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Effects of *Ecklonia cava* as fucoidan-rich algae on growth performance, nutrient digestibility, intestinal morphology and caecal microflora in weanling pigs

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Objective: In the present study, role of increasing levels of *Ecklonia cava* (seaweed) supplementation in diets was investigated on growth performance, coefficient of total tract apparent digestibility (CTTAD) of nutrients, serum immunoglobulins, cecal microflora and intestinal morphology of weanling pigs.
Methods: A total of 200 weaned pigs (Landrace×Yorkshire×Duroc; initial body weight 7.08±0.15 kg) were randomly allotted to 4 treatments on the basis of body weight. There were 5 replicate pens in each treatment including 10 pigs of each. Treatments were divided by dietary *Ecklonia cava* supplementation levels (0%, 0.05%, 0.1%, or 0.15%) in growing-finishing diets. There were 2 diet formulation phases throughout the experiment. The pigs were offered the diets *ad libitum* for the entire period of experiment in meal form.
Results: The pigs fed with increasing dietary concentrations of *Ecklonia cava* had linear increase ($p<0.05$) in the overall average daily gain, however, there were no significant differences in gain to feed ratio, CTTAD of dry matter and crude protein at both phase I and phase II. Digestibility of gross energy was linearly improved ($p<0.05$) in phase II. At day 28, pigs fed *Ecklonia cava* had greater (linear, $p<0.05$) *Lactobacillus* spp., fewer *Escherichia coli* (*E. coli*) spp. (linear, $p<0.05$) and a tendency to have fewer cecal *Clostridium* spp. ($p = 0.077$). The total anaerobic bacteria were not affected with supplementation of *Ecklonia cava* in diets. Polynomial contrasts analysis revealed that villus height of the ileum exhibited a linear increase ($p<0.05$) in response with the increase in the level of dietary *Ecklonia cava*. However, villus height of duodenum and jejunum, crypt depth, villus height to crypt depth ratio of different segments of the intestine were not affected.
Conclusion: The results suggest that *Ecklonia cava* had beneficial effects on the growth performance, cecal microflora, and intestinal morphology of weanling pigs.

Keywords: Weanling Pigs, *Ecklonia cava*, Performance, Gut Microbiota, Gut Morphology

INTRODUCTION

During the weaning transition undesirable stress accompanied by various changes in the piglet's gastrointestinal environment result in reduced voluntary feed intake [1], which subsequently increases susceptibility to intestinal dysfunction and weaning related disorders [2]. The widespread use of in-feed antibiotics, at both therapeutic and sub-therapeutic levels, increases public health scares [3]. The underlying mechanisms for the growth-promoting effect of antibiotics are expected to relate to gastrointestinal tract, particularly in the small intestine, at the bacteriological, physiological and immunological level [2]. Dietary manipulations post-weaning have the ability to improve weaning associated with intestinal dysfunction.

Marine algae is a good source of biomass, of which, a few types have been identified as rich sources of structurally diverse bioactive compounds with great pharmaceutical and biomedical potential. Presently these identified types are mainly being used for consumption by humans. It is estimated that two-thirds of the earth's biomass is comprised of more than 25,000 species of algae [4]. That statistic shows the potential for algae to be used as animal feed additives due to their vast biodiversity [5] and has recently been discussed as a source of bioactive compounds due to their biological properties [6,7]. *Ecklonia cava* (*E. cava*) is among one of the types of seaweed, which is found mainly in South Korea, Japan and China and widely used in human food because of their therapeutic effects. It is a highly resilient brown algae that grows in clear waters [8] and is well known for having fucoidans and polyphenolic compounds (phlorotannins). Fucoidans are branched polysaccharide sulfate esters extracted from the cell wall of marine algae, containing l-fucose as one of the major monosaccharides [9] and they have been shown to have antibacterial [6], immunomodulating [10] and antioxidant [11] activities. Previous researches suggested that *E. cava* can be used as environment friendly substitutes for antibiotics in diets to improve growth in fishes [12] and the immune systems of animals [13]. However, as per our knowledge, not much work has been done to utilize *E. cava* as feed additive for pigs. In this study, non-antibiotic feed additives are being investigated.

It is hypothesized that the inclusion of *E. cava* will improve the performance of weanling pigs by modifying the gastrointestinal tract microflora, digestibility and immunity. Therefore, the aim of the present experiment is to evaluate the effect of varying dietary inclusion levels of *E. cava* on the growth performance, coefficient of total tract apparent digestibility (CTTAD) of nutrients, serum immunoglobulins, cecal microflora and intestinal morphology of weanling pigs.

MATERIALS AND METHODS

The protocol for the present experiment was approved by the Institute of Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. The experiment was conducted at the facility of Kangwon National University farm. All the pigs (Landrace×Yorkshire×Duroc) were housed in partially slatted concrete floor pens (pen size 1.90 m×2.54 m) which were equipped with self-feeder and nipple drinker to allow *ad libitum* access to feed and water.

Ecklonia cava collection and processing

The seaweed *E. cava* was directly collected from the sea near Jeju Island, washed three times with water to remove the impurities and then dried. The stem was then removed and only leaves were ground to make a particle size of about 100 μm. This 100 μm powder form of *E. cava* was directly used in the feed. The rate of fucoidan, proportion of monosaccharaides and chemical composition (Table 2) were measured using the previously reported methods [14].

Animals and experimental design

A total of 200 weaned pigs (Landrace×Yorkshire×Duroc; average initial body weight (BW): 7.08±0.15 kg) were randomly allotted to four treatments on the basis of BW and sex (all male or all female). There were five replicate pens in each treatment with 10 pigs per pen. Treatments were divided by dietary *E. cava* supplementation levels (0%, 0.05%, 0.1%, or 0.15%) in growing-finishing diets. There were 2 diet formulation phases throughout the experiment (Table 1). The diets were prepared in meal form and were formulated to contain 14.22 MJ/kg metabolism energy (ME) and 1.53% lysine (phase I; Table 1) and 14.02 MJ/kg ME and 14.0 g/kg lysine (phase II; Table 1). All diets met or exceeded the nutrient requirements as recommended by [15].

Table 1. Ingredient and chemical composition of basal diets (as-fed basis)[1)]

Item	Phase I (d 0 to 14)	Phase II (d 15 to 28)
Ingredient (g/kg)		
Corn	341.3	500.8
Whey powder	200.0	153.8
Fish meal	50.0	30.0
SBM dehulled	232.8	229.2
SPC	50.0	30.0
Soy oil	36.4	30.5
MCP	3.1	7.0
Limestone	6.4	8.6
Salt	2.0	2.0
DL-Methionine (980 g/kg)	1.4	1.1
L-Lysine (780 g/kg)	1.6	2.0
Vitamin premix[2)]	2.0	2.0
Mineral premix[3)]	2.5	2.5
Choline-chloride (500 g/kg)	0.5	0.5
Lactose	70.0	-
Total	100.00	100.00
Chemical composition (%)		
ME (MJ/kg)	14.22	14.02
CP	230.0	210.0
Calcium	8.0	8.0
Available phosphorous	4.6	4.6
Lysine	15.3	14.0
Met+Cys	8.7	7.9
Lactose	200.0	100.0

SBM, soybean meal; SPC, soy protein concentrate; MCP, mono calcium phosphate; ME, metabolism energy; CP, crude proten; Met+Cys, methionine+cysteine.

1) The dietary treatments were: Control (basal diet) and basal diet supplemented with 0, 0.5, 1.0, and 1.5g/kg *Ecklonia cava* respectively.

2) Supplied per kilogram of diet: 20,000 IU vitamin A, 4,200 IU vitamin D_3, 10 IU vitamin E, 5.6 mg vitamin K_3, 2.8 mg vitamin B_1, 5.5 mg vitamin B_2, 4.2 mg vitamin B_6, 0.042 mg vitamin B_{12}, 14 mg pantothenic acid, 42 mg niacin, 0.105 mg biotin, 1.05 mg folic acid.

3) Supplied per kilogram of diet: 50 mg Fe, 0.20 mg Co, 30 mg Cu, 30 mg Mn, 20 mg Zn, 0.35 mg I, 0.25 mg Se.

Table 2. Chemical composition of *Ecklonia cava*

Items	
Component (%)	
Moisture	8.89±0.30
Crude protein	11.30±0.63
Crude fiber	31.00±0.72
Crude lipid	1.25±0.19
Crude ash	15.60±0.57
Carbohydrate	32.47±0.57
Sulfate	7.88±0.70
Fucoidan	11.2±0.60
Proportion of monosaccharides (%)	
Fucose	47.01±1.24
Rhamnose	1.45±0.38
Galactose	22.05±0.27
Glucose	9.71±0.10
Mannose	4.74±0.65
Xylose	13.96±1.02

Sample preparation and measurements

The pigs were weighed individually at the beginning of the trial, and then at d 14 and d 28 respectively. Consumption of the feed was recorded at the end of each phase. On the bases of these records, average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) were calculated.

To evaluate the digestibility, 2.5 g/kg of chromium was added in the diets as indigestible marker and the diets were fed to pigs during last seven days of each phase. Fecal grab samples were collected randomly from four pigs of each pen during the last three days of each phase. Feces were pooled and dried in an air forced drying oven at 60°C for 72 h and ground in a Wiley laboratory mill (Thomas Model 4 Wiley Mill, Thomas scientific, Swedesboro, NJ, USA) using a 1-mm screen for chemical analysis.

To analyze the concentrations of immunoglobulins (IgG, IgA, and IgM), 10 mL blood sample was collected from two randomly selected pigs from each pen at d 14 and d 28 of the experiment. The sampling was done by jugular vein puncture using a disposable vacutainer tube without anticoagulants (Becton Dickinson, Franklin, NJ, USA). The blood samples were then centrifuged (3,000×g for 15 min 4°C) for serum separation which were then stored at –20°C until analysis.

To study the effect of diets on the small intestinal morphology and cecal microflora, pigs from each treatment (two per replicate) reflecting average BW were selected and sacrificed by electrocution at the end of the experiment (d 28). The cecum contents were collected in sterilized plastic bottles and stored at 7°C for bacterial analysis. The intestinal samples from the region of duodenum, jejunum and ileum were also collected after removing their contents and flushing with physiological saline. The samples were submerged in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 30 g/L glutaraldehyde, 20 g/L paraformaldehyde and 15 g/L acrolein and then brought to the laboratory for studying the morphological changes.

Chemical and microbial analyses

Experimental diets and excreta samples were analyzed in triplicate for dry matter (DM, method 930.15; 16), crude protein (CP, method 990.03; 16), ash (method 942.05; 16), calcium and phosphorus (method 985.01; 16). The gross energy (GE) of diets and feces were measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL, USA), and chromium concentration was determined with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of [17]. Amino acid composition of feed samples was determined by HPLC (Waters 486, Waters Corp., Milford, MA, USA) after acid hydrolysis [18]. The methionine and cystine were determined following oxidation with performic acid [19]. The concentrations of serum IgG, IgA, and IgM were analyzed using radial immune-diffusion kits (Tripple J Farms, Bellingham, WA, USA).

The microbiological assay of cecal samples was carried out by the procedure suggested by [20]. One gram of mixed content was diluted with 9 mL of Butterfields phosphate buffer solution, followed by further serial dilutions in Butterfields phosphate buffer dilution solution. Duplicate plates were then inoculated with 0.1 mL sample and incubated. The microbial groups enumerated were total anaerobic bacteria (TAB, plate count agar, Difco Laboratories, Detroit, MI, USA), *Lactobacillus* spp. (MRS agar+0.200 g/L NaN3+0.500 g/L L-cystine hydrochloride monohydrate) and *Clostridium* spp. (Tryptose sulphite cycloserine agar, Oxoid, Hampshire, UK). The anaerobic conditions during the assay of total anaerobic bacteria and *Clostridium* spp. were created by using gas-pak anaerobic system (BBL, No. 260678, Difco, Detroit, MI, USA). The microbial populations were log transformed before statistical analysis.

Small intestinal morphology

Three cross-sections for each intestinal sample were prepared after staining with azure A and eosin using standard paraffin embedding procedures. A total of 10 intact and well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height was measured from the tip to the villus crypt junction. Crypt depth was measured as the depth between adjacent villi. All morphological measurements (villus height or crypt depth) were made in 10 μm increments by using an image processing and analysis system (Optimus software version 6.5, Media Cybergenetics, North Reading, MA, USA).

Statistical analyses

The data were analyzed as a randomized complete block design using the general linear model procedure of SAS [21]. The linear and quadratic contrasts were used to compare effect of increasing dietary *E. cava* levels (0, 0.5, 1.0, and 1.5 g/kg). The pen was

Table 3. Effects of supplemental *Ecklonia cava* on growth performance of weanling pigs

Items	Ecklonia cava (%)				SEM	p-values	
	0	0.05	0.10	0.15		Linear	Quadratic
Phase I (d 0 to 14)							
ADG (g)	294	297	311	306	8.03	0.184	0.671
ADFI (g)	455	458	468	463	11.30	0.507	0.747
G:F	0.64	0.65	0.66	0.66	0.01	0.171	0.579
Phase II (d 14 to 28)							
ADG (g)	394	397	426	414	11.55	0.104	0.540
ADFI (g)	645	646	661	662	15.38	0.350	0.989
G:F	0.61	0.61	0.65	0.63	0.01	0.248	0.437
Overall (d 0 to 28)							
ADG (g)	344	347	368	360	7.12	0.045	0.459
ADFI (g)	550	552	565	563	8.17	0.188	0.834
G:F	0.62	0.63	0.65	0.64	0.01	0.111	0.227

SEM, standard error of means; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

used as the experimental unit for the analysis of all the parameters. Probability values of ≤0.05 were considered as significant.

RESULTS

Growth performance

As shown in Table 3, during the phase I and II, there was no significant effects of dietary *E. cava* on ADG, ADFI, and G:F. A linear response to increasing dietary *E. cava* was demonstrated for the overall ADG (p<0.05). However, dietary *E. cava* did not affect the overall ADFI and G:F.

Digestibility

Polynomial contrasts analysis showed no significant differences in the CTTAD of GE and CP at both the phases (d 14 and d 28), but the digestibility of GE in the second phase linearly improved (p<0.05; Table 4).

Cecal microbial population

The Pigs supplemented with *E. cava* in diets showed no effect on the total anaerobic bacteria populations. However, the population of *Lactobacillus* spp. was linearly increased (p<0.05; Table 5) in pigs supplemented with increasing *E. cava* levels in diets. The population of *E. coli* were linearly reduced (p<0.05; Table 5) and population of *Clostridium* spp. tended to decrease (p = 0.077) linearly in *E. cava* supplemented pigs.

Table 4. Effects of supplemental *Ecklonia cava* on apparent total tract digestibility (%) of nutrients in weanling pigs

Item	Ecklonia cava (%)				SEM	p-values	
	0	0.05	0.10	0.15		Linear	Quadratic
D 14							
DM	81.50	81.68	82.07	81.94	1.08	0.730	0.891
GE	81.33	82.03	82.49	82.79	0.71	0.152	0.773
CP	73.02	73.25	73.56	73.20	0.73	0.796	0.687
D 28							
DM	81.65	81.85	82.08	82.03	1.01	0.769	0.905
GE	81.14	82.69	83.86	82.99	0.73	0.042	0.093
CP	73.86	73.95	74.07	74.21	0.68	0.721	1.000

SEM, standard error of means; DM, dry matter; GE, gross energy; CP, crude protein.

Serum immunoglobulins

Increasing *E. cava* level in the diets had no significant effect on serum level of IgG, IgA, and IgM (Table 6) in both phases.

Intestinal morphology

The diets supplemented with increasing levels of *E. cava* had higher villus height (p<0.05; Table 7) in ileum, but villus height was not affected in other sections of intestine. Crypt depth and villus height to crypt depth ratio was unaffected (Table 7) by supplementation of *E. cava* in diets.

DISCUSSION

In the recent years, efforts are being made to select new alternatives for feed additives. Seaweeds due to their enormous biodiversity and availability, have the potential to be used for this purpose [4,5]. A few of the seaweeds have been tried as feed additives to some extent [22,23], however, the focus of the present study to evaluate the effect of a rich fucoidan (*E. cava*) supplementation in weanling pigs was significant and meaningful. There was a linear increase in the ADG of the weanling pigs with increasing levels of *E. cava*. This is in line with the earlier reports of [24] who reported inconsistent, but minimal, performance improvement and increase in ADFI after feeding different seaweeds in pig diet. In contrast, [25] reported the addition of rich fucoidan seaweed to well digestible diets did not enhance performances of weaned piglets.

Table 5. Effects of supplemental *Ecklonia cava* on caecal microbial populations (Log_{10} CFU/g) in weanling pigs (d 28)

Items	Ecklonia cava (%)				SEM	p-value	
	0	0.05	0.10	0.15		Linear	Quadratic
Total anaerobic bacteria	8.72	8.71	8.67	8.68	0.15	0.806	0.975
Lactobacillus spp.	8.35	8.45	8.64	8.61	0.11	0.049	0.508
Clostridium spp.	7.52	7.48	7.25	7.30	0.12	0.077	0.693
Escherichia coli	6.44	6.34	6.15	6.18	0.10	0.043	0.524

CFU, colony-forming unit; SEM, standard error of means.

Table 6. Effects of supplemental *Ecklonia cava* on serum immunoglobulins (mg/mL) of weanling pigs

Items	Ecklonia cava (%)				SEM	p-value	
	0	0.05	0.10	0.15		Linear	Quadratic
D 14							
IgG	6.41	6.40	6.42	6.39	0.13	0.923	0.946
IgA	0.38	0.35	0.37	0.36	0.04	0.737	0.764
IgM	0.83	0.84	0.86	0.87	0.03	0.281	0.972
D 28							
IgG	6.34	6.40	6.38	6.41	0.11	0.745	0.927
IgA	0.33	0.35	0.36	0.34	0.04	0.889	0.605
IgM	0.84	0.86	0.87	0.88	0.03	0.385	0.899

SEM, standard error of means; Ig, immunoglobulin.

This variation in the results may be due to difference in the seaweed variety and the concentration that was used in the diets. In the present study, ADG was observed to be almost 6% higher in 1 g/kg *E. cava* supplemented diets in comparison to the control diet. The earlier study of [26] reported a 10% increase in the BW gain when the diet was supplemented with algae. The greater ADG in pigs fed the *E. cava* in diets in the present study might be associated with the improvement in GE digestibility and villi height.

In the present study, the digestibility of GE in the second phase was improved. This is in line with the earlier reports of [27] that reported higher digestibility of GE in rich fucoidan seaweed supplemented diets. Increase in the digestibility in the present study, was also accompanied by a slight increase in villi height. A critical role of villi height in the absorption of intestinal nutrients has been reported [28]. Thus, it is more likely that change in the height of villi could be under the influence of the *E. cava* in the diets that might be responsible for the increase in nutrient uptake and thus resulted in higher digestibility. [29] studied the nutrient digestibility after dietary supplementation of polysaccharides and found a significant interaction between laminaran and fucoidan supplementation on digestibility of GE. Improvement in the digestibility of nutrients and growth performance could be the result of the decrease of harmful bacteria such as *E. coli* and the increase of friendly bacteria such as *Lactobacillus* in weanling pigs' microbiota. *Lactobacilli* are able to produce polysaccharide depolymerases and glycosidases, which can improve the digestibility of nutrients by degrading the structural polysaccharide in plant cell wall [30].

Table 7. Effects of supplemental *Ecklonia cava* on small intestinal morphology of weanling pigs (d 28)

Items	Ecklonia cava (%)				SEM	p-values	
	0	0.05	0.10	0.15		Linear	Quadratic
Villus height (μm)							
Duodenum	438	444	451	446	12.08	0.591	0.713
Jejunum	402	407	413	404	9.32	0.811	0.454
Ileum	309	314	324	322	5.12	0.044	0.459
Crypt depth (μm)							
Duodenum	271	260	254	258	9.83	0.494	0.837
Jejunum	249	258	253	260	11.59	0.617	0.913
Ileum	197	200	208	206	5.43	0.167	0.661
VH:CD							
Duodenum	1.64	1.71	1.70	1.76	0.08	0.372	0.949
Jejunum	1.63	1.59	1.65	1.58	0.09	0.809	0.878
Ileum	1.57	1.58	1.59	1.57	0.04	0.925	0.843

SEM, standard error of means ; VH:CD, villus height:crypt depth ratio.

In the present study, supplementation of increasing levels of *E. cava* did not affect the immune status of the piglets. It has been previously reported that polysaccharides have immunomodulatory activities, and the presence of polysaccharides such as fucoidan in *E. cava* would most likely be responsible for the immunomodulatory activities [31]. However, the significant effect on immunoglobulins observed in previous work could not be confirmed in the current experiment, it might be because of low dosage of *E. cava* and health status of piglets in this study.

Ecklonia cava, a good source of Fucoidan, a sulfated polysaccharides [27] contains almost 10% of dry mass of total algae weight. In the present study increasing level of *E. cava* supplementation reduced the *Clostridium* spp. and *E. coli* population. This could be due to the presence of sulfated polysaccharides that are well known for their bacterial and viral inhibiting activities [22]. In previous studies, [6] also reported a promising reduction in the coliform populations in the cecum and colon of weaning pigs after supplementing fucoidan extract in diets. Moreover, in earlier studies, [32] reported that polysaccharides can act as prebiotics. Phlorotanin is another important ingredient of *E. cava* with promising effects against *E. coli* [33]. In this study, the number of *Lactobacillus* spp. increased linearly. This could be due to the prebiotic effects of *E. cava* that may have stimulated the growth of beneficial bacteria in the digestive track and would have exerted growth promoting and health improving effects on the weanling pigs. Moreover, the ratio between these bacterial groups has traditionally been considered as an index of gut health, with a high index related to a greater resistance to intestinal disorders [34]. Therefore, *E. cava* may offer a dietary means to modulate the gut environment to adjust the intestine microbiota and decrease the risk of diarrhoea in the absence of in-feed antibiotics.

In the present study the villus height in Ileum was linearly improved with increasing levels of *E. cava* supplementation in diets. The linear increase might have aided the nutrient digestion by enhancing the surface area for absorption. The epithelial turnover and villus height have a direct correlation with the nutrient absorption. A similar result was also reported in the previous study when pigs were offered seaweed extracted polysaccharides (laminaran and fucoidan), the villus height and villus height to crypt depth ratio improved [35]. The higher cecal *lactobacillus* in probiotic-supplemented diet indicated greater villus height and villus height to crypt depth ratio in the different small intestinal segments resulted in a greater absorption surface and greater digestibility of nutrients such as crude protein, dry matter and GE [36]. Moreover, the suppression of pathogenic bacteria by favorable bacteria results in the better villus height and villus height to crypt depth ratio [36]. The aid for the favorable microorganisms and hindered growth of unfavorable microorganisms could be one of the reasons behind the better gut morphology.

CONCLUSION

In conclusion, effects of *E. cava* on the digestibility of nutrients and intestine morphology were minimal; however, it had a great effect on growth performance and intestinal microbiota. Trends for increased digestibility and villi height will encourage us to try other levels of *E. cava* as effective feed additive on pigs in the future studies.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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