RESEARCH ARTICLE

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Effects of phytogenic feed additives in growing and finishing pigs under different stocking density

Hyun Ah Cho[1#], Min Ho Song[2#] Ji Hwan Lee[3#], Han Jin Oh[1], Jae Woo An[1], Se Yeon Chang[1], Dong Cheol Song[1], Seung Yeol Cho[4], Dong Jun Kim[4], Mi Suk Kim[4], Hyeun Bum Kim[5]*, Jin Ho Cho[1]*

[1]*Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea*
[2]*Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Korea*
[3]*Department of Poultry Science, University of Georgia (UGA), Athens, GA 30602, USA*
[4]*Eugene-Bio, Suwon 16675, Korea*
[5]*Department of Animal Resources Science, Dankook University, Cheonan 31116, Korea*

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#These authors contributed equally to this work.

***Corresponding author**
Hyeun Bum Kim
Department of Animal Resources Science, Dankook University, Cheonan 31116, Korea.
Tel: +82-41-550-3652
E-mail: hbkim@dankook.ac.kr

Jin Ho Cho
Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea.
Tel: +82-43-261-2544
E-mail: jinhcho@chungbuk.ac.kr

ORCID
Hyun Ah Cho
https://orcid.org/0000-0003-3469-6715
Min Ho Song
https://orcid.org/0000-0002-4515-5212
Ji Hwan Lee
https://orcid.org/0000-0001-8161-4853

Abstract

This study was to investigate effects of different phytogenic feed additives (PFA) in grower finishing pigs with stressed by high stocking density. A total of 84 growing pigs ([Landrace × Yorkshire] × Duroc) with initial body weight (BW) of 28.23 ± 0.21 kg were used for 10 weeks (4 replicate pens with 3 pigs per pen). The dietary treatment consisted of basal diets in animal welfare density (positive control [PC]), basal diet in high stocking density (negative control [NC]), NC + 0.04% bitter citrus extract (PT1), NC + 0.01% microencapsulated blend of thymol & carvacrol (PT2), NC + 0.10% mixture of 40% bitter citrus extract and 10% microencapsulated blend of thymol and carvacrol (PT3), NC + 0.04% premixture of grape seed and grape marc extract, green tea and hops (PT4), and NC + 0.10% fenugreek seed powder (PT5). The reduction of space allowance significantly decreased ($p < 0.05$) growth performance (average daily gain, average daily feed intake, feed efficiency) and nutrient digestibility (dry matter, crude protein). Also, the fecal score of NC group increased ($p < 0.05$) compared with other groups. In blood profiles, lymphocyte decreased ($p < 0.05$), and neutrophil, cortisol, TNF- α increased ($p < 0.05$) when pigs were in high stocking density. Basic behaviors (feed intake, standing, lying) were inactive ($p < 0.05$) and singularity behavior (biting) were increased ($p < 0.05$) under high stocking density. However, PFA groups alleviated the negative effects such as reducing growth performance, nutrient digestibility, increasing stress indicators in blood and animal behavior. In conclusion, PFA groups improved the health of pigs with stressed by high stocking density and PT3 is the most effective.

Keywords: Pig, Robustness, Additive, Stress, Plant extract, High stocking density

INTRODUCTION

Recently, there has been increased interest in using natural and safe feed additives to enhance robustness for pigs [1,2]. Phytogenic feed additives (PFA) are plant-derived compounds such as leaves, bark, seeds, roots, flowers, twigs, tree herbs, and fruits [3]. According to the European Council, PFAs

Han Jin Oh
https://orcid.org/0000-0002-3396-483X
Jae Woo An
https://orcid.org/0000-0002-5602-5499
Se Yeon Chang
https://orcid.org/0000-0002-5238-2982
Dong Cheol Song
https://orcid.org/0000-0002-5704-603X
Seung Yeol Cho
https://orcid.org/0000-0003-3853-1053
Dong Jun Kim
https://orcid.org/0000-0002-1420-0527
Mi Suk Kim
https://orcid.org/0000-0002-9177-8701
Hyeun Bum Kim
https://orcid.org/0000-0003-1366-6090
Jin Ho Cho
https://orcid.org/0000-0001-7151-0778

Competing interests
No potential conflict of interest relevant to this article was reported.

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Cho HA, Song MH, Kim HB, Cho JH.
Data curation: Cho HA, Song MH, Lee JH.
Formal analysis: Cho HA, Song MH, Lee JH.
Investigation: Oh HJ, An JW, Chang SY, Song DC, Cho SY, Kim DJ, Kim MS.
Writing - original draft: Cho HA, Song MH.
Writing - review & editing: Cho HA, Song MH, Lee JH, Oh HJ, An JW, Chang SY, Song DC, Cho SY, Kim DJ, Kim MS, Kim HB, Cho JH.

Ethics approval and consent to participate
The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval CBNUA-1530-21-01).

can be categorized as sensory and flavoring compounds and generally feels safe as substitutes for antibiotics [3]. PFAs have been recognized as the latest feed additives and antibiotics alternatives for livestock [4,5]. Previous studies have reported that PFA complex including sunflower, thyme, and garlic can improve growth performance in monogastric animals [6–8]. Mahima et al. [9] have also reported that dietary PFA supplementation has immunomodulatory effects such as immunoglobulin secretion, cytokine, lymphocyte expression, phagocytosis, and histamine release. Essential oils such as thymol, cavacrol, cymene, terpinene reduce the pathogenic microbial load, but also promote digestive enzymes thereby affecting nutrient digestibility [10–12]. Other studies have shown dietary herbs (i.e., onion, fenugreek seed, and anise seed) enhanced economical efficiency to farms by improving the growth and health of mono-gastric animals [7,13,14]. High stocking density is the most significant caused by inducing stress during growing-finishing periods. Stress caused high stocking density can reduce feed intake, thereby causing low body weight (BW) gain [15–17]. Also, this stress can increase aggressive and negative social behavior such as fighting, feeder occupying, tail biting [18,19] and the incidence of body lesions [20–22]. Supplementaion of *Scutellaria baicalensis* L. roots mitigated negative behavior caused by heat stress in mono-gastric animals [23,24]. However, studies on the relationship between high stocking density and PFA have not been reported. In addition, there are few studies searching for effective PFA against stress derived from high stocking density. Therefore, the objective of this study was to explore effective PFA against environmental stress and the exact mechanism alleviated by PFA in a stress situation for grower-finishing pigs.

MARTERIAL AND METHODS

The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval CBNUA-1530-21-01).

Preparation of phytogenic feed additives

PFA1 is a bitter citrus extract (BioFlavex® GC, HTBA, Beniel, Spain) that is rich in 25%–27% naringin and 11%–15% neohesperidin. PFA2 is a microencapsulated blend of thymol and carvacrol (AviPower® 2, VetAgro SpA, Reggio, Emmilia, Italy) that contains 7% of thymol and 7% carcacrol. PFA3 is a mixture of PFA1, PFA2 and excipient in ratio of 4:1:5. It contains 0.7% thymol, 0.7% carvacrol, 10%–10.8% naringin and 4.4%–6% neohesperidin. PFA4 is a premixture of grape seed & grape marc extract, green tea and hops (AntaOx® FlavoSyn, DR. Eckel GmbH, Niederzissen, Germany) containing more than 10% of flavonoids. PFA5 is fenugreek seed powder containing 12% saponin (Fenugreek Seed Powder, P&D Export, Jaguar, India). All PFAs materials were provided by EUGENE BIO (Suwon, Korea).

Animals, housing, and experimental design

A total of 84 crossbred LYD ([Landrace × Yorkshire] × Duroc) mixed-sex growing pigs at 10 weeks of age (average BW 28.23 ± 2.89kg) were used in a 10-week feeding trial. Pigs were allotted to one of seven treatments in a completely randomized block design based on initial BW. Treatments were as follow: positive control–(PC; basal diet in animal welfare density), negative control (NC; basal diet in high stocking density), PT1 (basal diet with 0.05% PFA1 in high stocking density), PT2 (basal diet with 0.04% PFA2 in high stocking density), PT3 (basal diet with 0.10% PFA3 in high stocking density), PT4 (basal diet with 0.04% PFA4 in high stocking density), PT5 (basal diet with 0.05% PFA5 in high stocking density). All pigs were housed in an environmentally controlled

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room. There are two types of room area. In growing pig periods, animal welfare stocking density is 0.55m^2/pig, high stocking density is 0.40 m^2/pig and in finshing pig periods, animal welfare stocking density is 1.00 m^2/pig, high stocking density is 0.60 m^2/pig. There are 4 replicate pens with 3 pigs per pen during the experiment period. Basal diet was mostly consisted with corn and soybean meal and were formulated to meet or exceed National Research Council [25] recommendations (Table 1). During the experimental period, each pen was equipped with a self-feeder and nipple drinker to allow *ad libitum* access to feed and water.

Sampling and measuremets

Growth performance

To calculate average daily gain (ADG), pig's BW was individually measured at the 09:00 on an empty stomach at start of grower (0 weeks), end of grower and start of finisher (4 weeks), end of the finisher (10 weeks). Feed intake and wasted feed were recorded daily to calculate average daily intake (ADFI). Feed efficiency (G:F) was calculated by ratio of body weight gain and feed intake.

Nutrient digestibility

Apparent total tract digestibility (ATTD) of dry matter (DM) and nitrogen (N) were estimated using 0.2% of chromic oxide as an inert indicator [26]. Crude proteins (CP) were measured from the N. Pigs were fed diets mixed with chromic oxide on 4th week and 10th week. Fresh fecal grab

Table 1. Ingredients and chemical composition of the basal experimental diets (as fed basis)

Items	Grower (0–4 w)	Finisher (4–10 w)
Ingredients (%)		
Corn	65.10	72.38
Soybean meal	23.90	17.40
Wheat bran	7.00	6.00
Soybean oil	1.00	1.00
L-Lysine	0.10	0.28
DL-Methionine	0.04	0.04
L-Threonine	0.03	0.03
Dicalcium phosphate	1.00	1.00
Limestone	1.20	1.25
Salt	0.50	0.50
Vitamin premix[1)]	0.08	0.08
Mineral premix[2)]	0.05	0.05
Calculated composition		
ME (kcal/kg)	3,276	3,284
Crude protein (%)	18.00	15.50
Lysine (%)	1.01	0.97
Methionine (%)	0.33	0.29
Calcium (%)	0.78	0.76
Phosphorus (%)	0.62	0.58

[1)]Provided per kilogram of complete diet: 20,000 IU of vitamin A, 4,000 IU of vitamin D_3, 80 IU of vitamin E, 16mg of vitamin K_3, 4 mg of thiamine, 20mg of riboflavin, 6 mg of pyridoxine, 0.08 mg of vitamin B_{12}, 120 mg of niacin, 50 mg of Ca-Pantothenate, 2 mg of folic acid, 0.08 mg of biotin.

[2)]Provided per kilogram of complete diet: 12.5 mg of manganese, 179 mg of zinc, 140 mg of copper, 0.5 mg of iodine, 0.4 mg of selenium.

ME, metabolizable energy.

samples collected via rectal massage from each pig, and these samples were stored in a freezer at −20℃ until analyzed. All feed and fecal samples were analyzed for DM and N following the procedures outlined by the AOAC [27] methods. N was determined with a Kjeltec 2300 nitrogen analyzer (Foss Tecator AB, Hoeganaes Sweden) and Chromium was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan) following the method described by Williams et al. [28]. The ATTD of DM and N were calculated with indirect ratio methods using the following formula: Coefficient of ATTD = [1− {(Nf × Cd)/(Nd × Cf)}] × 100. Where: Nf = nutrient concentration in faeces (% DM), Nd= nutrient concentration in diet (% DM), Cf = chromium concentration in faeces (% DM), Cd = chromium concentration in diets (% DM).

Fecal score

During experiment, each pig fecal score was measured by same person before daily feeding. The fecal was scored according to its moisture content and shape. Normal feces are 0-point, soft feces are 1-point, mild diarrhea are 2-point and severe diarrhea are 3-point [29]. The score was calculated by averaging each group with the average value of the daily fecal score of each pig.

Blood sample

For the serum profile, at each pen, one pig was randomly selected to collect blood samples through venipuncture at the end of 4th week, and 10th week. At the time of collection, blood samples were collected both whole blood and serum in nonheparinized tubes and vacuum tubes containing K_3EDTA (Becton Dickinson Vacutainer systems, Franklin Lake, NJ, USA), respectively. White blood cells (WBC) and WBC including lymphocyte, neutrophil, basophil concentration in whole blood were measured using an automatic blood analyzer (ADVIA 120, Bayer, Whippany, NJ, USA). After collection, serum samples were centrifuged 12,500×g for 15 min at 4℃. Samples were stored at −20℃ in the refrigerator until analysis. Serum cortisol levels were assessed using enzyme-linked immunosorbent assay kits (LDN GmbH & Co., Nordhorn, Germany) following to the manufacturer's protocol. Tumor necrosis factor-alpha (TNF-α) and interleukine-6 (IL-6) concentration was analyzed with ELISA kit (Quantikine, R&D systems, Minneapolis, MN, USA) and they were measured at 450 nm.

Pig behavior

Collection of each pig image data was recorded by using six-day/night infrared cameras (QNB-7080 RH, Hanwha, Seoul, Korea) installed 3m above each pen. A total of 28 pig behaviors were analyzed by randomly selecting one pig from each pen. Observers collected data based on results of Yang et al. [30], and only one person made all observations and video analysis to see consistent results. The pig behavior analysis was classified for the following criteria (A) Feed intake: the act of eating with the head in the feed bin, or similar behavior. (B) Standing: the act of standing still with the forelimbs and hindlimbs extended perpendicular to the floor, or similar behavior. (C) Lying: the act of lying with the whole body on the floor, lying with the head, front legs, hind legs and abdomen all touching the floor. (D) Sitting: Two front legs are spread vertically to the floor, two rear legs and two hips are sitting on the floor, like a dog sitting on the floor, or something like that. (E) Drinking water: the act of drinking water for 10 seconds by putting your mouth in a drinking nipple. (F) Posture transition (lying → standing) A behavior that changes from lying down to standing, in which the two front legs are stretched first, and the hind legs are naturally stretched out. (G): Posture transition (standing → lying): A behavior that changes from a standing behavior to a lying behavior, in which the two front legs are bent to the floor first, and then the two hind legs are naturally folded and lying down. (H) Rooting: the act of repeating similar behaviors, such as

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scratches, itching, or something on the nose and front legs. (I) Biting: The act of biting another pig's ears, mouth, and tail with teeth and then biting again or doing similar things.

Statistical analysis

All data were analyzed by one-way ANOVA using SPSS software (ver. 20.0, IBM, Armonk, NY, USA), and the differences among treatments were examined by Tukey's multiple range test, which were considered to be significant at $p < 0.05$, unless otherwise stated.

RESULTS

Growth performance

There was no difference among treatment groups in the initial BW of pigs (Table 2). During the growing period (0–4 weeks), PT3 group significantly increased ($p < 0.05$) ADG and G:F ratio than NC group. During the finishing period (4–10 weeks), NC group significantly decreased ($p < 0.05$) ADG and ADFI than PC group. PFA groups ADG significantly higher ($p < 0.05$) than NC group. The PT3-PT4 groups ADFI significantly higher ($p < 0.05$) than NC group. During entire experimental period (0–10 weeks), NC group significantly decreased ($p < 0.05$) ADG, ADFI and G:F ratio than PC group. PFA groups significantly higher ($p < 0.05$) ADFI than NC group. The PT3-PT4 groups significantly increased ($p < 0.05$) ADG and G:F ratio than NC group.

Nutrient digestibility

During the growing period (0–4 weeks), the ATTD of DM significantly increased ($p < 0.05$) in PT1-PT3 groups compared PC group (Table 3). The ATTD of CP significantly decreased ($p < 0.05$) in NC group compared to PC group. However, PFA groups significantly increased ($p < 0.05$)

Table 2. Effects of different phytogenic feed additives on growth performance in growing-finishing pigs with stressed by stocking density[1)]

Items	PC[2)]	NC	PT1	PT2	PT3	PT4	PT5	SEM	*p*-value
BW (kg)									
Initial	28.00	27.53	27.64	28.97	28.62	28.48	27.88	0.309	0.868
4 w	56.30^{ab}	53.23^{b}	53.77^{b}	57.43^{ab}	59.35^{a}	57.04^{ab}	56.15^{ab}	0.592	0.083
Final	110.63^{a}	96.72^{c}	97.23^{c}	101.37^{bc}	109.43^{a}	106.41^{ab}	102.39^{bc}	0.767	< 0.001
0–4 w									
ADG kg	0.98^{ab}	0.89^{b}	0.90^{b}	0.98^{ab}	1.06^{a}	0.98^{ab}	0.98^{ab}	0.014	< 0.001
ADFI (kg)	1.98^{bc}	2.03^{abc}	1.94^{c}	2.01^{abc}	2.06^{ab}	2.08^{a}	2.03^{ab}	0.012	< 0.001
G:F	0.49^{ab}	0.44^{b}	0.46^{ab}	0.49^{ab}	0.51^{a}	0.47^{ab}	0.48^{ab}	0.006	< 0.001
4–10 w									
ADG (kg)	1.26^{a}	1.01^{c}	1.01^{c}	1.02^{c}	1.16^{ab}	1.15^{b}	1.08^{bc}	0.013	< 0.001
ADFI (kg)	2.92^{a}	2.54^{c}	2.82^{ab}	2.82^{ab}	2.88^{ab}	2.79^{b}	2.77^{b}	0.016	< 0.001
G:F	0.43^{a}	0.40^{abc}	0.36^{c}	0.36^{c}	0.41^{ab}	0.41^{ab}	0.39^{bc}	0.004	< 0.001
Overall period									
ADG (kg)	1.16^{a}	0.97^{e}	0.98^{e}	1.02^{de}	1.14^{ab}	1.1^{bc}	1.05^{cd}	0.009	< 0.001
ADFI (kg)	2.58^{a}	2.29^{c}	2.41^{b}	2.43^{b}	2.46^{b}	2.43^{b}	2.41^{b}	0.012	< 0.001
G:F	0.45^{ab}	0.43^{cd}	0.41^{d}	0.42^{cd}	0.46^{a}	0.45^{ab}	0.44^{bc}	0.004	< 0.001

[1)]Each value is the mean value of 4 replicates.

[2)]PC, basal diet in animal welfare density; NC, basal diet in high stocking density; PT1, basal diet with PFA1 in high stocking density; PT2, basal diet with PFA2 in high stocking density; PT3, basal diet with PFA3 in high stocking density; PT4, basal diet with PFA4 in high stocking density; PT5, basal diet with PFA5 in high stocking density.

[a–e]Means within column with different superscripts differ significantly ($p < 0.05$).

BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency; PFA, phytogenic feed additives.

Table 3. Effects of different phytogenic feed additives on nutrient digestibility in growing-finishing pigs with stressed by stocking density[1)]

Items	PC[2)]	NC	PT1	PT2	PT3	PT4	PT5	SEM	*p*-value
4 week									
DM (%)	85.55[a]	84.02[b]	85.72[a]	85.71[a]	86.14[a]	85.43[ab]	85.46[ab]	0.143	0.03
CP (%)	73.35[a]	69.45[b]	73.35[a]	73.58[a]	74.47[a]	73.16[a]	72.88[a]	0.271	0.01
10 week									
DM (%)	85.85[a]	83.17[b]	85.84[a]	85.75[a]	86.93[a]	86.15[a]	86.11[a]	0.179	0.01
CP (%)	67.98[bc]	64.75[c]	70.58[ab]	70.65[ab]	72.87[a]	71.37[a]	71.22[ab]	0.398	0.01

[1)]Each value is the mean value of 4 replicates.

[2)]PC, basal diet in animal welfare density; NC, basal diet in high stocking density; PT1, basal diet with PFA1 in high stockingdensity; PT2, basal diet with PFA2 in high stocking density; PT3, basal diet with PFA3 in high stocking density; PT4, basal diet with PFA4 in high stocking density; PT5, basal diet with PFA5 in high stocking density.

[a–c]Means within column with different superscripts differ significantly ($p < 0.05$).

DM, dry matter; CP, crude protein; PFA, phytogenic feed additives.

CP digestibility than NC group. During the finishing period (4–10 weeks), PFA groups decreased ($p < 0.05$) ATTD of DM and CP compared to NC group. The PT3-PT4 groups CP digestibility numerically increased ($p < 0.05$) than other PFA groups.

Fecal score

During the growing period (0–4 weeks), NC group showed significantly higher ($p < 0.05$) fecal score than PC group (Table 4). However, PFA groups significantly decreased ($p < 0.05$) fecal score compared to NC group. During finishing period (4–10 weeks), the difference of diarrhea incidence was not observed among all treatment groups.

Blood profile

During the growing period (0–4 weeks), there were no significant differences ($p > 0.05$) on WBC, Basophil, and IL-6 among treatment groups (Table 5). The NC group significantly decreased ($p < 0.05$) lymphocyte and increased ($p < 0.05$) neutrophil, cortisol, and TNF-α level in blood compared with PC group. However, PFA groups significantly alleviated ($p < 0.05$) these negative effects by stress with stocking density and was similar with the level of PC group. During the finishing period (4–10 weeks), there were no significant differences ($p > 0.05$) on WBC among treatment groups. NC group significantly decreased ($p < 0.05$) lymphocyte and significantly increased ($p < 0.05$) neutrophil, cortisol, and TNF- α level in blood compared to PC group. However, PFA groups significantly increased ($p < 0.05$) lymphocyte and significantly decreased ($p < 0.05$) neutrophil, cortisol, and TNF- α compared with NC group. PT3 group showed ($p < 0.05$) the lowest results in neutrophil, cortisol, and IL-6 among PFA groups.

Table 4. Effects of different phytogenic feed additives on fecal score in growing pigs with stressed by stocking density[1)]

Items	PC[2)]	NC	PT1	PT2	PT3	PT4	PT5	SEM	*p*-value
4 week									
Fecal score[3)]	0.26[b]	0.76[a]	0.33[b]	0.31[b]	0.30[b]	0.29[b]	0.28[b]	0.017	0.02

[1)]Each value is the mean value of 4 replicates.

[2)]PC, basal diet in animal welfare density; NC, basal diet in high stocking density; PT1, basal diet with PFA1 in high stocking density; PT2, basal diet with PFA2 in high stocking density; PT3, basal diet with PFA3 in high stocking density; PT4, basal diet with PFA4 in high stocking density; PT5, basal diet with PFA5 in high stocking density.

[3)]Fecal score was deterrmined as follow : 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea.

[a,b]Means within column with different superscripts differ significantly ($p < 0.05$).

PFA, phytogenic feed additives.

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Table 5. Effects of different phytogenic feed additives on blood profile in growing-finishing pigs with stressed by stocking density[1)]

Items	PC[2)]	NC	PT1	PT2	PT3	PT4	PT5	SEM	*p*-value
4 week									
WBC (10^3/μL)	23.03	23.47	23.18	23.21	22.72	22.99	23.04	0.155	0.93
Lymphocyte (%)	45.31^a	36.9^b	46.77^b	47.47^{ab}	46.37^b	41.70^a	47.80^a	0.506	0.01
Neutrophil (%)	39.43^{bc}	48.07^a	39.63^{bc}	38.53^{bc}	36.97^c	39.83^b	40.03^b	0.436	0.03
Basophil (%)	0.65	0.70	0.67	0.70	0.63	0.70	0.63	0.027	0.98
Cortisol (μg/dL)	1.82^c	3.47^a	2.60^b	2.19^{bc}	1.92^c	2.74^b	2.78^b	0.069	0.01
TNF-α (pg/mL)	61.90^b	73.13^a	62.63^b	62.77^b	61.93^b	62.67^b	62.40^b	0.506	0.01
IL-6 (pg/mL)	72.58	72.20	72.50	72.00	72.37	72.13	72.33	0.254	0.99
10 week									
WBC (10^3/μL)	17.74	17.76	17.85	17.64	17.76	17.63	17.72	0.148	0.99
Lymphocyte (%)	43.40^a	35.90^b	45.40^a	46.57^a	44.37^a	45.63^a	46.87^a	0.492	0.01
Neutrophil (%)	42.08^b	52.37^a	41.20^b	40.83^b	42.03^b	42.20^b	44.13^b	0.499	0.01
Basophil (%)	0.68^{ab}	0.70^a	0.70^a	0.63^{abc}	0.50^{bc}	0.47^c	0.47^c	0.019	0.01
Cortisol (μg/dL)	0.72^b	2.40^a	0.61^{bc}	0.64^{bc}	0.51^c	0.67^b	0.61^{bc}	0.070	0.01
TNF-α (pg/mL)	86.70^b	99.83^a	85.73^b	85.80^b	84.90^b	85.70^b	85.83^b	0.596	0.01
IL-6 (pg/mL)	80.80^{ab}	81.77^a	80.37^{ab}	80.70^{ab}	80.30^b	80.43^{ab}	80.53^{ab}	0.132	0.04

[1)]Each value is the mean value of 4 replicates.

[2)]PC, basal diet in animal welfare density; NC, basal diet in high stocking density; PT1, basal diet with PFA1 in high stocking density; PT2, basal diet with PFA2 in high stocking density; PT3, basal diet with PFA3 in high stocking density; PT4, basal diet with PFA4 in high stocking density; PT5, basal diet with PFA5 in high stocking density.

[a–c]Means within column with different superscripts differ significantly ($p < 0.05$).

WBC, white blood cell; TNF- α, tumor necrosis factor-α; IL-6, Interleukin-6; PFA, phytogenic feed additives.

Table 6. Effects of different phytogenic feed additives on behavior changes in growing pigs with stressed by stocking density[1)]

	PC[2)]	NC	PT1	PT2	PT3	PT4	PT5	SEM	*p*-value
Basic behavior (min/hour)									
Feed intake	4.03	4.01	4.03	4.02	4.10	4.10	4.05	0.009	0.02
Standing	7.05	7.11	6.99	7.12	7.01	6.98	7.13	0.030	0.74
Lying	44.58	44.16	44.39	44.51	44.09	44.66	44.29	0.106	0.78
Sitting	4.34	4.72	4.59	4.35	4.80	4.26	4.53	0.087	0.64
Singularity behavior (count/hour)									
Drink water	5.04	5.19	5.15	5.10	5.11	5.14	5.12	0.015	0.23
Rooting	1.08	1.10	1.11	1.04	1.12	1.06	1.03	0.014	0.46
Posture transition (lying-sitting)	3.54	3.49	3.50	3.48	3.44	3.44	3.51	0.020	0.86
Posture transition (sitting-lying)	3.53	3.48	3.50	3.48	3.43	3.45	3.50	0.015	0.64
Biting	0.18^b	0.23^a	0.21^{ab}	0.18^b	0.15^c	0.17^b	0.18^b	0.05	< 0.001

[1)]Each value is the mean value of 4 replicates.

[2)]PC, basal diet in animal welfare density; NC, basal diet in high stocking density; PT1, basal diet with PFA1 in high stocking density; PT2, basal diet with PFA2 in high stocking density; PT3, basal diet with PFA3 in high stocking density; PT4, basal diet with PFA4 in high stocking density; PT5, basal diet with PFA5 in high stocking density.

[a,b]Means within column with different superscripts differ significantly ($p < 0.05$).

PFA, phytogenic feed additives.

Animal behavior

The effects of different PFA on animal behavior were shown in Tables 6, 7, and Fig. 1. During the growing period (0–4 weeks), there are no siginificant differences ($p > 0.05$) in basic behavior and most of singularity behavior. The NC group had significantly higher ($p < 0.05$) biting frequency

Table 7. Effects of different phytogenic feed additives on behavior changes in finishing pigs with stressed by stocking density[1)]

	PC[2)]	NC	PT1	PT2	PT3	PT4	PT5	SEM	*p*-value
Basic behavior (min/hour)									
Feed intake	4.63^{a}	4.37^{b}	4.67^{a}	4.59^{a}	4.61^{a}	4.67^{a}	4.61^{a}	0.020	< 0.001
Standing	6.77^{a}	6.32^{c}	6.38^{c}	6.44^{bc}	6.71^{a}	6.56^{ab}	6.61^{ab}	0.031	< 0.001
Lying	44.88^{c}	45.51^{a}	45.21^{b}	45.18^{b}	44.98^{c}	44.99^{c}	44.96^{bc}	0.041	< 0.001
Sitting	3.72	3.80	3.74	3.79	3.70	3.78	3.82	0.012	0.75
Singularity behavior (count/hour)									
Drink water	5.34	5.28	5.27	5.30	5.44	5.38	5.29	0.026	0.59
Rooting	1.12	1.08	1.11	1.09	1.21	1.19	1.15	0.021	0.58
Posture transition (lying-sitting)	3.78	3.43	3.49	3.58	3.71	3.68	3.69	0.035	0.60
Posture transition (sitting-lying)	3.77	3.41	3.50	3.60	3.70	3.67	3.68	0.045	0.37
Biting	0.16	0.23	0.18	0.15	0.17	0.16	0.18	0.008	0.20

[1)] Each value is the mean value of 4 replicates.

[2)] PC, basal diet in animal welfare density; NC, basal diet in high stocking density; PT1, basal diet with PFA1 in high stocking density; PT2, basal diet with PFA2 in high stocking density; PT3, basal diet with PFA3 in high stocking density; PT4, basal diet with PFA4 in high stocking density; PT5, basal diet with PFA5 in high stocking density.

[a–c] Means within column with different superscripts differ significantly ($p < 0.05$).

PFA, phytogenic feed additives.

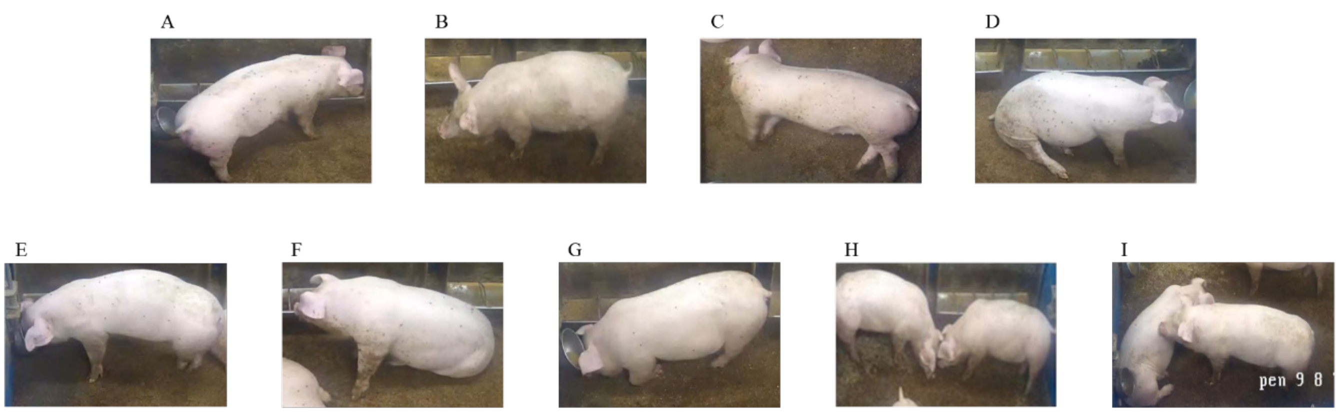

Fig. 1. Classification of pig behavior changes. (A) Feed intake, (B) standing, (C) lying, (D) sitting, (E) drink water, (F) posture transition (lying → standing), (G) posture transition (standing → lying), (H) rooting, (I) biting.

than PC group. However, PFA groups had significantly alleviated ($p < 0.05$) biting frequency compared with NC group. Among PFA groups, PT3 group showed the lowest biting frequency. During the finishing period (4–10 weeks), NC group showed ($p < 0.05$) more lying time and less feed intake and standing time than PC group. Feed intake time significantly increased ($p < 0.05$) in PFA groups than NC group. Standing time significantly increased ($p < 0.05$) in PT2-PT5 groups than NC group. Lying time significantly decreased ($p < 0.05$) in PFA groups than NC group. Especially, PT3-PT5 groups showed similar result with PC group. In singularity behavior, there are no significant difference ($p > 0.05$) in treatment groups. But NC group showed numerically high number of biting than other treatment groups.

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DISCUSSION

Growth performance

High stocking density can disturb the movement of animals due to limited feeding environment (space, feeders, and drinkers). Moreover, high stocking density can interfere with airflow and generate heat energy [12]. It can result in difficulty in evacuating body temperature, poor air quality, reduced access to feed and water, and poor performance of animals due to increased ammonia levels [31–33]. High heat energy and poor air quality are known to cause heat stress and adverse effects on growth rate, feed consumption, mortality, and health [34–36]. Similarly, our study showed that pigs under high stocking density (i.e., 0.40 m^2/ growing pig, 0.60 m^2/finishing pig) had reduced ADG and ADFI by 16.38% and 11.24%, respectively, than those under welfare density (i.e., 0.55 m^2/ growing pig, 1.0 m^2/finishing pig) during the whole period (grower: 28–56 kg, finisher: 56–103 kg). Spicer and Aherne [37] have also reported that daily gain and daily feed are reduced 8.47% and 13.15%, respectively, when group size is decreased from 0.72 m^2/pig to 0.35 m^2/pig. Stress-induced heat and high stocking densities can reduce growth performance by damaging cellular structure, increasing intracellular water imbalance, and increasing free radical concentration [38]. However, our study revealed that pigs under high stocking density with supplementation of PFA showed improvement (i.e., BW decreased 11.61%) in growth performance compared to those in the unsupplemented group. Many researchers reported that dietary supplementation of PFA such as Korean pine extract, cinnamon, turmeric, essential oils, and rosemary can improve growth performance with reducing stress response [38–41]. In our study, PFA supplementation under our high stocking density showed no difference in ADFI between treatments in the growing period, but significantly increased with PFA supplementation in the finishing period This is consistent with previous studies suggesting that PFA is effective for intake when supplied long-term [42]. Moreover, PFA3 could improve the flavor of feed and increase the palatability of feed intake in pigs [43–46]. Therefore, using natural products with polyphenols (suitable structure for free radical scavenging activity) can effectively alleviate stress caused by low space allowance and heat through their antioxidant activity with improved low feed intake, thereby increasing growth performance.

Nutrient digestibility

High stocking density can negatively affect nutrient digestibility and growth performance. During the whole experiment periods, nutrient digestibility (DM, CP) showed improvement in the treatment group added with PFA than that in the control group without PFA under high stocking density. PFA can also enhance nutrient digestibility and absorption [47,48]. It has been reported that the addition of essential oils to monogastric animals can enhance the activity of trypsin, maltase and pancreatic amylase and increases glucose absorption in the small intestine [49]. Therefore, the addition of PFA can stimulate the secretion of mucus in the intestine, thereby reducing the adhesion of pathogens and stabilizing intestinal microbial symbiosis [50]. It can be seen that improved digestive tract function is associated with increased nutrient digestibility. It can also be said that the antibacterial action of PFA contributes to the increase of nutrient digestibility. PFAs such as carvacrol, thymol, anetol, oregano, anise, and citrus essential oil have antibacterial activity against intestinal microbes when ingested. Among them, phenolic substances are the most active compounds [51,52]. PT2 and PT3 have a phenolic structure in our experiment. It was shown that the digestibility of DM and CP was higher than the high stocking density throughout the experiment period. Fiesel et al. [53] reported an increase in nutrient digestibility due to the antioxidant effect of polyphenols and an increased absorbable surface of the intestine. As the experiment progressed, the digestibility deviation of DM and CP increased according to the presence or absence of PFA in the feed under high stocking density. In this experiment, it was confirmed that the digestibility was gradually improved when PFA was used, leading to improved performance of pigs. In particular, it was found that the digestibility was significantly improved by flavonoids, a common component of PT3-PT5 additives. A previous study has shown that flavonoids have DM and CP synergistic effects [54]. Therefore, it can be concluded that the use of flavonoid additives can increase the digestibility of nutrients, as it can improve nutrient availability by boosting immunity and antibacterial action in pigs.

Fecal score

In high stocking density, the frequency of diarrhea was increased during the growing period, although it showed no significant difference during the finishing period. Many studies have found that diarrhea in pigs is more likely to be induced by stress [55–57]. Actually, the frequency of diarrhea is increased in weaned pigs during stress [58]. Diarrhea has been found intermittently in growing pigs [59]. When pigs get stressed, their immunity is lower and pathogens in the intestine are activated. Intestinal pathogens can suppress unnecessary energy loss such as reduced feed intake and G:F ratio known to interfere with immune system activation. In addition, intestinal pathogens can inhibit homeostasis of the epithelial barrier, causing secretory diarrhea due to intestinal damage through osmotic stress or inflammatory diarrhea by increasing inflammatory cytokines. However, in our study, the frequency of diarrhea was significantly reduced when PFAs were fed to pigs in a stressful situation. These results indicate that PFA can improve fecal status by improving intestinal health, and further studies on fecal microflora should be conducted. When pigs are fed with natural products reduces the frequency of diarrhea due to stress as the natural product's antibacterial action improves intestinal health and increased digestibility [60,61]. Many researchers have checked diarrhea scores of weaning pigs, but not those of growing to finishing pigs. In the present study, complete diarrhea was not found even in the growing period, although a lot of soft feces were observed for pigs under a high stocking density condition. The difference between growing period and finishing period is that as pigs grow, their immune system gets better, and their gut health improves. Therefore, we can confirm a meaningful diarrhea score even in pigs during the growing period. Thus, it is necessary to check the status of feces.

Blood profile

In the present study, there were no significant differences in blood profile between the entire experiment period WBC or growing period basophil and IL-6 of pigs between treatment groups. However, pigs fed with PFAs under a high stocking density condition had better blood results than those without addition of additives under a high stocking density condition. Pigs with high stocking density are subjected to critical psychological, social, and environmental stresses. High stocking density can also cause chronic severe stress that affects immunity and health [62]. Lymphocytes show various immunological responses including modulation of immune defense and immunoglobulin [63]. In our study, lymphocytes were decreased during stress situation, but returned to PC levels when PFAs were added. According to Dhabhar [64], in stressful situations, lymphocyte counts are decreased due to changes induced by trafficking or redistribution of lymphocytes to other body compartments of glucocorticoids. This result was similar to our study. In our study, the number of neutrophils was increased when pigs were stressed. This number was then decreased after supplementation with PFA in our study. It has been reported that stressful situations cause decreasing lymphocytes and increasing neutrophils in the blood [65]. As a result, it was possible to confirm the indirect change caused by supplementation of PFA to relieve stress. Cortisol, a steroid hormone, or glucocorticoid produced by the adrenal gland and released in

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response to stress, is often used as a physiological marker to quantify animal stress [66]. It is well known that cortisol can regulate intermediary metabolism, immunity, and growth [67,68]. A poor welfare situation can cause animals to be extremely stressed. In this study, cortisol level was increased under high stocking density compared with animal welfare density (space decreased in growing pig 27.27%, in finshing pig 40%). This result was agreement with the results of Jang et al. [69] that reported decreasing space allowance (decreasing 28.13%) induced increasing cortisol level (2.3 to 4 μg/dL).

However, PFAs supplementation alleviated high cortisol level in blood caused by high stocking density. Li et al. [39] observed that flavonoids, which are physiologically active substances of PFA, down-regulated immune responses by mediated viruses and the T-cell, thereby reducing psychological stress. This observation suggests that PFA mitigates the increased cortisol concentration by high stocking density.

Pro-inflammatory cytokines such as TNF-α and IL-6 are potential outputs of the cellular immune system and can indirectly reflect immune responses due to the activation of T-cells [70,71]. This study showed that high stocking density increased pro-inflammatory cytokine level. These results suggest that the environmental stress caused by a limited space allowance can induce a cellular immune response. When stressed out, pro-inflammatory cytokines are secreted to promote cortisol secretion and suppress growth hormone secretion [72,73]. Excessive pro-inflammatory cytokines can induce fever, inflammation, tissue destruction [74], and in some cases, even shock and death [75]. Thus, the immune system is activated due to high stocking stress, which shifts nutrient distribution priorities from growth to host defense [70,72,76].

In addition, TNF-α and IL-6 content is reduced through improved gut microbiota, antioxidant, and anti-inflammatory effects, due to improved digestibility of nutrients, alleviating stress response, and strengthening immunity [77,78]. However, PFA was effective methods to alleviate negative effects of a high stocking density in our study. Other researchers also reported essential oil and herb extract reduced pro-inflammatory cytokines [79,80].

Therefore, PFA is effective in relieving stress, and PT3 group showed the highest effect among PFAs group. The reason the PT3 group outperformed the others was due to the construction of the PFA group. Flavonoids and terpenoids (carvacrol and thymol) may protect cells from the harmful effects of autoxidation.

Animal behavior

A high stocking density equates to a reduced floor space allowance. Decreasing floor space allowance per pig increases the frequency of contact, social tension, and aggression [81–84]. In addition, when heat production per unit floor area is increased, heat stress will occur and induce oxidative stress [22,85]. If this stress is not well managed in pigs, it can increase their susceptibility to stress and hence reduce their immune and health status. Throughout our study, animal behavior at high stocking density improved when fed with PFA. The biting frequency was increased in NC but decreased after PFA treatment similar to PC. Among all treatment groups, PT3 group showed the lowest biting frequency. Greene et al. [38] has reported that biting as a representative form of aggressive behavior can occur in pigs under chronic stress. This is consistent with our study. When ingesting phenolic compounds as components of PT3 group, it is possible to restore redox homeostasis and prevent oxidative stress by improving the activity of antioxidant enzymes SOD, CAT, GPx, and GR [86]. Therefore, the effect of adding PFA3 not only can help pigs cope with biting behavior caused by stress, but also can overcome it. During the finishing period, basic behaviors (eating, standing, lying down) were more active when fed with PFA added in high stocking density. In addition, the feed intake increased during PFA feeding in growth performance.

Feed intake is an important indicator because it is related to body weight, ADG, ADFI, and G: F ratio. Pigs with a high stocking density face difficulty in feeding due to competition in the feeder. In this study, PC group showed less time than other treatments in feed intake. Therefore, the number of trips to the feeder is directly related to intake and can affect growth performance. Also, standing and lying time were similar to NC group. They were more active than PC group. Especially, PT3-PT5 groups are more activated than others. Pearce and Paterson [87] have reported that observation of the behavior of standing motionless in a narrow space is a behavior that pigs do to cope with stress at a high stocking density. As stress increased, the amount of physical activity decrease. It can be seen that when the standing time decrease, the lying time increases at the same time. This indicates that there is a close relationship between basic behavior and growth performance. Through this experiment, it can be seen that when pigs get stressed, their basic behaviors (standing, lying, and feeding) were affected at the same time.

CONCLUSION

Dietary supplementation of PFA improves the growth performance, nutrient digestibility, immunity, fecal score, and animal behavior in grower-finishing pigs. As a result, lymphocytes, neutrophils, cortisol, IL-6, and TNF- α in the blood, bites, and basic behaviors were improved, indicating that stress was reduced and strengthened. The diarrhea index improved because of getting healthier, which means less damage to the intestines and increased digestibility. Due to these positive effects, growth performance was improved, and it was found that PFA is an effective additive for stress due to high stocking density. Among them, the most effective and additional advantages were found when using PFA3 (mixture of PFA1 40%, PFA2 10% and excipient 50%) rather than using PFA1 (bitter citrus extract) and PFA2 (microencapsulated blend of thymol and carvacrol) separately.

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