Effects of dietary microalgae, probiotics, and *Poncirus trifoliata* peel extract as feed additives on growth performance and gut health in piglets

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사료 내 미세조류, 생균제 및 탱자 껍질 추출물의 첨가가 자돈의 생산성 및 장 건강에 미치는 효과

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Abstract

An investigation was done to evaluate the effects of adding microalgae, probiotics and *Poncirus trifoliata* peel extract on the growth performance, intestinal histology, and immune characteristics in piglets. A total of 60 piglets were assigned to 4 treatments in 3 replications with 5 piglets as a replicate in this experiment for 28 days. The dietary treatments were control group (basal diet), microalgae group (basal diet + *Parachlorella* spp 0.5%), probiotics group (basal diet + *Lactobacillus paracasei* subsp. Tolerans 0.1% of the diet) and peel extract group (basal diet + *Poncirus trifoliata* peel extract 0.2%). The results showed that diets supplemented with microalgae, probiotics and *Poncirus trifoliata* peel extract had insignificant effects on growth performance of piglets. Dietary probiotics tended to increase the villus height to crypt depth ratio. Interleukin-2 and Interleukin-10 of serum was highest in peel extract group. It is concluded that dietary probiotics and *Poncirus trifoliata* peel extract improves gut health and immunity in piglets.

1. Introduction

Feed additives as dietary ingredients can be advantageous to domestic animals by improving animal health and productivity.

Microalgae are a possible new source of nutrients and health additives for animal feed formulations. In addition, microalgae have been used as feed additives, which improve gut health due to the presence of natural ingredients with antibacterial, anti-inflammation, and antioxidant properties [1]. As the first probiotics to be issued as a feed additive, *Lactobacillus* spp. are considered an excellent alternative to antibiotics and have been used as a feed additive owing to their safety and various positive effects [2]. *Poncirus trifoliata* is “non-comestible” fruit, because of its intense bitterness caused by the presence of poncirin [3]. In other words, *Poncirus trifoliata* is locally abundant and hitherto unexploited pectin source.

Although feed additives are attracting attention for use in the pig industry, there are few studies on gut health and immunity of microalgae, probiotics, *Poncirus trifoliata* peel extract and in piglets. Therefore, the aim of this study was to determine the effect of dietary microalgae, probiotics, and *Poncirus trifoliata* peel extract on the growth performance, intestinal histology, and immune characteristics of the piglets.

2. Materials and methods

2.1 Animals, experimental design, and diets

A total of 60 piglets [(Landrace x Yorkshire) x Duroc] with average initial body weight of 8.4 kg were assigned to 4 treatments with 3 replicates of 5 piglets in a completely randomized design for 28 days. The four groups of dietary treatment were as follows: (1) CON, basal diet; (2) MA(microalgae), basal diet + *Parachlorella* spp 0.5%; (3) PRO(probiotics), basal diet + *Lactobacillus paracasei* subsp. Tolerans 0.1%; (4)

PE(peel extract), basal diet + *Poncirus trifoliata* peel extract 0.2%. The basal diet was formulated to meet or exceed the dietary requirements of the growing pigs (Table 1). All piglets were allowed ad libitum access to feeding and water during the experiments.

[Table 1] Ingredients and chemical composition of the experimental diets (as-fed basis)

Item	ratio, %
Ingredients	
Corn	67.40
Soybean meal	25.00
Molasses	3.00
Soybean oil	2.00
Limesotne	0.80
L-Lysine HCl	0.10
Dicalcium phosphate	0.90
Salt	0.30
Vitamin-mineral premix[1]	0.50
Calculated compositions	
Metabolizable energy, kcal/kg	3,334
Crude protein	17.88
SID[2] lysine	0.86
SID methionine+cysteine	0.50
SID tryptophan	0.18
SID threonine	0.54
Calcium	0.60
Phosphorus	0.53
STTD[3] phosphorus	0.31

[1]The values supplied per kilogram of premix feed concentrations: Vit A 5,000,000 IU; Vit D_3 1,000,000 IU; Vit E 1,000 mg; Vit B_1 150 mg; Vit B_2 300 mg; Vit B_{12} 1,500 mg; Niacin amide 1,500 mg; DL-calcium pantothenate 1,000 mg; Folic acid 200 mg; Vit H 10 mg; Choline chloride 2,000 mg; Mn 3,800 mg; Zn 1,500 mg; Fe 4000 mg; Cu 500 mg; I 250 mg; Co 100 mg; and Mg 200 mg. [2]SID, standardized ileal digestible. [3]STTD, standardized total tract digestible.

2.2 Sampling and measurements

Pigs were weighed individually on days 0 and 28, and feed intake was measured to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). The feed efficiency (G:F) was calculated as the ratio of body weight gain to feed intake.

On the last day, blood samples were collected from all pigs into serum-separating tubes and allowed to clot at room temperature for 1 h. Serum was obtained after centrifugation at 1,800 × g for 15 min and frozen at −20℃ for analysis of serum characteristics. All pigs were then slaughtered and 5-cm long segments from the central part of the jejunum of each pig were washed and fixed in 10% buffered formalin for studying the intestinal morphology.

2.3 Jejunal morphology

The fixed samples were dehydrated, embedded in paraffin, and cut into 5-μm-thick sections using a rotary microtome (Leica RM 2245, Leica Biosystems, Tokyo, Japan). The sections were fixed on glass slides and stained with hematoxylin and eosin. The stained slides were scanned and images were captured to measure the villus height (VH), crypt depth (CD), and VH:CD ratio of a minimum of 10 well-orientated villi in each jejunal segment using ImageJ (National Institutes of Health, Bethesda, MD, USA).

2.4 Serum cytokine

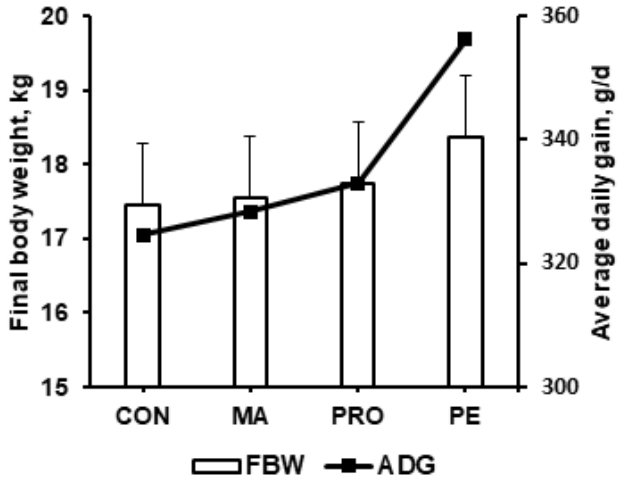
The TNF-α ELISA kit (PTA00; R&D Systems, Inc. Minneapolis, MN, USA), IL-2 ELISA kit (CSB-E06783p; Cusabio Inc., Wuhan, China), and IL-10 ELISA kits (P1000; R&D System Inc. Minneapolis, MN, USA) was used to determine serum concentrations of TNF-α, IL-2, and IL-10.

2.5 Statistical analysis

Data analysis was performed using the general linear model (GLM) procedure in SAS software (SAS Institute, Cary, NC, USA), with individual pigs as the experimental unit. Tukey's multiple comparison test was conducted to determine statistical differences among treatments. The significance level was pre-set at $p < 0.05$, and tendency was declared at $p < 0.10$.

3. Results and discussion

None of the dietary microalgae, probiotics, and *Poncirus trifoliata* peel extract affected growth performance, including body weight, average daily gain, average daily feed intake, gain to feed ratio. The effects of dietary microalgae, probiotics, and *Poncirus trifoliata* peel extract on final body weight and average daily gain are presented in Figure 1 ($p > 0.05$).



[Fig. 1] Effects of dietary microalgae, probiotics and *Poncirus trifoliata* peel extract as feed additives on final body weight and average daily gain in piglets

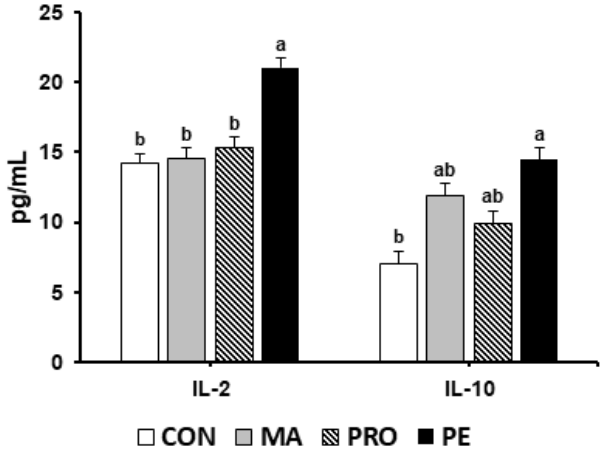
The effects of dietary microalgae, probiotics, and *Poncirus trifoliata* peel extract on jejunum morphology are presented in Table 2. Dietary probiotics tended to increase the villus height to crypt depth ratio compared with the control; however, statistical significance was not found (p = 0.078).

[Table 2] Effects of dietary microalgae, probiotics and *Poncirus trifoliata* peel extract as feed additives on jejunum morphology in piglets

Item[1]	CON	MA	PRO	PE	SEM	P-value
VH, μm	412.35	402.69	457.73	406.71	22.25	0.296
CD, μm	217.21	236.73	221.71	249.77	12.71	0.291
VH:CD	1.97	1.74	2.10	1.68	0.12	0.078

[1]VH, villus height; CD, crypt depth; VH:CD, villus height to crypt depth ratio. [2]SEM, pooled standard error of the means.

The effects of dietary microalgae, probiotics, and *Poncirus trifoliata* peel extract on serum cytokine are presented in Figure 2. The TNF-α concentration in serum was not altered by dietary feed additives. Concentration of IL-2 and IL-10 was the highest in the PE group (p < 0.05).



[Fig. 2] Effects of dietary microalgae, probiotics and *Poncirus trifoliata* peel extract as feed additives on serum cytokine (IL-2, IL-10) in piglets The a,b indicates significance at p < 0.05

In conclusion, dietary microalgae, probiotics, and *Poncirus trifoliata* peel extract had no effect on growth performance. On the other hands, dietary probiotics and *Poncirus trifoliata* peel extract were effective in increasing villus height to crypt depth ratio and serum cytokine. Collectively, our study suggests that dietary probiotics and *Poncirus trifoliata* peel extract can be used as the functional supplement to affect gut health and immunity in piglets.

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