to meet or exceed the nutrient requirements recommended by NRC (1998). Pigs were housed in an environmentally controlled, slatted-floor facility in 36 adjacent pens (1.8×1.8 m) at the pig farm of Dankook University. All pigs were provided with *ad libitum* access to feed and water through a self-feeder and nipple drinker, respectively, throughout the experiment. The target room temperature and humidity were 25°C and 60%, respectively.

Sampling and measurements

Pig weights were measured at the beginning and the end of the experiment period, feed consumption were also recorded on a pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain:feed ratio (G:F). Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 d prior to fecal collection at the 6th wk for calculation of DM, N, and energy digestibility. Fecal grab samples were collected at random from at least 2 pigs in each pen (1 gilt and 1 barrow). All feed and feces samples were stored immediately at -20°C until analysis. All the feed and fecal samples were freeze-dried and finely ground to be able to pass through a 1-mm screen. The determination of DM (method 934.01), N (method 955.04),

Table 1. Composition of the basal experimental diets (as-fed basis)³

Items	Content
Ingredients (g/kg)	
Corn	553.8
Soybean meal	334.3
Molasses	25.0
Animal fat	53.3
Difluorinated phosphate	19.3
Limestone	7.8
L-lysine HCl	1.7
Trace mineral premix ¹	1.0
Vitamin premix ²	1.2
Salt	2.0
DL-methionine	0.3
Choline chloride	0.3
Chemical composition	
ME (kcal/kg)	3,360
Crude protein (%)	20.00
Lysine (%)	1.30
Calcium (%)	0.90
Phosphorus (%)	0.80
Analyzed composition	
GE (kcal/kg)	4,112
Crude protein (%)	19.65
Calcium (%)	0.87
Phosphorus (%)	0.76

¹ Provided per kg of complete diet: 12.5 mg Mn, 179 mg Zn, 140 mg Cu, 0.5 mg I and 0.4 mg Se.

² Provided per kg of complete diet: 20,000 IU of vitamin A; 4,000 IU of vitamin D₃; 80 IU of vitamin E; 16 mg of vitamin K₃; 4 mg of thiamine, 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B₁₂; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.

³ The additive was included in the diets by replacing the same amount of corn.

and energy digestibility were conducted in accordance with the methods established by the AOAC (2000). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the apparent total tract digestibility (ATTD) of DM, N, and energy were calculated using indirect methods described by Williams et al. (1962).

Two pigs were randomly selected from each pen (1 gilt and 1 barrow) and bled via jugular venipuncture at the beginning of the experiment (0 d). The same pigs were bled at the end (35 d) of the experiment. Blood samples were collected into vacuum tubes containing K₃EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to obtain whole blood. The red blood cells (RBC), white blood cells (WBC) and lymphocyte counts of whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA).

On 21 and 35 d, fecal samples were collected from 2

Table 2. Effect of fermented chlorella supplementation on growth performance in growing pigs¹

Items	NC	PC	FC01	FC02	SE ²	p-value
Body weight (kg)						
Initial	26.63	26.57	26.53	26.57	1.41	0.215
Final	52.84	54.37	54.04	53.28	1.57	0.183
Growth performance						
ADG (g)	624 ^b	662 ^a	655 ^a	636 ^{ab}	12	0.013
ADFI (g)	1,472	1,495	1,479	1,525	43	0.236
G/F	0.424	0.443	0.443	0.417	0.013	0.118

¹ NC = Basal diet; PC = NC+antibiotics; FC01 = NC+0.1% fermented *chlorella*; FC02 = NC+0.2% fermented *chlorella*. ² Standard error.

^{a,b} Means in the same row with different superscripts differ ($p < 0.05$).

pigs (1 gilt and 1 barrow) randomly selected from each pen by massaging the rectum, after which they were pooled on a pen basis and placed on ice for transportation to the laboratory, where analysis was immediately carried out. 1 g of fresh sample from each pen was then diluted with 9 ml of 0.1% peptone broth (Becton, Dickinson and Co.) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

For the analysis of the fecal NH₃ and H₂S, fresh feces were collected from 2 pigs in each pen on the last 2 d of the experiment. The total sampled feces was then thawed and homogenized, after which the stock feces were stored in 2.6-L plastic boxes with a small hole in the middle of one side that was sealed with adhesive plaster. The samples were allowed to ferment for 1 d at room temperature (25°C), after which 100 ml of the headspace air was sampled from approximately 2.0 cm above the fecal sample. Concentration of NH₃ and H₂S were measured within the scope of 5.0 to 100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0 to 20.0 ppm (4LK, detector tube; Gastec Corp.). After collection, box was resealed with adhesive plaster to measure the fecal noxious content at d 3 and 5 as aforementioned. Prior to measurement, the fecal samples were manually shaken for

approximately 30 s to disrupt any crust formation on the surface of the fecal sample and to homogenize the samples. Data of the fecal noxious gas content were presented as the mean value.

Statistical analyses

Before conducting statistical analysis of the microbial counts, the value was transformed logarithmically. All data were subjected to statistical analysis in a randomized complete block design using the GLM procedures (SAS Inst. Inc., Cary, NC, USA), with the pen serving as the experimental unit. The initial BW was used as a covariate for the ADFI and ADG, and the initial values were used as a covariate for the blood profile. Duncan's multiple range test was used to compare the means of the treatments. Variability in the data was expressed as the pooled standard error (SE) and a $p < 0.05$ was considered to be statistically significant.

RESULTS

Growth performance

The inclusion of PC and FC01 treatment led to a higher ($p < 0.05$) ADG than those fed NC group (Table 2). No difference ($p > 0.05$) was observed on the body weight, ADFI and G:F ratio during the whole experiment.

Nutrient digestibility and blood characteristics

No difference ($p > 0.05$) was observed on the apparent total tract digestibility of N and energy at the end of the experiment (Table 3). Pig fed the PC and FC01 treatment led to a higher ($p < 0.05$) DM digestibility compared with NC group, whereas the inclusion of FC02 treatment did not

Table 3. Effect of fermented chlorella supplementation on nutrient digestibility in growing pigs¹

Items (%)	NC	PC	FC01	FC02	SE ²	p-value
Dry matter	76.04 ^b	79.03 ^a	78.61 ^a	77.92 ^{ab}	1.25	0.021
Nitrogen	78.87	79.30	78.37	78.55	1.97	0.238
Energy	75.74	78.22	76.94	76.50	1.45	0.143

¹ NC = Basal diet; PC = NC+antibiotics; FC01 = NC+0.1% fermented *chlorella*; FC02 = NC+0.2% fermented *chlorella*. ² Standard error.

^{a,b} Means in the same row with different superscripts differ ($p < 0.05$).

Table 4. Effect of fermented chlorella supplementation on blood profiles in growing pigs¹

Items	NC	PC	FC01	FC02	SE ²	p-value
RBC (10 ⁶ /μl)	7.77	7.61	7.59	7.70	0.31	0.152
WBC (10 ³ /μl)	19.3	19.5	19.3	19.3	0.30	0.315
Lymphocyte (%)	47.5	47.7	49.5	50.2	4.87	0.169

¹ NC = Basal diet; PC = NC+antibiotics; FC01 = NC+0.1% fermented *chlorella*; FC02 = NC+0.2% fermented *chlorella*. ² Standard error.

Table 5. Effect of fermented chlorella supplementation on fecal microbial in growing pigs¹

Items (log ₁₀ cfu/g)	NC	PC	FC01	FC02	SE ²	p-value
<i>E. coli</i>	6.79 ^a	6.40 ^b	6.31 ^b	6.21 ^b	0.19	0.021
<i>Lactobacillus</i>	7.51 ^a	7.43 ^a	7.78 ^b	7.96 ^b	0.16	0.016

¹ NC = Basal diet; PC = NC+antibiotics; FC01 = NC+0.1% fermented *chlorella*; FC02 = NC+0.2% fermented *chlorella*. ² Standard error.

^{a,b} Means in the same row with different superscripts differ (p<0.05).

(p>0.05) increase the DM at the end of the experiment. Moreover, blood characteristics were not affected (p<0.05) by the treatment throughout the experiment (Table 4).

Fecal microbial shedding and fecal noxious gas content

The inclusion of PC treatment reduced the fecal *E. coli* (p<0.05) compared with NC treatment (Table 5), whereas the *lactobacillus* were not affected. Dietary FC supplementation led to a significant (p<0.05) higher *lactobacillus* concentration and lower *E. coli* concentration than the NC treatment. Moreover, pig fed FC or PC treatment reduced (p<0.05) the fecal NH₃ and H₂S content compared with those fed NC treatment (Table 6).

DISCUSSION

Effect of antibiotics supplementation

It is evident from the current study that pig fed antibiotic supplemented diet led to a higher ADG than those fed the NC treatment. In agreement with our study, Yan et al. (2012c) also reported an increased growth performance with antibiotics supplementation, and suggested that the reason for the improved growth performance is likely to be the increased nutrient digestibility. Similar in our study, an increased DM digestibility was also observed with the antibiotic supplementation. It is well accepted that dietary antibiotic supplementation could decrease the pathogenic bacteria in the intestine, and subsequently increased the nutrient digestibility of pigs. Nagy and Fekete (1999) also suggested that *E. coli* in the intestine may decrease the nutrient digestibility and growth performance in farm animals. In this study, the inclusion of antibiotic

significantly reduced the *E. coli* concentration compared with basal diet, which reflected the increased nutrient digestibility and confirmed the beneficial effect of antibiotics supplementation in growing pigs.

Effect of FC supplementation

In the current study, a marginally positive effect on the ADG was observed with the FC supplementation at the level of 1% compared with NC group. It has been suggested that the *Chlorella* contained growth promoting substance such as S-nucleotide adenosyl peptide complex, which may affect the growth performance and digestibility of the animals (Han et al., 2002). Halama (1990) also suggested there are many kind of unknown compounds such as polysaccharides, phenolic substances and aromatic compound in the algae, which are nutritional and ecological important to the animals. Therefore, it is suitable to hypothesize that the inclusion of *Chlorella* could improve the growth performance of the pigs. Moreover, some recent studies also confirmed that FC supplementation improved production performance in laying hens (Zheng et al., 2012). Obviously, our results indicated that pig fed FC01 treatment led to a higher ADG than those fed NC treatment, which confirmed the beneficial effect of the FC in pigs. Besides, the inclusion of FC01 led to a higher DM digestibility than the NC treatment, which is in agreement with Keijiro (2011), who suggested that rat fed fermented *C. vulgaris* supplemented diet led to a higher nutrient digestibility than those fed non-fermented *chlorella*. Furthermore, Hasegawa et al. (2000) had previously suggested that *C. Vulgaris* could protect the mice from the immune-suppressive action of stress and following gastric ulcer formation. Justo et al.

Table 6. Effect of fermented chlorella supplementation on fecal noxious gas content in growing pigs¹

Items (%)	NC	PC	FC01	FC02	SE ²	p-value
NH ₃	5.91 ^a	3.89 ^b	4.21 ^b	3.76 ^b	0.81	0.015
H ₂ S	7.94 ^a	5.25 ^b	5.40 ^b	5.01 ^b	1.21	0.007

¹ NC = Basal diet; PC = NC+antibiotics; FC01 = NC+0.1% fermented *chlorella*; FC02 = NC+0.2% fermented *chlorella*. ² Standard error.

^{a,b} Means in the same row with different superscripts differ (p<0.05).

(2001) also demonstrated the immune modulating and anti-cancer properties of the microalgae *C. Vulgaris* in mice. Therefore, the improved immune statuses could also explain the beneficial effect observed on the growth performance and nutrient digestibility in the current study. However, to the best of knowledge, this is first study concerning the effect of FC supplementation in pig, no study could be compared with the current study. Further study is still necessary to investigate the effect and mechanism how the FC affect the animal before putting the FC into practice utilization.

It is well accepted that gastrointestinal microflora play an important roles in animal production because intestine is the largest nutrient adsorption part in pigs; our previous study in pigs also concluded that fecal microbial is highly associated with the production performance of the pigs (Yan et al., 2012c). Therefore, fecal microbial shedding was investigated to evaluate the FC supplementation in pigs. In the current study, our results indicated that pigs fed FC supplemented diet led to a lower *E. coli* concentration but higher *lactobacillus* concentration than those fed NC group. Lin (1969) had previously suggested that the inclusion of *Chlorella* growth factor could increase the *lactobacillus* concentration by up to 400% *in vitro*, and confirmed the beneficial effect of *C. Vulgaris* on the *lactobacilli* diversity *in vivo* (Janczyk et al., 2009). Rania and Hala (2008) also suggested that the extract of *Chlorella* had antibacterial activities against *E. coli* because of the present of alkaloids and lipopolysaccharides. Therefore, we hypothesized that the FC supplementation might increase the gut health and subsequently explain the apparent total tract digestibility and growth performance in this study.

In terms of the fecal noxious gas content, the inclusion of FC significantly decreased the fecal noxious gas content compared with NC group. Ferket et al. (2002) have previously suggested that the ultimately fecal noxious gas emission of animals is related to intestinal microflora, especially the harmful intestinal bacteria population. Our previous study also suggested that a lower fecal noxious gas content could be observed in pigs by manipulating the microflora in the gastrointestinal tract of pigs (Yan et al., 2012c). Besides, several studies also suggested that the fecal noxious gas content is related to the nutrient digestibility (Yan et al., 2010b; Yan et al., 2011b), because the increased digestibility may allow less substrate for the microbial fermentation in the large intestine, which consequently decrease the fecal noxious gas content. In our study, pig fed the FC supplemented diet led to a better balanced microflora in the intestine and higher nutrient digestibility than those fed the NC group. Therefore, we hypothesized that the reason for the reduction in fecal noxious gas content may be the results of increased nutrient digestibility and the benefited microflora in the

gastrointestinal tract of pigs.

CONCLUSION

In conclusion, our results indicated that the inclusion of fermented *chlorella* at the level of 0.1% could improve the growth performance, nutrient digestibility, fecal microbial shedding (lower *E. coli* and higher *lactobacillus*), and decrease the fecal noxious gas emission in growing pigs when compared with the NC group. Dietary FC could be considered as a good source in growing pig because of its growth promoting effect.

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