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Improvement of weanling pigs immune status and metabolic condition using ultraweak light

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Abstract

Weaning stress is the most common issue in swine farms, which increases mortality and morbidity. The use of artificial light is an option for modifying the immune system and metabolic pathways. This study aimed to evaluate the influence of ultraweak light (Photonia) on growth performance, immune system and metabolism of weanling pigs, and the carry-over effect on the growth performance in postweanling growing stages. A total of 30 weaned pigs with an average initial body weight of 7.06 ± 0.11 kg (age: 21 days) were allotted two treatments (Control and Photonia) with 15 replicates. The pelleted form diets were prepared for pigs in three phases including phase 1 (Days 0–14), phase 2 (Days 15–28) and phase 3 (Days 29–48). The gain-to-feed ratio (G:F) of pigs was significantly greater in the Photonia treatment. On Day 28, a higher concentration of immunoglobin A (IgA) ($p < 0.01$) and IgG ($p < 0.01$) was observed in the Photonia pigs. On Day 48, the Photonia treatment showed a greater serum IgA ($p < 0.01$) and IgG ($p < 0.05$). The concentration of interleukin (IL)-6 was decreased ($p < 0.05$) in the Photonia treatment. At Day 48, the concentrations of tumour necrotic factor-α, IL-1β and IL-6 in serum were decreased ($p < 0.05$) in pigs in the Photonia treatment. Metabolic pathways analysis showed that the Photonia treatment increased the D-glutamine, D-glutamate, alanine, aspartate, glutamate and phenylalanine compared with the control treatment. In conclusion, the use of Photonia for weanling pigs is recommended due to improved G:F, immune status and activation of amino acids metabolic pathways including D-glutamine, D-glutamate, alanine, aspartate, glutamate and phenylalanine.

KEYWORDS

artificial light, immune system, immunoglobulin, irradiation, metabolites

Chang Beon Lee and Abdolreza Hosseindoust contributed equally to this study.

1 | INTRODUCTION

Pork is a popular protein source for humans and weaning stress is a serious issue in pigs' life due to separating from the mother sow, changing feed form and entering a new environment (Moturi et al., 2021). Therefore, weanling pigs are so vulnerable to any stressors, particularly immune-related factors (Hosseindoust et al., 2017; Lee et al., 2016). Several production technologies have already focused on improving traditional systems including air quality, nutrient availability, and housing type (Kim et al., 2016). Besides the abovementioned factors, the source and duration of lighting are key factors in swine production to enhance the welfare and optimize the genetic potential of growth.

Developing rearing systems improves pigs' welfare and health. Artificial lights in different intensities and wavelengths influence growth performance, physiology, immunity and welfare (Oh & Jeong, 2019; Sharideh & Zaghari, 2017; Zamanizad et al., 2019). The selection of proper light may improve immune response and alleviate the adverse effect of stress in pigs. It has already been demonstrated that visible light in different wavelength ranges has biological effects on vasodilation, wound healing, removing depression, circadian abnormality, immune status and depression in human-based studies (Gavish et al., 2008; Lugongolo et al., 2017; Van Tran et al., 2021). The light-emitting diode (LED) with a wavelength of 610–710 nm showed anti-inflammatory, tissue biostimulation and analgesic characteristics (Van Tran et al., 2021). However, the use of artificial light and its role in pigs' welfare has been mostly ignored. To extend these results to pigs, the biological effect of visible light has to be examined from the scale of wavelength and irradiation power. Monochromatic LED is cost-efficient and environmentally friendly compared with laser irradiation (Lim et al., 2009; Van Tran et al., 2021). Recently, there is an increasing trend to use LED-based lamps in several countries in Europe, the United States and Korea. LED irradiation has recently been applied in modern poultry (Sharideh & Zaghari, 2017; Zamanizad et al., 2019) with positive effects on chickens body weight (BW), circadian activity, immune function and behaviour of broiler chickens (Liu et al., 2018; Rozenboim et al., 2004). An additional clue to the immune response of LED irradiation can be related to T-cell differentiation and proliferation, which affects the messenger RNA (mRNA) expression level of proinflammatory cytokines such as tumour necrotic factor (TNF-α), interleukin (IL)-1β and IL-6 (Hosseindoust et al., 2020; Lim et al., 2009). Irradiated polychromatic visible polarized light in the range of 540–780 nm increased helper T lymphocyte number and total lymphocytes count in humans (Lim et al., 2009). Secretions of several cytokines are associated with the activation of helper T-cells (Lim et al., 2009). The inflammatory reaction of TNF-α and IL-6 has angiogenesis and destructive effects by contributing to oxidative stress and reactive oxygen species production (Hosseindoust et al., 2020). Besides the immunological effects, the metabolism of vitamin D (Kolp et al., 2017) and several metabolites can be affected by waves and change the metabolical pathways (Salah et al., 2020). Based on the influence of LED waves on immune status and metabolite production, it can be hypothesized that LED wave exposure illustrates the stimulatory effect on the growth performance and health of pigs during weaning stress. The current study was designed to evaluate the effects of Photonia LED irradiation in 500 and 780 nm wavelengths on growth performance, blood parameters, inflammatory cytokines, immunoglobulins, hair cortisol and blood metabolites.

2 | MATERIALS AND METHODS

2.1 | Irradiation procedure

The light source, Photonia™ (Biolight Corporation) based on LED was irradiated. Its optical characteristics were adjusted by passive optical components and the resulting spectrum was measured by a spectrometric instrument (CAS 140 CT; Instrument Systems GmbH). The partially polarized light with a wavelength range from 500 to 780 nm was realized and situated at a height of 2.1 m from the floor to cover all the corners. The measured spectral irradiance at the peak wavelength of 703 nm and irradiance are 5.1×10^{-11} W/cm^2 nm and 5.2×10^{-9} W/cm^2 respectively. Pigs were allowed to move freely and subjected to be under the influence of the light source on the whole skin. Each subjected pig was irradiated the whole day (24 h). The irradiation process in the experiment was continued for 48 days.

2.2 | Animal and experimental design

A total of 30 weaned pigs (Landrace × Yorkshire × Duroc) with an average initial BW of 7.06 ± 0.11 kg (age: 21 days) were distributed among two treatments based on BW. There were two treatments as Photonia and Control. Each treatment included 15 cages with 1 piglet per cage. The pelleted form diets were prepared for pigs in three phases including phase 1 (Days 0–14), phase 2 (Days 15–28) and phase 3 (Days 29–48). The diet formula (Table 1) was according to the nutrient requirements of the National Research Council (2012). The pens were metal cages with a plastic floor (1.2 m × 2.4 m). The average temperature was adjusted to 30°C at the start of the study and was gradually reduced to 25°C from Day 8 onward. The humidity of the barn ranged from 61% to 66%. All pens were equipped with a low-pressure nipple drinker and self-feeder to provide constant ad libitum access to water and feed.

2.3 | Experimental sampling

BW was measured on the second, fourth, and seventh weeks. Growth performance (average daily gain [ADG]), feed consumption (average daily feed intake [ADFI]) and gain-to-feed ratio (G:F) were calculated at the end of each phase, considering the mortalities. At the end of each phase, all pigs were subjected to blood sampling via jugular vein using anticoagulants-free vacutainer tubes (Becton Dickinson). The samples were centrifuged (15 min, 3000*g* and 4°C) and stored at −20°C to be used for haematological parameters analysis.

TABLE 1 Formula and chemical composition of basal diets (as-fed basis).

Items	Phase 1 (Days 1–14)	Phase 2 (Days 14–28)	Phase 3 (Days 29–48)
Ingredient (%)			
Corn	43.33	47.83	54.95
Fish meal	5.00	3.00	3.00
Dehulled soybean meal	24.49	29.49	26.03
Whey powder	17.00	15.38	11.25
Soybean oil	3.38	1.60	1.50
Monocalcium phosphate	0.40	0.36	0.48
Limestone	0.77	0.83	0.92
Salt	0.30	0.30	0.30
DL-methionine	0.14	0.08	0.37
L-lysine	0.33	0.28	0.37
L-threonine	0.14	0.13	0.13
L-tryptophan	0.22	0.17	0.15
Vitamin premix[a]	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25
Choline chloride	0.05	0.05	0.05
Lactose	3.95	–	–
Total	100	100	100
Chemical composition (%)			
Metabolisable energy (kcal/kg)	3400	3350	3300
Crude protein	23.00	21.00	19.00
Calcium	0.80	0.70	0.66
Available phosphorus	0.42	0.36	0.33
SID lysine	1.35	1.23	1.23
SID methionine + cystine	0.74	0.68	0.63

Abbreviation: SID, standardized ileal digestible.

[a]Supplied per kilogram of diet: 16,000 IU vitamin A, 3000 IU vitamin D3, 40 IU vitamin E, 5.0 mg vitamin K3, 5.0 mg vitamin B1, 20 mg vitamin B2, 4 mg vitamin B6, 0.08 mg vitamin B12, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.

[b]Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.

2.4 | Chemical and immunological analyses

The crude protein (method number 990.03) and dry matte (method number 930.15) analysis of diets were performed in triplicate according to the Association of Official Analytical Chemists (2007). The count of white blood cells (WBC), lymphocytes, neutrophils, monocytes, red blood cells, eosinophils, and basophils was performed by Hemavet® Hematology System (CDC Technologies). The concentrations of immunoglobin G (IgG; k3231094, Komabiotech), IgA (k3231092 Komabiotech), IL-1β, TNF-α, and IL-6 were calculated using ELISA kits (Invitrogen; Thermo Fisher Scientific).

2.5 | Hair cortisol

Hair cortisol content was determined as described previously (Nejad et al., 2019). In brief, the forehead of pigs was selected for hair sampling on the day of 0, 28 and 48. The collected samples were kept in aluminium foil and placed in polypropylene tubes (HM Hyundai Micro) until dry at room temperature. The 5 mL isopropyl alcohol was added to each tube to wash the samples (three times) in order to remove contaminations, then dried at 23 ± 1°C for 7 days. The prepared samples were subjected to hair cortisol extraction using methanol dilution via ELISA kit according to instructions (Cayman Chemical).

2.6 | Metabolomics sample analysis

The collected faecal samples (from all pigs) were immediately sealed and placed in ice; then transferred to the laboratory within 90 min. According to He et al. (2019), 500 µL distilled water was added to 100 mg of faeces, and was vortexed for 60 s. The extraction was performed with the addition of 1000 µL methanol and vortexed for 30 s. The samples were transferred to an ultrasound machine to be incubated at 25°C for 10 min, then 30 min incubation on ice to be prepared for centrifugation (5000 r/min; 5°C; 15 min). All the supernatants were moved to new centrifuge tubes (2 mL) and dried. The dried samples were mixed with 60 µL of methoxyamine solution in pyridine and vortexed (30 s) to be allowed for the reaction for 120 min at 37°C. The samples were ground by a grinding machine at 70 Hz for 60 s, and vortexed for 40 s. All samples were centrifuged at 5000 r/min at 5°C for 15 min. The supernatants were placed in new sample bottles to be analyzed by gas chromatography–mass spectrometry (GC-MS) using Agilent7890A/5975C (Agilent Technologies).

2.7 | Statistical analyses

The statistical analysis between the Control and Photonia treatments was performed using the *t* test (SAS Institute) with the pig as the experimental unit. The difference between means was considered significant when probability values were less than 0.05. For metabolites analysis, the raw data from the detected metabolites were normalized to ($^{13}C_2$)-myristic acid, and compared with the standard reference (http://srdata.nist.gov/gateway/) according to He et al. (2019). The statistical analysis of untargeted metabolomics was performed with the SAS software. The metabolites with variable importance projection value higher than 1.0 and *p* values of 0.05 were considered metabolites that could discriminate between the

TABLE 2 Effects of Photonia on growth performance in weanling pigs.

Item	Control	Photonia	SEM	*p* Value
Initial body weight (kg)	7.07	7.05	0.09	0.992
Final body weight (kg)	31.70	34.24	1.23	0.08
Day 14				
ADG (g)	371	395	12.26	0.204
ADFI (g)	512	522	8.89	0.447
G:F	0.73	0.76	0.02	0.27
Day 28				
ADG (g)	455	521	21.88	0.065
ADFI (g)	679	736	22.94	0.121
G:F	0.67	0.71	0.01	0.039
Day 48				
ADG (g)	653	715	41.92	0.178
ADFI (g)	1,145	1,181	69.38	0.622
G:F	0.57	0.61	0.01	0.018
Overall				
ADG (g)	513	565	26.85	0.088
ADFI (g)	825	859	29.5	0.28
G:F	0.62	0.66	0.02	0.113

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

Control and Photonia treatments. The MetaboAnalyst 5.0 was used to evaluate the impact of Photonia on metabolic pathways and metabolite set enrichment (http://www.metaboanalyst.ca/faces/ModuleView.xhtml).

3 | RESULTS

3.1 | Growth performance

The effect of Photonia on growth performance is shown in Table 2. There was no dead piglet and there was no stressful condition during the whole experimental phase (days 0–48). There was no significant difference in ADG, ADFI, and G:F until Day 14. The ADG and ADFI were not affected by Photonia on days 28 and 48; however, the G:F of pigs was significantly greater ($p < 0.05$) in the Photonia treatment. The overall result showed no difference in ADG, ADFI, and G:F.

3.2 | Blood metabolites, immunoglobulins and cytokine concentrations

There were no differences in blood WBC, lymphocyte, neutrophils, monocytes, eosinophils and basophils among the treatments (Table 3). At Day 28, a higher concentration of IgA ($p < 0.01$) and IgG ($p < 0.01$) were observed in the Photonia pigs (Table 4). At Day 48, the Photonia treatment showed a greater serum IgA ($p < 0.01$) and IgG ($p < 0.05$) compared with the control. The exposure of pigs to Photonia did not affect the concentration of TNF-α and IL-1β at Day 28; however, the concentration of IL-6 was decreased ($p < 0.05$) in the Photonia treatment (Table 5). At Day 48, the concentrations of TNF-α, IL-1β and IL-6 in serum were decreased ($p < 0.05$) in pigs in the Photonia treatment.

TABLE 3 Effects of Photonia on blood profile in weanling pigs.

Item	Control	Photonia	SEM	*p* Value
Day 14				
WBC	25.82	24.86	0.57	0.166
Lymphocytes	61.69	62.47	0.81	0.395
Neutrophils	32.73	34.57	1.06	0.153
Monocytes	6.40	5.53	1.24	0.523
Eosinophils	1.56	1.53	0.11	0.778
Basophils	0.11	0.10	0.04	0.869
Day 28				
WBC	24.91	26.01	2.01	0.614
Lymphocytes	63.57	64.50	0.39	0.074
Neutrophils	36.07	37.52	0.90	0.186
Monocytes	5.60	5.70	0.83	0.910
Eosinophils	1.90	1.73	0.11	0.189
Basophils	0.28	0.26	0.10	0.856

Abbreviations: SEM, standard error of means; WBC, white blood cells.

TABLE 4 Effects of Photonia on serum immunoglobulin levels in weanling pigs.

Item	Control	Photonia	SEM	*p* Value
Day 28 (ng/mL)				
IgA	8.72	10.28	0.29	0.001
IgG	22.59	26.12	0.68	0.001
Day 48 (ng/mL)				
IgA	33.76	58.41	1.08	<0.001
IgG	41.56	45.15	1.48	0.036

Abbreviations: IgA, immunoglobulin-A; IgG, immunoglobulin-G; SEM, standard error of means.

3.3 | Hair cortisol

The effect of Photonia on hair cortisol is shown in Table 6. There were no hair cortisol differences between treatments at day 0, 28, and 48.

TABLE 5 Effects of Photonia on serum cytokine levels in weanling pigs.

Item	Control	Photonia	SEM	*p* Value
Day 28 (pg/mL)				
TNF-α	239	224	29.61	0.628
IL-1β	17.17	14.33	2.39	0.263
IL-6	179	171	2.81	0.023
Day 48 (pg/mL)				
TNF-α	474	428	19.69	0.043
IL-1β	186	155	8.32	0.005
IL-6	323	290	12.11	0.021

Abbreviations: IL-1β, Interleukin-1β; IL-6, interleukin-6; SEM, standard error of means; TNF-α, tumour necrosis factor-α.

TABLE 6 Effects of Photonia on hair cortisol level of weanling pigs.

Cortisol, pg/mL	Control	Photonia	SEM	*p* Value
Day 0	63.51	61.30	7.14	0.763
Day 28	80.05	88.26	11.36	0.491
Day 48	124	151	17.78	0.162

Abbreviation: SEM, standard error of mean.

3.4 | Metabolomics

To analyze the metabolic profile related to Photonia irradiation, a comprehensive metabolome analysis with GC-MS was applied to evaluate the relative abundance of metabolites in the serum. We detected differences in 27 metabolites between the Control and Photonia treatments (Figure 1). The concentrations of Acetate, anethole, gaba, 2-methyl-Z,Z-3,13-octadecadienol, 1-aminopropanol-(2), butyric acid, L-glutamate, vitamin K1, ethanol, iron, hydroxyoxime, tetraacetyl-D-xylonic nitrile, dimethicone, 2-[(trimethylsilyl)oxy]tetradecanoic acid, oxalic acid, N-methylnicotinamide, pyridine, 3-acetoxypentadecane, gamma-aminobutyric acid, acetamide, spermidine, and Pnb-001 were increased in the Photonia treatment, and the concentrations of ethyl 9-octadecenoate, 4-hydroxyphenylethanol, trifluoroacetic acid, *N*-acetyl-D-serine, acetylarylamine were greater in the Control treatment. Principal component analysis segregated the data into two groups based on the principal component 1 (PC1) value (84.7% of the variance; Figure 2), showing that PC1 captured the Photonia variance in the data. According to the change in metabolite levels, metabolic pathways analysis identified the Photonia treatment influenced the D-glutamine, D-glutamate, alanine, aspartate, glutamate and phenylalanine compared with the Control treatment (Figure 3).

4 | DISCUSSION

The growth rate of pigs during the weaning period is a big challenge in pork production. The use of artificial lighting can be a tool to be used in swine production that targets to increase feed consumption and growth performance. LED irradiation is characterized by several physical parameters such as power density, wavelength and duration of irradiation. However, to date, the relationship between the abovementioned factors and immunity or growth response in farm animals remains unclear. The high variability in reports of previous researchers in human-based studies can be in part a result of the difference in suitable parameters. There is a lack of biophotonic research on pigs' growth performance; however, several researchers concluded that LED light improves the growth performance of poultry (Liu et al., 2018; Sharideh & Zaghari, 2017). The greater G:F and a tendency for higher ADG of pigs in the current study may be associated with improved immune status and amino acids metabolism pathways.

Immunoglobulins, produced by WBC, play an important role in immune reactions by the destruction of specific antigens from bacteria or viruses (Asadi & Ferrara, 2021; Balan et al., 2019). Blood immunoglobulin isotypes have been associated with susceptibility to infection (Asadi & Ferrara, 2021; Balan et al., 2019). IgA is the main isotype of immunoglobulin at mucosal surfaces with an important role in protecting the animal against infection (Balan et al., 2019; Lee et al., 2007). The greater IgA and IgG in the Photonia treatment may be responsible for reducing the susceptibility of weaned pigs to diarrhoea and bacterial infections. Then, it may have long-term health-promoting effects through the protection of the intestine and immune status. The reason behind the greater immunoglobulin is unclear, however, unlike UV light, which did not penetrate the hypodermic area, the LED with 710 nm wavelength is able to reach to epidermal layer and interact with circulating lymphocytes in order to affect immunoglobulin production (Van Tran et al., 2021). The therapeutic influence of LED during the acute inflammation period is related to mitochondria activation and stimulating electron transfer along the inner mitochondrial membrane (Van Tran et al., 2021). This reaction increases ATP production resulting in the activation of DNA and RNA replication as well as protein synthesis, and finally cell proliferation (Silveira et al., 2009). Therefore, Photonia can affect immunoglobulin production indirectly by activation of WBC (Lim et al., 2009). Moreover, it has been reported that the porcine immunoglobulin isotypes function is under the influence of protein and amino acids structure including glutamate (Crawley & Wilkie, 2003). The upregulation of amino acid pathways including glutamate may facilitate the production of immunoglobulin in Photonia treatment.

The infrared LED has a therapeutic application because of its effect on oxidative stress and immune-related factors (Oh & Jeong, 2019; Zamanizad et al., 2019). In the current study, there was no statistical difference between the control and Photonia in the stimulation of the innate immune system until Day 28, showing that

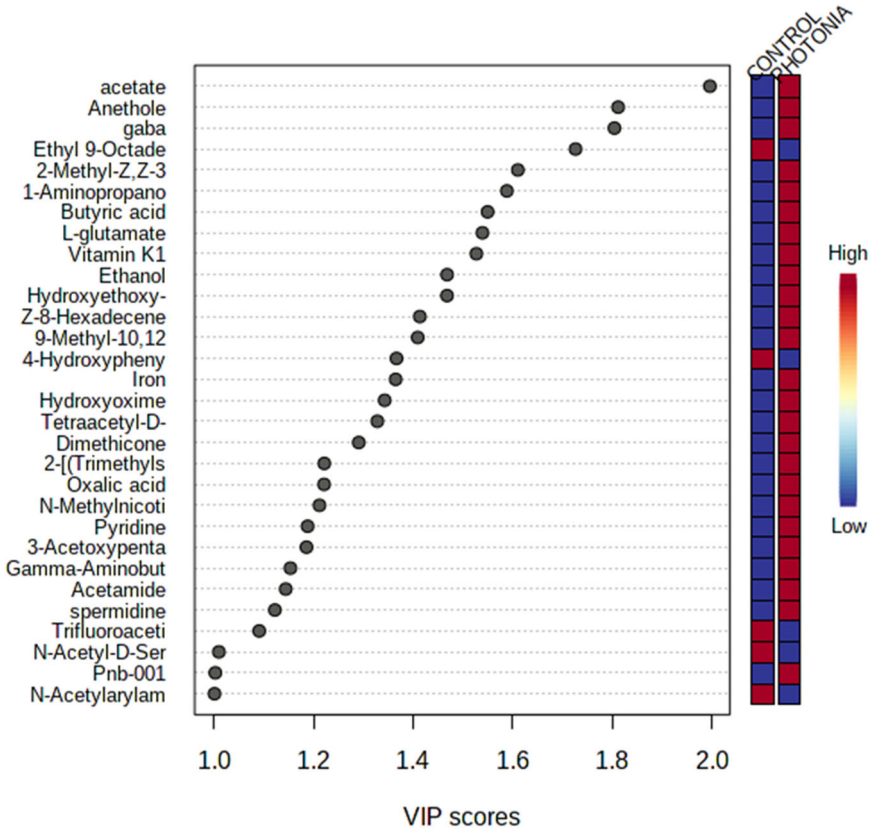

FIGURE 1 Top 27 Significant compounds. Metabolites are accountable for class discrimination with Visual Infusion Phlebitis (VIP) score > 1 between the Photonia and Control treatments. [Color figure can be viewed at wileyonlinelibrary.com]

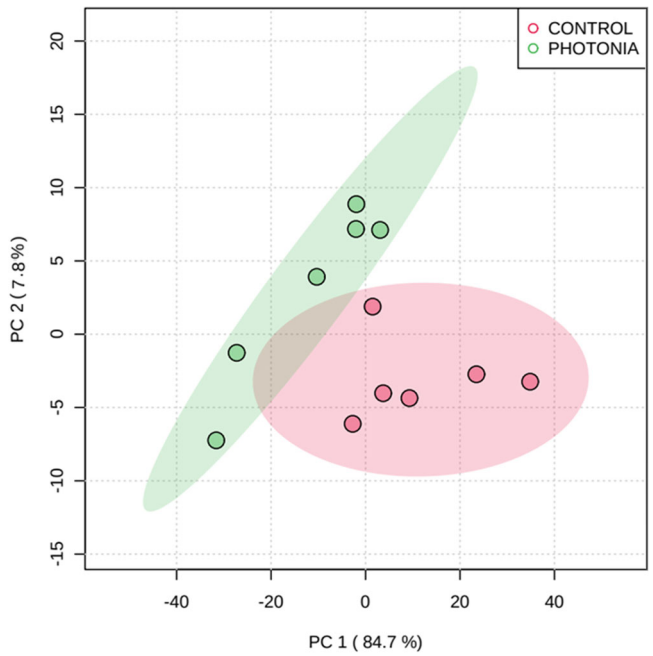

FIGURE 2 Partial least squares projection to latent structures and discriminant analysis based on the serum compounds data. PC, principal component. [Color figure can be viewed at wileyonlinelibrary.com]

Photonia has to be applied for a prolonged period. The TNF-α and IL-1β are classic indicators of innate immune system activation (Hosseindoust et al., 2020). Low-level laser therapy irradiation not only reduces the secretion of IL-1β, TNF-α and interferon (IFN)-γ but also decreases mRNA level and cell proliferation (Lim et al., 2009). Inflammation reactions increase by TNF-α, which contributes to the production of IL-6 in the activation of the acute phase of inflammatory responses (Oliveira et al., 2013). The effects of Photonia on the production of cytokines may depend on the length of exposure. In this study, irradiation of 5.24×10^{-9} W/cm^2 was used to evaluate the anti-inflammatory effects. The positive effect of laser irradiation on anti-inflammatory or immunosuppressants was also noted in human-based experiments (Gavish et al., 2008). However, there is a lack of studies on the immunomodulating and anti-inflammatory roles of biophotons in farm animals. Low levels of IL-1β and IL-6 could regulate immune responses by decreasing inflammatory or immunosuppressant effects (Oliveira et al., 2013). We also speculate that the decreased TNF-α, IL-1β and IL-6 in the irradiated piglets could be because of the upregulation of D-glutamate and phenylalanine metabolic pathways. D-glutamate and phenylalanine decrease the production of several immune-related cytokines (Bhandage et al., 2018). Furthermore, various reports have demonstrated that the release of nitrogen oxide values was exclusively increased by the use of biophoton at λ = 780 nm (Gavish et al., 2008). The result of this study revealed that exposure of pigs to Photonia greatly decreased innate immune stimulation by reducing cytokines production.

The radiation dose and efficiency of tissue metabolism can be evaluated by a multiparametric approach for biomarker discovery. Among the approaches, metabolomics is yet a practical method that includes multiple promising factors to provide a wide range of

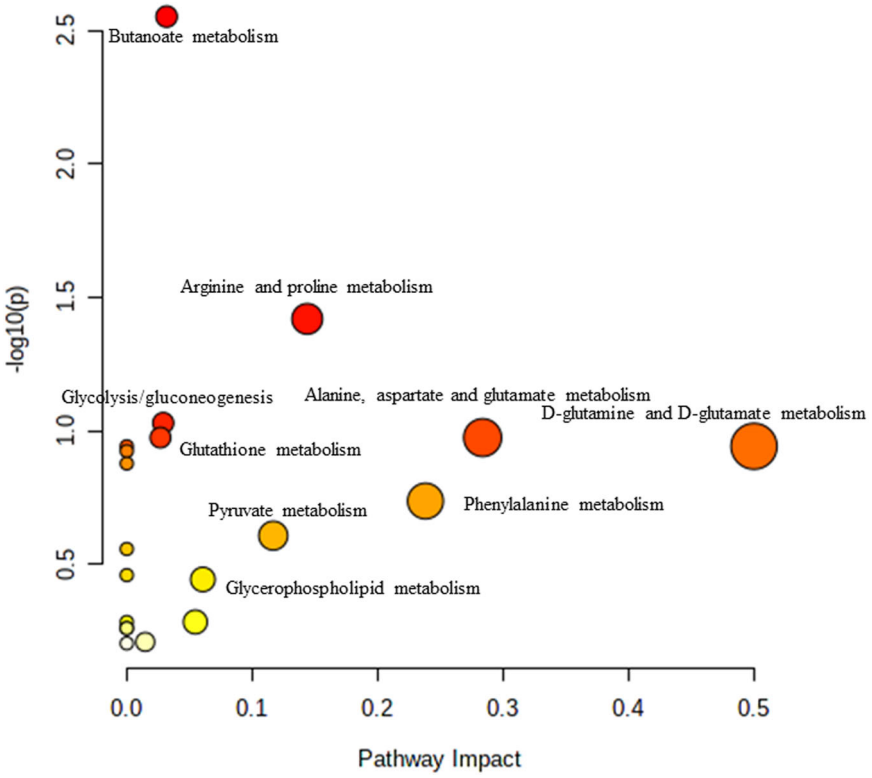

FIGURE 3 Metabolome view map of the differential metabolites (Visual Infusion Phlebitis score > 1, $p < 0.05$) identified in the serum of pigs between the Photonia and Control treatments. The *x*-axis represents the pathway impact and the *y*-axis represents the pathway enrichment. The node colour is based on its *p* value, and the node radius is determined based on the pathway impact values. Larger sizes and darker colours represent higher pathway enrichment and impact values respectively. [Color figure can be viewed at wileyonlinelibrary.com]

small-size molecules in order to reflect the physiological status in a cellular frame. The tendency ($p = 0.09$) of ADG difference between the Photonia and Control group may be related to energy and protein metabolism. The increase of anethole in the blood may be associated with lower anti-inflammatory responses. It has been shown that anethole has high anti-inflammatory and antioxidant effects (Samadi-Noshahr et al., 2021) in mice models. The anethole decreases the production of inflammatory cytokines such as TNF-α and IL-6 (Kim et al., 2017), which is in agreement with the results in the current study. In addition, γ-aminobutyric acid (GABA) is best known as an extracellular signalling molecule which mainly produced in the brain, blood and pancreatic islets and secreted by the pancreatic islet β cells. GABA is a major inhibitory neurotransmitter in regulating 47 kinds of cytokines production including IL-1β, IFN-γ, TNF-α, IL-6 and IL-12 through the cluster of differentiation T4+ cells regulation in humans (Bhandage et al., 2018). The second step is a pointed procedure that clusters the gathered information to determine the pathways. Six amino acid metabolic pathways were influenced by Photonia treatment, and these pathways may play key roles in protecting pigs against inflammatory effects and greater growth performance. These comprise glutamate, phenylalanine, alanine and aspartate metabolism. The higher availability of amino acids encourages protein biosynthesis. The improved amino acid pathways and the potential role of reducing weaning stress are discussed below. Glutamine is an important metabolite in the pathway of D-glutamine, D-glutamate, alanine and aspartate (He et al., 2016), which was observed in the Photonia pigs. Moreover, glutamine is the most abundant building block amino acid for protein synthesis (Wu et al., 2016) that can be responsible for greater ADG in the Photonia treatment. The production of GABA triggers D-glutamate production by activation of GABA transaminase in mitochondria (Bhandage et al., 2018). The high levels of glutamine in the body's amino acid pool increase immunomodulatory effects and macrophage inflammatory responses (Wu et al., 2016). As inflammation is a common issue in pigs during the weaning period with the release of TNF-α, IL-1β and IL-6, the upregulated D-glutamate pathway in pigs in the Photonia treatment may be responsible for the reduction of inflammatory cytokines production. The antioxidant role of D-glutamine and D-glutamate metabolism has already been confirmed, reducing oxidative stress and free radical injury (Bhandage et al., 2018). In addition, the glucogenic roles of glutamine and alanine can be important in supplying glucose in a negative energy balance (Oliveira & Rodrigues, 2021). Phenylalanine metabolism, a substrate of tyrosine, was altered in the Photonia treatment. Tyrosine is associated with several functions including immunity and energy metabolism. The upregulated amino acid pathways may be responsible for a greater G:F ratio and immune status. The results of the current study were achieved based on the experimental situation. Further, studies are required to evaluate the effects of Photonia on a farm scale.

5 | CONCLUSION

In summary, the G:F ratio of weanling pigs was improved by using Photonia. The exposure of pigs to Photonia improved immune status by increasing the concentration of nontargeted immunoglobins and

decreasing acute phase inflammatory cytokines. The positive effects of Photonia were reflected in the activation of amino acid metabolically pathways including D-glutamine, D-glutamate, alanine, aspartate, glutamate and phenylalanine.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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