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Article Title (within 20 words without abbreviations)	Effects of different stocking density and various phytogenic feed additives dosage levels on growing-finishing pigs
Running Title (within 10 words)	Effects of stoking density and phytogenic feed additives on pig
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Abstract

This study was to investigate the effects of different phytogenic feed additives (PFA) dosage levels in growing-finishing pigs stressed by high stocking density. A total of 72 mix sexed 12 weeks growing pigs [(Landrace × Yorkshire) × Duroc] with initial body weight (BW) of 49.28 ± 4.58 kg were used for 8 weeks. There were 3 replicate pens in each treatment group, with 3 pigs per pen. The dietary treatment groups consisted of basal diets in animal welfare density (NC, negative control), basal diet in high stocking density (PC, positive control), PC + 0.04% essential oil (ES1), PC + 0.08% essential oil (ES2), PC + 0.10% bitter citrus extract & essential oil (CES1), PC + 0.20% bitter citrus extract & essential oil (CES2), PC + 0.05% grape pomace extract (GP1), PC + 0.10% grape pomace extract (GP2). The reduction of space allowance decreased ($p < 0.05$) average daily gain, feed efficiency and digestibility of dry matter, crude protein, and gross energy. Also, the fecal score of PC groups increased ($p < 0.05$) compared with other groups. Basic behaviors (feed intake, standing, lying) were inactive ($p < 0.05$) and singularity behavior (biting) was increased ($p < 0.10$) under high stocking density. There was no difference in blood profile. However, the supplementation of PFA alleviated the negative effects such as reduced growth performance, nutrient digestibility, some increasing stress indicators in blood (cortisol) and animal behavior (biting). In conclusion the negative effect of high stocking density was most effectively mitigated by normal dosage of mixture of bitter citrus extract and essential oil additive (CES1).

Keywords: pig, dosage, additive, stress, plant extract, stocking density

Introduction

Improving the stress resilience of livestock is critical to profitable meat production. It also addresses ethical, animal welfare and sustainability issues. However, pigs are still stocked at a high density for effective management, improvement of space utilization, and profitability [1]. High stocking density can cause the absence of living and feeding space, generation of heat energy, and interference with airflow, thereby causing reduced access to feed and water due to heat stress and poor air quality caused by noxious gas [2]. Consequently, pigs with high stocking density are affected by severe environmental and psychological stress. Such stress can adversely affect the health of pigs [3,4]. The high stocking density can cause heat stress, which increases oxidative stress in the body [5]. If this stress is not well managed in pigs, it can increase susceptibility to stress and hence reduce immune and health status. Phytogenic feed additives (PFA) such as herbs, spices, and their extracts are broadly defined as plant-derived bioactive compounds. They are often supplemented into animal diets [6]. Many studies have reported positive effects of PFA on animal health and growth performance under various stress environments [7,8]. However, their function and impact can vary depending on plant origin, extraction method, formulation. Only few studies have compared different dosage of PFA with various additive. In addition, studies searching for effective PFA against stress derived from high stocking density are limited. Therefore, this study aims to investigate effects of phytogenic feed additives dosage levels on growth performance, nutrient digestibility, blood profile, and animal behavior of growing-finishing pigs in different stocking density.

Material 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-1642-21-02).

Preparation of phytogenic feed additives

Three types of additives with two dosage levels (normal, double) were used in this experiment. ES is essential oil. It contains microencapsulated blend of 7% thymol and 7% carvacrol (AviPower® 2, VetAgro SpA, Reggio, Emmilia, Italy). Bitter citrus extract (BioFlavex®, HTBA, Beniel, Spain) contains 25-27% naringin and 11-15% neohesperdin. CES is mixture of bitter citrus extract, essential oil, and excipient in ratio of 1:4:5. It contains 0.7% thymol, 0.7% carvacrol, 10 ~ 10.8% naringin and 4.4 ~ 6% neohesperidin. GP is grape pomace extract. It contains premixture of grape seed & grape marc extract, green tea and hops (AntaOx®FlavoSyn, DR. Eckel GmbH, Niederzissen, Germany) containing more than 10% of flavonoids. All PFAs materials were provided by EUGENE BIO Co., (Suwon, South Korea).

Animals, housing, and experimental design

In total, 12 weeks of age 72 mixed-sex growing pigs ([Landrace × Yorkshire] × Duroc) with average initial body weight (BW) of 49.28 ± 4.58 kg were used in 10-week feeding trial. The experiment was performed with 8 treatment groups according to 3 types of PFA, 2 dosage levels, and 2 types of stocking density. Pigs were allotted to one of eight treatments in a completely randomized block design based on initial body weight (BW). Treatments were as follows: NC (negative control; basal diet in animal welfare density), PC (positive control; basal diet in high stocking density), ES1 (basal diet with 0.04% essential oil in high stocking density), ES2 (basal diet with 0.08% essential oil in high stocking density), CES1 (basal diet with 0.1% bitter citrus extract & essential oil in high stocking density), CES2 (basal diet with 0.2% bitter citrus extract & essential oil in high stocking density), GP1(basal diet with 0.05% grape pomace extract in high stocking density), GP2 (basal diet with 0.10% grape pomace extract in high stocking density). Each treatment had three replicates per treatment with three pigs. All of the pigs were kept in one of two types of environmental-controlled rooms NC set the animal welfare density during the whole experimental period and the remaining treatment groups were set based on animal welfare standard decreasing 20% in growing period and decreasing 40% in finishing period. During growing pig periods, animal welfare stocking density is 0.55m^2/pig and high stocking density is 0.40 m^2/pig. During finishing pig periods, animal welfare stocking density is 1.00 m^2/pig, and high stocking density is 0.60 m^2/pig. The diets were formulated to meet or exceed the National Research Council [9] recommendation for growing-finishing pigs (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.

Growth performance

To calculate average daily gain (ADG), pig's BW was individually measured at 09:00 on an empty stomach at the start of grower (0 weeks), end of grower and start of finisher (2 weeks), and end of the finisher (8 weeks). During the experiment, each pig 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. 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 [10]. The score was calculated by averaging each group with the average value of the daily fecal score of each pig.

Nutrient digestibility

For nutrient digestibility, 0.2% of chromium oxide (Cr_2O_3) in the test feed was added as an indicator at 2 and 10 weeks after the start of the test, and minutes were collected by anal massage after feeding from 3 days before sample collection. The collected minutes were dried in a dryer at 60℃. for 72 hours, then pulverized with a Willey mill and used for analysis. Powder samples were analyzed for dry matter (DM), crude protein (CP) and total energy (GE). DM analyzed samples for 16 hours in an oven at 105 °C according to the AOAC method [11]. CP was calculated by multiplying the sample by 6.25 by titrating N according to the Kjeldahl method. GE was analyzed using a bomb calorimeter (model 12361, Parr Instrument Company, Moline, IL, USA).

Blood sample

For blood analysis, 1 pig per pen was randomly selected at 2 weeks and 10 weeks of the experiment, and blood was collected through the jugular vein. Immediately after blood collection, blood was dispensed into EDTA-treated tubes and serum separation tubes. The blood dispensed into the serum tube was stored in a -20°C freezer from which serum was separated through centrifugation at 3,000 rpm, 20 min, and 4℃ until analysis. Blood leukocytes-based hemocytometry was performed using an automated hematology analyzer (ADVIA120; Bayer, Tarrytown, NY) and serum cortisol levels were determined using an enzyme-linked immunosorbent assay kit (LDN GmbH & Co., Nordhorn, Germany) according to the manufacturer's protocol. was evaluated using Tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) concentrations were analyzed with an ELISA kit (Quantikine, R&D systems, Minneapolis, MN, USA) and measured at 450 nm.

Pig behavior

Collection of each pig image data was recorded by using one- day/night infrared cameras (QNB-7080 RH, Hanwha, Seoul, Korea) installed 3m above each pen. A total of 24 pig behaviors were analyzed by randomly selecting one pig from each pen. Observers gathered data based on Yang et al. [12] findings, and only one person was responsible for all observations and video analysis to ensure consistency. 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 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 SPSS software (ver. 20.0; IBM, USA) orthogonal contrasts were used to compare possible relationships between treatments using the PROC procedure using a general linear model and the differences among treatments were examined by Tukey's multiple range test, which was considered to be significant at $P < 0.05$, unless otherwise stated.

Results

Growth performance

The effects of different stocking density and phytogenic feed additives dosage levels on growth performance were shown in Table 2. There was no difference between groups in the initial and 2nd weeks BW of pigs. At weeks 0-2 (growing phase), PC group significantly decreased (p < 0.05) ADG, G:F ratio, and significantly increased (p < 0.05) frequency of diarrhea compared to NC group. Compared with the PC group, CES1 group and CES2 group significantly increased (p < 0.05)G:F ratio.

At weeks 2-8 (finishing period), PC group significantly decreased (p < 0.05) ADG and G:F ratio compared to NC group. Compared with PC group, CES1 group significantly increased (p < 0.05) ADG, 8^{th} weeks BW and G:F ratio, and CES2 group significantly increased (p < 0.05) only G:F ratio.

During the entire experimental period (0-8 weeks), PC group significantly decreased (p < 0.05) ADG and G:F ratio compared to NC group. Pigs fed with supplementation of PFA except for ES2significantly increased (p < 0.05) G:F ratio compared to PC group. Among them, CES1 and CES2 group G:F ratio increased similarly to the NC group. In the case of ADG, CES1 and GP1 group significantly increased (p < 0.05) than PC group.

Nutrient digestibility

The effects of different stocking density and phytogenic feed additives dosage levels on nutrient digestibility were shown in Table 3. At 2 weeks, the ATTD of DM and CP was significantly decreased ($p < 0.05$) in the PC group compared to NC group. Supplementation of PFA significantly improved ($p < 0.05$) digestibility of DM and CP compared to PC group. There was no significant difference in GE digestibility between NC group and PC group. The CES1 group showed the highest GE digestibility than other groups including NC ($p < 0.05$).

At week 8, the ATTD of DM, CP and GE in the PC group was significantly decreased ($p < 0.05$) compared to the NC group. Supplementation of PFA groups significantly improved digestibility of DM, CP and GE compared to PC group. In particular, the CES1 and CES2 group showed similar digestibility to the NC group.

Blood profile

The effects of different stocking density and phytogenic feed additives dosage levels on blood profile were shown in Table 4. At week 2, there were no significant difference between NC group and PC group in blood profile. In the case of IL-6, GP2 group significantly decreased ($p < 0.05$) compared to PC group.

At week 8, CES1 group cortisol level significantly decreased ($p < 0.05$) compared to other supplementation of PFA groups. In the case of TNF-α, PC group significantly increased ($p < 0.05$) than NC group. CES, GP groups significantly alleviated ($p < 0.05$) TNF-α level than PC group.

Pig behavior

The effects of different stocking density and phytogenic feed additives dosage levels on animal behavior were shown in Table 5, Table 6 and, Figure 1. During growing pig period (week2), PC group significantly decreased ($p < 0.05$) feed intake time and increased ($p < 0.05$) standing time than NC group. Compared to PC group, CES1 group significantly increased ($p < 0.05$) feed intake time and CES2 group significantly increased ($p < 0.05$) feed intake time and decreased ($p < 0.05$) standing time. CES1 group showed lower biting frequency ($p < 0.05$) than PC group and similar with NC group.

During finishing pig period (week8), PC group significantly decreased ($p < 0.05$) feed intake time and significantly increased ($p < 0.05$) lying time than NC group. Supplementation of PFA groups significantly increased ($p < 0.05$) feed intake time than PC group and similar with NC group. CES, GP group significantly decreased ($p < 0.05$) lying time than PC group and similar with NC group. In singularity behavior, there are no significant ($p > 0.05$) result showed. PC group showed a tendency to increased frequency of biting compared to other groups ($0.05 < p < 0.10$) and rooting also showed an increasing contrasting effect.

Discussion

Pigs are social animals and live together in cages. However, as stated in the EU, pigs should be prohibited from being kept at excessive stocking densities for the protection and optimal growth of pigs [13]. A high stocking density is known to impede the movement of pigs due to limited space. It also makes them become competitive. However, because of the profit on the farm, the optimal stocking density is not kept. Therefore, we experimented with the use of various dosages of phytogenic feed additives to mitigate the negative effects of high stocking density.

Due to the negative effect of high stocking density, growth performance (ADG and ADFI) is reduced compared to pig raised with an optimal density considering animal welfare [14]. High stocking density can induce a high-temperature environment, making pigs increase heat loss and decrease heat production to remain homoeothermic [15]. Many studies have reported that eating, digestion, and absorption of nutrients can generate heat energy [16]. Thus, pigs exposed to high temperatures reduced heat by decreasing feed intake and increasing water intake [16,17]. Similarly, our study revealed decreases of ADG by 35%, ADFI by 17%, G:F ratio by 12% during growing period with decreases of ADG by 18% and G:F ratio by 16% during the finishing period in pigs under high stocking density than in pigs under optimal density considering animal welfare. The differences according to the additive dosage level were shown in mixture of bitter citrus extract and essential oil groups (CES). During finishing periods, CES1 showed similar feed efficiency with CES2, but significantly increased in body weight (6.60%). Because additives change the flavor by changing the compounds in the feed [18,19]. So, CES2 ADFI reduced (9.97%) than CES1 which also affected ADG (11.19%). Therefore, it can be seen that increasing the amount of feed additive doesn't make increasing growth performance [20,21].

The goal of pig production in the pig industry is to grow pigs quickly and healthily. Pigs under optimal density considering animal welfare and pigs under high stocking density fed with PFA showed similar feed efficiency (i.e., NC: 0.45, T3: 0.44), although their weights were numerically different (i.e., NC: 121.19kg, T3: 115.13kg). Stress caused by a high stocking density was associated with a decrease in feed intake [3]. Therefore, growth performance improvement with the addition of PFA under a high stocking situation was due to improved nutrient digestibility, not feed intake. Also, the relationship between PFA addition dosage level and growth performance does not increase proportionally. Consequently, it appeared that PFA could mitigate the negative effect of high stocking density on growth performance, although it could not completely offset such negative effect.

The frequency of diarrhea at high stocking density was increased during growing periods (i.e., NC: 0.36; PC:0.88). However, there was no diarrhea during finishing periods. Under high stocking density, pigs exposed to heat stress show decreased intestinal integrity and immunity with reduced digestive capacity [22,23]. The lower immunity causes an inflammatory response, which destroys the integrity of the intestine, increases gut permeability, impairs the absorptive functionality of the intestine, and causes diarrhea [24]. To reduce diarrhea, feed additives can be used to promote intestinal development [25]. In our study, the use of PFA in a high stocking density condition decreased diarrhea to a level similar to that of pigs raised under optimal density considering animal welfare. Several previous studies have reported that citrus compounds, essential oils, and grape pomace used in our experiments could reduce diarrhea [26-28]. The reason why diarrhea showed high frequency only during the growing period, but not during the finishing period, might be because the immune system is more complete as pigs grow with the improvement of intestinal health. In addition, there was no significant difference in the frequency of diarrhea according to the dosage of the additive, although there was a numerical difference in that the frequency of diarrhea decreased as the dosage of the additive decreased (i.e., Normal dosage compared to double dosage: ES group decreased 31.25%, CES1 group decreased 5.26%, GP group decreased 9.09%).

Increasing stocking density negatively affected nutrient digestibility in our whole study. Actually, many studies have shown that high stocking density can reduce nutrient digestibility [29,30]. In our study, PFA addition increased digestibility of DM and CP during the 2^{nd} week and digestibility of DM, CP, and GE during the 8^{th} week than non-PFA addition. The increase in nutrient digestibility of pigs fed with PFA showed the same result as a decrease in the frequency of diarrhea. This nutrient digestibility improvement might be due to stimulation of saccharase, amylase, and phosphatase activities [31]. Nutrient digestibility under high stocking density recovered more during finishing phase than during the growing phase in our study. The effect can be seen as an increase in immunity and resistance to intestinal disease due to the development of the digestive system as pigs grow [32]. In particular, it can be seen that the 2^{nd} week CES1 group and the 8^{th} week CES groups in which mixture of bitter citrus extract and essential oil were added returned to NC level. Both bitter citrus extract and essential oil are composed of phenolics known to possess antioxidant, antimicrobial, and anti-inflammatory activities. They can improve gut health and immunity. When fed with phenolic compounds, fermentation in the feed occurs well, leading to changes in the intestinal surface area and increased nutrient absorption due to digestive enzyme activity [33]. The DM and GE digestibility of CES2 increased sharply (i.e. DM: 1.98%; GE: 4.61%) at 8^{th} weeks compared to 2^{nd} weeks. It appears that PFA is more effective when it is supplied in long-term [34]. However, there are not many studies on feeding dosage levels of PFA under high stocking density. Thus, more research is needed. The reason why the nutrient digestibility of grape pomace extract added groups (GP1, GP2) were increased, although it did not recover to the NC group level due to saponin, an anti-nutrient in animal diets [35].

Immunity and health status can be reduced when stress is not well managed. Excessive stressors can increase the concentration of reactive oxygen species (ROS), leading to lipid peroxidation and oxidative damage to cell membranes. Lack of sufficient antioxidants to eliminate ROS will lead to oxidative damage and inflammation [36]. In addition, stress can induce the production of various inflammatory cytokines by activating the immune system of the gastrointestinal tract, an immune organ that constitutes more than 70% of nutrient metabolism and immune cells in the body. In fact, in our experiment, the intestinal environment deteriorated in high stocking density compared to that under optimal density considering animal welfare (i.e. Fecal score decreased 59.09%). Thus, the frequency of diarrhea was increased, the nutrient digestibility was lowered, and the growth performance was impaired.

Several studies have reported worsening blood profiles due to stress [35,37]. Immune markers TNF-α and IL-6 are decreased under stress. Under stressful conditions, pro-inflammatory cytokines are secreted to promote cortisol secretion and suppress growth hormone secretion [38]. Excessive pro-inflammatory cytokines can induce fever, inflammation, tissue destruction [39], shock, and even death in some cases [40].In the current study, there was no significant difference in blood profile according to the concentration of stocking density except for 8^{th} week TNF-α (i.e. NC: 22.77; PC: 78.67). However, the addition of PFA alleviated negative results of some blood parameters (2 weeks WBC and 8 week cortisol). Among pro-inflammatory cytokines, compared to PC group treatment, IL-6 decreased in the GP2 group at the 2^{nd} week, and TNF-α decreased in the CES, GP groups at the 8^{th} week. The reason for the decrease is that thymol, one of the components of essential oil, has anti-inflammatory action. It can reduce the expression of pro-inflammatory cytokines [41]. It can also inhibit maturation of dendritic cells and activation of T-cell proliferation, which play a major role in promoting immune responses *in vitro*. Another ingredient, carvacrol, has high antioxidant properties. The hydroxyl group (OH^-) connected to the aromatic ring can accumulate free radicals to reduce tissue damage and cell function. Carvacrol, an antioxidant, has antioxidant activity to protect cells [42]. Grape phenolic compounds can trap and destroy free radicals with antioxidant properties. Grape seed polyphenols are very sensitive to oxygen, light, acids, and alkalis, but relatively less sensitive to heat. Therefore, grape phenol compounds are effective against heat stress caused by high stocking density [43].

Stocking density can increase the frequency of contact, thus increasing social tension and aggression [4,44]. It can also increase heat production per area, which can boost thermal stress [3]. High stocking density is known to cause heat exhaustion, which can increase sweating, panting, and water demand, which in turn can increase water intake [45]. However, in our study, the NC group with the least heat stress had the most water intake than PC group (growing period: 1.94%; finishing period: 0.35%). More research is needed in the future to clarify this. Aggression is a sign of competition for controlling a resource of special significance [46]. Several studies have shown that heat stress increases lying and aggression behavior of pigs [47,48]. Our study showed similar results. During the finishing period, the PC group of pigs tended to spend more time lying down (1.29%) with more aggressive biting (9.52%) compared to pigs in the NC group. Supplementation of PFA reduced the lying time to a level similar to that of the NC group. During the growing period, the PC group (0.74 count/hour) showed increased number of bites compared to the NC group (0.65 count/hour) and the CES1 group (0.64 count/hour) showed fewer number of bites with a level similar to the NC group. In the current study, growth performance increased with PFA supplementation. Going to the feeder is related to feed intake, which affects BW, ADG, ADFI, and G:F ratio. Both grower and finisher showed reduced feed intake times due to stress of competition in the feeder. In particular, if mixture of bitter citrus extract and essential oil (CES) were added during the growing period, the feed intake time was similar to that of the NC group (i.e. CES1: 4.46 min/hour; CES2: 4.49 min/hour NC: 4.55 min/hour). This might be because essential oils & bitter citrus extract reduced oxidative stress due to their powerful antioxidant effects. It has also been reported that the addition of essential oils can improve negative behavior of rats [49]. Therefore, the most effective way to deal with aggression is to add 0.10% of essential oil and citrus. Pearce & Paterson [50] have reported that pigs' immobile sitting or standing in confined spaces is a way to cope with crowded stress. During the finishing period, the NC group coped well with stress by standing longer than the PC group in the present study. Thus, the increase in the frequency of aggressive behavior might be due to the sharper decrease in stocking density during the more extended period in finishing period than growing period.

Conclusion

Our goal in this experiment was to verify the effects of various plant feed additives with dosage level in pigs induced by environmental stress. Pigs have high stress levels, weakened immunity, and reduced growth performance in high stocking density situation. However, the addition of citrus extracts and essential oils mitigated the negative effects of high stocking density. Also, relationship between PFA addition dosage level and growth performance does not increase proportionally. In summary, the negative effect of high stocking density was most effectively mitigated by normal dosage of mixture of bitter citrus extract and essential oil additive (CES1).

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

Items	Grower 0-2w	Finisher 2-8w
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-Theronine	0.03	0.03
Dicalcium phosphate	1.00	1.00
Limestone	1.20	1.25
Salt	0.50	0.50
Vitamin premix[a]	0.08	0.08
Mineral premix[b]	0.05	0.05
Calculated composition		
ME (kcal kg^{-1})	3276	3284
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

Note: ME, metabolizable energy.

[a]Provided per kilogram of complete diet: 20 000 IU of vitamin A, 4000 IU of vitaminD3, 80 IU of vitamin E, 16mg of vitamin K3, 4mg of thiamine, 20mg 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, 0.08 mg of biotin.

[b]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.

Table 2. Effects of dosage level of phytogenic feed additives on growth performance in growing-finishing pigs with stressed by stocking density

Items	NC	High stocking density								SEM	P-value
		PC	ES1 (0.05%)	ES2 (0.10%)	CES1 (0.10%)	CES2 (0.20%)	GP1 (0.04%)	GP2 (0.08%)			
`BW											
Initial[4]	49.36	48.98	49.33	49.29	49.39	49.38	49.32	49.21		0.54	0.990
2W[1,4]	64.71	58.91	61.26	60.08	62.32	61.07	61.24	60.51		0.62	0.483
8W[1,2,4]	121.19^{a}	105.19^{c}	108.72^{bc}	107.39^{c}	115.13^{ab}	107.53^{c}	109.44^{bc}	108^{bc}		0.81	<0.001
0-2W											
ADG[1,2,3,4,5]	1.10^{a}	0.71^{c}	0.85^{bc}	0.77^{bc}	0.92^{b}	0.83^{bc}	0.85^{bc}	0.81^{bc}		0.02	<0.001
ADFI[1,3,4,6,7,8]	2.52^{a}	2.09^{c}	2.25^{b}	2.17^{bc}	2.11^{c}	1.92^{d}	2.12^{c}	2.10^{c}		0.02	<0.001
G:F[1,2,4,5,6,8]	0.44^{a}	0.34^{c}	0.38^{ab}	0.35^{b}	0.44^{a}	0.44^{a}	0.40^{ab}	0.38^{ab}		0.01	<0.001
Fecal score[1,2,3,4]	0.29^{b}	0.88^{a}	0.33^{b}	0.48^{b}	0.36^{b}	0.38^{b}	0.40^{b}	0.44^{b}		0.02	<0.001
2-8W											
ADG[1,4]	1.34^{a}	1.10^{b}	1.13^{b}	1.13^{b}	1.26^{a}	1.11^{b}	1.15^{b}	1.13^{b}		0.01	<0.001
ADFI[4,5,6,7]	3.63^{ab}	3.70^{a}b	3.45^{ab}	3.79^{a}	3.51^{ab}	3.16c	3.46^{bc}	3.46b		0.03	<0.001
G:F[1,2,4,5,6,7,8]	0.37^{a}	0.30c	0.32^{bc}	0.30^{c}	0.36^{ab}	0.36^{ab}	0.33^{abc}	0.33^{bc}		0.01	<0.001
0-8W											
ADG[1,2,3,4,5,8]	1.28^{a}	1.00^{d}	1.06^{cd}	1.04^{cd}	1.17^{b}	1.04^{d}	1.08^{c}	1.05^{cd}		0.01	<0.001
ADFI[4,5,6,7]	3.35^{abc}	3.30^{a}	3.21^{abc}	3.38^{ab}	3.16^{bc}	2.85^{d}	3.12^{c}	3.13^{c}		0.03	<0.001
G:F[1,2,4,5,6,7,8]	0.45^{a}	0.30^{e}	0.39^{cd}	0.36^{de}	0.44^{ab}	0.43^{ab}	0.41^{bc}	0.40^{cd}		0.00	<0.001

Abbreviation: NC, basal diet in animal welfare density; PC, basal diet in high stocking density;ES1, basal diet with essential oil 0.05% in high stocking density; ES2, basal diet with essential oil 0.10% in high stocking density; CES1, basal diet with mixture of bitter citrus extract and essential oil 0.10% in high stocking density; CES24, basal diet with mixture of bitter citrus extract and essential oil 0.20% in high stocking density; GP1, basal diet with grape pomace extract 0.04% in high stocking density; GP2, basal diet with grape pomace extract 0.08% in high stocking density; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency; Fecal score was determined as follow : 0, Normal feces; 1, Soft feces; 2, Mild diarrhea; 3, Severe diarrhea; SEM, standard error of means.

[a-e]Means with different letters are significantly differ ($p < 0.05$) or tend to differ ($0.05 \leq p < 0.10$).

[1]contrast: NC vs PC ($p<0.05$). [2]contrast: PC vs other treatments ($p < 0.05$). [3]contrast: PC vs Essential oils ($p < 0.05$). [4]contrast: PC vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [5]contrast: PC vs Grape pomace extract ($p < 0.05$). [6]contrast: Essential oils vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [7]contrast: Essential oils vs Grape pomace extract ($p < 0.05$). [8]contrast: mixture of bitter citrus extract and essential oil vs Grape pomace extract ($p < 0.05$).

Table 3. Effects of dosage level of phytogenic feed additives on nutrient digestibility in growing-finishing pigs with stressed by stocking density

Items	NC	High stocking density							SEM	P-value
		PC	ES1 (0.05%)	ES2 (0.10%)	CES1 (0.10%)	CES2 (0.20%)	GP1 (0.04%)	GP2 (0.08%)		
2 week										
DM [1,2,3,4,5,6,7,8]	85.17[ab]	81.65[d]	84.08[bc]	83.34[c]	86.4[a]	83.31[c]	84.98[ab]	85.43[ab]	0.31	<0.001
CP [1,2,3,4,5,8]	75.58[a]	70.91[c]	74.42[ab]	73.39[b]	74.77[ab]	74.55[ab]	73.46[b]	73.70[b]	0.20	<0.001
GE [1,2,3,4,5,8]	78.79[bc]	76.74[cd]	78.64[bc]	77.04[bcd]	81.07[a]	74.98[d]	78.88[b]	78.85[b]	0.38	<0.001
8 week										
DM [1,2,3,4,5,7,8]	85.67[a]	80.15[d]	82.88[c]	84.46[ab]	85.24[ab]	84.96[ab]	84.26[b]	82.28[c]	0.23	<0.001
CP [1,2,3,4,5,6,7]	76.67[a]	67.14[c]	70.19[b]	70.05[b]	76.78[a]	75.47[a]	71.04[b]	71.15[b]	0.41	<0.001
GE [1,2,3,4,5,7,8]	78.35[a]	69.65[c]	76.98[ab]	77.07[ab]	78.16[a]	78.44[a]	76.38[ab]	73.82[b]	0.64	<0.001

Abbreviation: NC, basal diet in animal welfare density; PC, basal diet in high stocking density; ES1, basal diet with essential oil 0.05% in high stocking density; ES2, basal diet with essential oil 0.10% in high stocking density; CES1, basal diet with mixture of bitter citrus extract and essential oil 0.10% in high stocking density; CES2, basal diet with mixture of bitter citrus extract and essential oil 0.20% in high stocking density; GP1, basal diet with grape pomace extract 0.04% in high stocking density; GP2, basal diet with grape pomace extract 0.08% in high stocking density; DM, dry matter; CP, crude protein; GE, gross energy; SEM, standard error of means.

[a-d]Means with different letters are significantly differ ($p < 0.05$) or tend to differ ($0.05 \leq p < 0.10$).

[1]contrast: NC vs PC ($p < 0.05$). [2]contrast: PC vs other treatments ($p < 0.05$). [3]contrast: PC vs Essential oils ($p < 0.05$). [4]contrast: PC vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [5]contrast: PC vs Grape pomace extract ($p < 0.05$). [6]contrast: Essential oils vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [7]contrast: Essential oils vs Grape pomace extract ($p < 0.05$). [8]contrast: mixture of bitter citrus extract and essential oil vs Grape pomace extract ($p < 0.05$).

Table 4. Effects of dosage level of phytogenic feed additives on blood profile in growing-finishing pigs with stressed by stocking density

Items	NC	High stocking density							SEM	P-value
		PC	ES1 (0.05%)	ES2 (Essential oil 0.10%)	CES1 (0.10%)	CES2 (0.20%)	GP1 (0.04%)	GP2 (0.08%)		
2 week										
WBC[5,7,8]	19.67^{c}	22.37^{bc}	19.39^{c}	22.7^{bc}	25.18^{ab}	20.08^{c}	27.51^{a}	23.34^{abc}	0.47	<0.001
Lymphocyte [2,3,4,5]	62.43^{a}	59.40^{a}	54.93^{ab}	47.30^{bc}	44.07^{c}	56.57^{ab}	47.50^{bc}	54.50^{ab}	1.00	<0.001
Neutrophil [2,3,4,6]	33.40^{d}	35.83^{d}	38.53^{cd}	46.7^{abc}	50.10^{a}	37.57^{d}	47.57^{ab}	39.00^{bcd}	0.96	<0.001
Basophil[1]	0.70^{a}	0.93^{a}	0.63^{ab}	0.90^{bcd}	1.20^{a}	0.67^{a}	0.63^{cd}	1.07^{abc}	0.04	0.001
Cortisol	2.70^{ab}	2.53^{ab}	2.83^{a}	1.84^{ab}	3.72^{ab}	2.64^{ab}	4.55^{b}	1.68^{a}	0.19	0.002
TNF- α	31.50^{b}	63.23^{ab}	120.8^{ab}	97.87^{ab}	38.37^{b}	94.7^{ab}	38.7^{b}	150.00^{a}	9.66	0.008
IL-6[2,4,5]	79.33^{abc}	120.10^{ab}	131.80^{a}	59.63^{bc}	57.63^{bc}	84.77^{abc}	95.20^{abc}	40.03^{c}	6.14	<0.001
8 week										
WBC	19.48^{ab}	21.58^{a}	20.30^{ab}	16.87^{b}	17.86^{ab}	17.84^{ab}	17.75^{ab}	19.15^{ab}	0.39	0.035
Lymphocyte	70.67	67.37	71.47	67.33	66.03	66.37	69.37	67.73	0.84	0.691
Neutrophil	26.47	29.97	25.53	28.70	29.97	31.20	27.40	28.83	0.84	0.729
Basophil	0.67	0.87	0.70	0.80	0.80	0.70	0.63	0.70	0.25	0.253
Cortisol[2,5]	4.13^{ab}	4.49^{a}	3.33^{abc}	2.48^{abc}	1.27^{c}	3.17^{abc}	2.02^{bc}	3.64^{ab}	0.21	<0.001
TNF- α [2,5]	22.77^{d}	78.67^{ab}	85.87^{a}	53.67^{bc}	32.83^{cd}	40.83^{cd}	33.10^{cd}	49.07^{c}	3.15	<0.001
IL-6[4,7]	47.30^{ab}	41.70^{ab}	51.93^{ab}	34.40^{b}	49.10^{ab}	46.90^{ab}	56.87^{a}	44.87^{ab}	1.53	0.012

Abbreviation: NC, basal diet in animal welfare density; PC, basal diet in high stocking density; ES1, basal diet with essential oil 0.05% in high stocking density; ES2, basal diet with essential oil 0.10% in high stocking density; CES1, basal diet with mixture of bitter citrus extract and essential oil 0.10% in high stocking density; CES2, basal diet with mixture of bitter citrus extract and essential oil 0.20% in high stocking density; GP1, basal diet with grape pomace extract 0.04% in high stocking density; GP2, basal diet with grape pomace extract 0.08% in high stocking density; WBC, white blood cell; TNF- α, tumor necrosis factor-α; IL-6, Interleukin-6; SEM, standard error of means.

$^{a\text{-}d}$Means with different letters are significantly differ ($p < 0.05$) or tend to differ ($0.05 \le p < 0.10$).

[1]contrast: NC vs PC ($p < 0.05$). [2]contrast: PC vs other treatments ($p < 0.05$). [3]contrast: PC vs Essential oils ($p < 0.05$). [4]contrast: PC vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [5]contrast: PC vs Grape pomace extract ($p < 0.05$). [6]contrast: Essential oils vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [7]contrast: Essential oils vs Grape pomace extract ($p < 0.05$). [8]contrast: mixture of bitter citrus extract and essential oil vs Grape pomace extract ($p < 0.05$).

Table 5. Effects of dosage level of phytogenic feed additives on behavior changes in growing pigs with stressed by stocking density

Items	NC	High stocking density							SEM	P-value
		PC	ES1 (0.05%)	ES2 (0.10%)	CES1 (0.10%)	CES2 (0.20%)	GP1 (0.04%)	GP2 (0.08%)		
Basic behavior (min/hour)										
Feed intake [1,3,4,5,6,8]	4.55^{a}	4.25^{c}	4.33^{bc}	4.30^{bc}	4.46^{ab}	4.49^{ab}	4.33^{bc}	4.36^{abc}	0.02	0.001
Standing [1,3,4,5,6,8]	6.57^{b}	7.42^{a}	6.94^{ab}	6.93^{ab}	6.83^{ab}	6.55^{b}	6.80^{ab}	7.11^{ab}	0.07	0.009
Lying [1,3,4,5,6,8]	45.45	43.90	44.42	44.43	45.31	44.43	44.60	44.53	0.15	0.069
Sitting [1,4,5,6]	3.43	4.44	4.32	4.35	3.52	4.40	4.27	4.00	0.11	0.045
Singularity behavior (count/hour)										
Drink water [4,8]	5.25	5.15	5.19	5.18	5.23	5.21	5.15	5.16	0.02	0.949
Rooting	2.06	2.09	2.12	2.08	2.05	2.06	2.07	2.08	0.18	0.996
Posture transition (lying-sitting) [1,3,4,5]	2.17	2.57	2.49	2.47	2.44	2.43	2.51	2.46	0.05	0.612
Posture transition (sitting-lying) [1,3,4,8]	2.18	2.56	2.49	2.48	2.43	2.41	2.52	2.47	0.04	0.613
Biting [1,2,3,4,5,7,8]	0.65^{b}	0.74^{a}	0.66^{b}	0.68^{ab}	0.64^{b}	0.67^{ab}	0.69^{ab}	0.70^{ab}	0.01	0.014

Abbreviation: NC, basal diet in animal welfare density; PC, basal diet in high stocking density; ES1, basal diet with essential oil 0.05% in high stocking density; ES2, basal diet with essential oil 0.10% in high stocking density; CES1, basal diet with mixture of bitter citrus extract and essential oil 0.10% in high stocking density; CES2, basal diet with mixture of bitter citrus extract and essential oil 0.20% in high stocking density; GP1, basal diet with grape pomace extract 0.04% in high stocking density; GP2, basal diet with grape pomace extract 0.08% in high stocking density; SEM, standard error of means.

$^{a-c}$Means with different letters are significantly differ ($p < 0.05$) or tend to differ ($0.05 \leq p < 0.10$).

[1]contrast: NC vs PC ($p < 0.05$). [2]contrast: PC vs other treatments ($p < 0.05$). [3]contrast: PC vs Essential oils ($p < 0.05$). [4]contrast: PC vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [5]contrast: PC vs Grape pomace extract ($p < 0.05$). [6]contrast: Essential oils vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [7]contrast: Essential oils vs Grape pomace extract($p < 0.05$). [8]contrast: mixture of bitter citrus extract and essential oilvs Grape pomace extract($p < 0.05$).

Table 6. Effects of dosage level of phytogenic feed additives on behavior changes in finishing pigs with stressed by stocking density

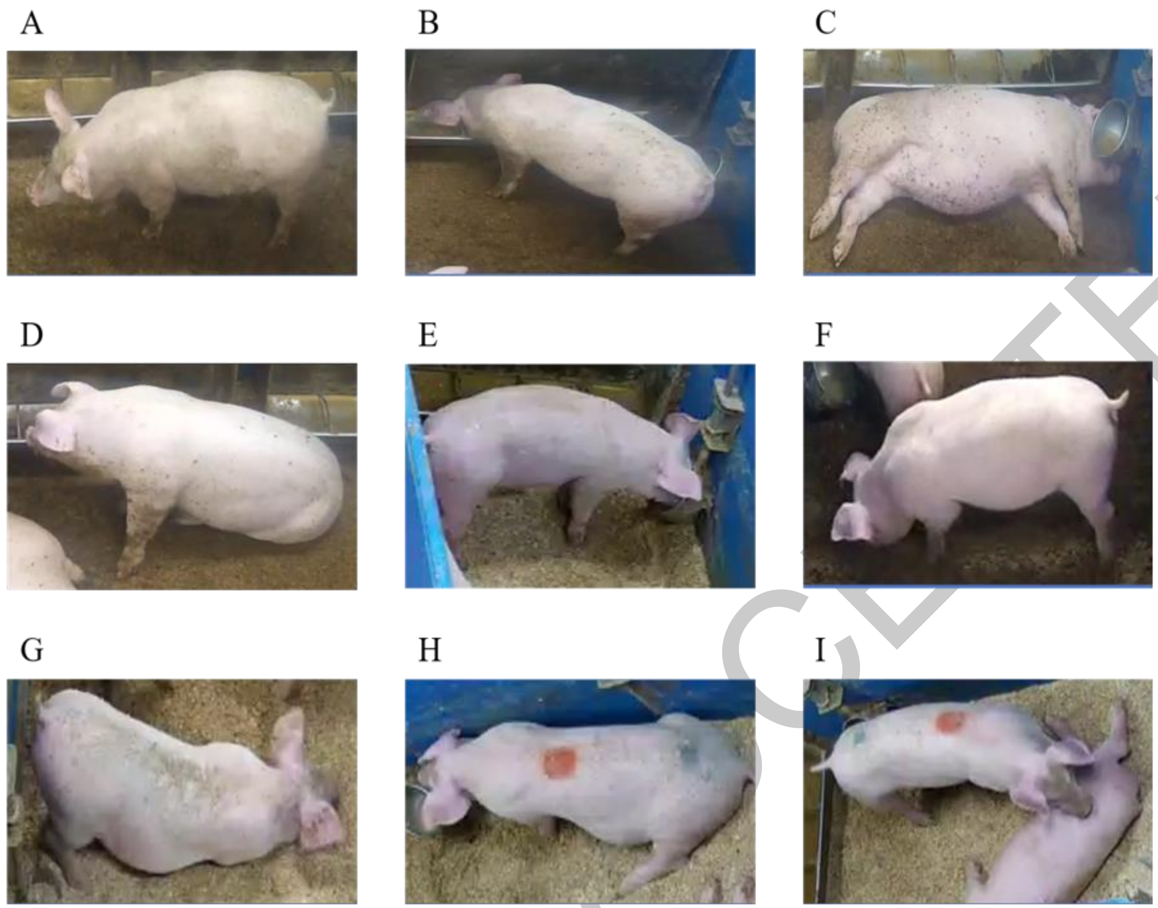
Items	NC	High stocking density							SEM	P-value
		PC	ES1 (0.05%)	ES2 (0.10%)	CES1 (0.10%)	CES2 (0.20%)	GP1 (0.04%)	GP2 (0.08%)		
Basic behavior (min/hour)										
Feed intake [1,3,4,5,6,8]	4.92^{a}	4.71^{b}	4.97^{a}	4.93^{a}	4.95^{a}	5.01^{a}	4.94^{a}	4.98^{a}	0.02	<0.001
Standing [1,4,5,6]	6.44^{a}	6.01^{b}	6.03^{b}	6.14^{ab}	6.27^{ab}	6.20^{ab}	6.40^{a}	6.21^{ab}	0.04	0.002
Lying [1,3,4,5,6,7]	45.10^{c}	45.68^{a}	45.46^{ab}	45.35^{bc}	45.15^{bc}	45.18^{bc}	45.17^{bc}	45.28^{bc}	0.04	<0.001
Sitting [1,5,6,7,8]	3.53	3.60	3.54	3.58	3.63	3.61	3.50	3.54	0.01	0.950
Singularity behavior (count/hour)										
Drink water[5]	5.66	5.64	5.52	5.70	5.61	5.65	5.56	5.69	0.03	0.712
Rooting [2,3,4,5,7]	1.41	1.27	1.43	1.30	1.34	1.47	1.45	1.40	0.02	0.231
Posture transition (lying-sitting) [1,4,5,6,7]	2.68	2.40	2.33	2.44	2.56	2.56	2.54	2.57	0.04	0.471
Posture transition (sitting-lying) [1,4,5,6,7]	2.67	2.39	2.33	2.45	2.55	2.57	2.52	2.56	0.05	0.716
Biting [1,2,3,4,5,6,7,8]	0.84	0.92	0.91	0.88	0.84	0.83	0.85	0.87	0.01	0.060

Abbreviation: NC, basal diet in animal welfare density; PC, basal diet in high stocking density; ES1, basal diet with essential oil 0.05% in high stocking density; ES2, basal diet with essential oil 0.10% in high stocking density; CES1, basal diet with mixture of bitter citrus extract and essential oil 0.10% in high stocking density; CES2, basal diet with mixture of bitter citrus extract and essential oil 0.20% in high stocking density; GP1, basal diet with grape pomace extract 0.04% in high stocking density; GP2, basal diet with grape pomace extract 0.08% in high stocking density; SEM, standard error of means.

$^{a-c}$Means with different letters are significantly differ ($p < 0.05$) or tend to differ ($0.05 \leq p < 0.10$).

[1]contrast: NC vs PC (p<0.05). [2]contrast: PC vs other treatments ($p < 0.05$). [3]contrast: PC vs Essential oils ($p < 0.05$). [4]contrast: PC vs mixture of bitter citrus extract and essential oil ($p < 0.05$). [5]contrast: PC vs Grape pomace extract($p < 0.05$). [6]contrast: Essential oils vs mixture of bitter citrus extract and essential oil($p < 0.05$). [7]contrast: Essential oils vs Grape pomace extract($p < 0.05$). [8]contrast: mixture of bitter citrus extract and essential oilvs Grape pomace extract($p < 0.05$).

Figure 1. Classification of pig behavior changes



Abbreviation: A, Feed intake; B, Standing; C, Lying; D, Sitting; E, Drink water; F, Rooting; G, Post transition (standing → lying); H, Post transition (lying→standing); I, Bitting.