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Growth Performance and Meat Quality of Growing Pigs Fed with Black Soldier Fly (*Hermetia illucens*) Larvae as Alternative Protein Source

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Abstract: Insects have been used as animal feed protein sources in livestock and poultry breeding, and their impact on pork quality needs to be studied. This experiment mainly explores the effect of adding black soldier flies to the feed on the growth performance and meat quality of pigs. All 24 weaned piglets were randomly divided into three groups, one group was given a normal diet as the control group (C), and the other two groups were supplemented with 4% (T1) and 8% (T2) black soldier flies as an alternative protein source, respectively. Pig growth performance and carcass traits were measured at the end of the 113-day experiment. After euthanizing the pigs, we used metabolomics to detect pig dorsal muscle and qPCR to detect gene expression in dorsal muscle and adipose tissue. For the average daily gain and backfat thickness, T2 group was significantly higher than T1 group and C group ($p < 0.05$). Intramuscular fat content was significantly elevated in the T1 and T2 groups ($p < 0.05$). The metabolomics results showed that there were significant differences in metabolites among the three groups ($p < 0.05$). The addition of black soldier flies could increase the content of some free amino acids, and the content of lipid metabolites also changed significantly ($p < 0.05$). The gene expression of type 1 muscle fibers in the T1 group and the PGC-1α gene expression in the T1 and T2 groups were significantly increased in the dorsal muscle ($p < 0.05$). The results of the present study showed that adding 4% black soldier fly instead of fish meal in the diet of growing pigs can significantly improve meat quality and supplementation of 8% black soldier flies has beneficial effects on growth performance of pigs.

Keywords: black soldier fly; piglets; skeletal muscle; meat quality; metabolomics

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1. Introduction

As the world's population grows, the demand for meat products is increasing. In recent years, the international community has begun to pay attention to the use of insects as animal feed as a means of addressing the global food issue brought on by climate change and population increase [1]. *Hermetia illucens* (Diptera: Stratiomyidae), the black soldier fly (BSF), is a true fly found in tropical and temperate regions worldwide. BSF is rich in crude protein (20–76 g/100 g) and fatty acids (2–50 g/100 g) [2]; its protein and fatty acid content is comparable to soybean meal and soybean oil [3]. Meanwhile, BSF contains large amounts of trace elements such as calcium, zinc, potassium, iron, manganese and phosphorus [4]. Because BSF is rich in nutritional value and contains antibacterial substances such as antimicrobial peptides and lauric acid [3,5], it is used in livestock and poultry breeding.

Many researchers have recently investigated the use of black soldier flies, prepupa powder, fat as protein or fat sources to replace corn, soybean meal and soybean oil partially or totally in animal feed. Broiler growth performance is improved when dried black soldier fly powder is used, and the content of saturated fatty acids in meat is increased [6–8]. The use of black soldier fly powder as a protein source in laying hens can improve egg quality dramatically [9,10]. Soybean meal and soybean oil were partially replaced with defatted black soldier fly larvae. The meat and egg quality of quails can be improved by feeding them with a black soldier fly diet [11]. Meanwhile, several researchers have studied the use of black soldier fly in the diet of fattening pigs. It is worth noting that the black soldier fly larvae's specific nutritional properties have an impact on animal health and may control intestinal microbiota [12]. On the growth of pre-fertilized pigs, many studies have focused on the diversity of intestinal flora and mucosal immunity of black soldier fly larvae [13]. Numerous studies have shown that the intramuscular fat content and fatty acid composition of meat have an important impact on meat quality [14]. The addition of black soldier fly larvae to fattening pig diets has recently been found to increase intramuscular fat content, fat-synthesis-related genes, and myosin heavy chain 2a type muscle fiber mRNA levels; however, the reasons for the changes have not been addressed [15]. There is still a scarcity of information on the properties of muscle fibers in fattening pigs in the developing stage, as well as how the black fly affects muscle fiber differentiation and metabolism in growing pigs.

Skeletal muscle myosin heavy chain (MyHC) subtypes are divided into types 1, 2a and 2b [16]. From the perspective of metabolic classification, type 1 muscle fibers mainly produce ATP by glycolysis to supply energy for skeletal muscle, and are called slow oxidation muscle fibers; type 2 muscle fibers, known as fast glycolytic muscle fibers, are mainly composed of glycolysis as the main energy supply mode [17]. Skeletal muscle with a different proportion of myosin heavy chain will have a vital impact on meat quality. The muscle with a higher proportion of type 1 muscle fiber has better quality and redder color, which is related to the fact that fatty acid oxidation requires a lot of oxygen and contains more myoglobin [18]. Local pig breeds in some parts of the world have better meat quality, which is also related to their special muscle fiber type composition, such as Rongchang pig in China [19].

In this study, muscle metabolomics was examined to investigate the effect of black soldier flies on the metabolite composition of growing pork. While short-chain fatty acids can regulate body metabolism through the role of intestinal flora [20,21], short-chain fatty acids in black soldier fly larvae regulate fatty acid metabolism by activating peroxisome proliferator-activated receptor γ (PPAR-γ) and sterol-regulatory element-binding proteins (SREBP-1) signaling pathways [22]. Transcription of acetyl coenzyme A carboxylase α (ACCα), hormone-sensitive lipase (HSL), fatty acid synthase (FAS), diacylglycerol acyltransferase 1 (DGAT1) and fatty acid transporter 1 (FATP1) is regulated downstream.

We hypothesize that including black soldier fly larvae in the diet of fattening pigs will alter the metabolic status of the pigs during their growth stage, alter the differentiation of different types of muscle fibers in the longissimus dorsi, and alter the rising pork meat quality.

2. Materials and Methods

All experimental procedures were pre-approved by the institutional ethical review committee/institutional review board in accordance with the Helsinki Declaration of 1975 on human experimentation, Office of Research, Zhongkai University of Agriculture and Engineering (20210713-17), Guangzhou, China.

2.1. Animal, Experiment Design and Diets

The purpose of this experiment was to see how varying quantities of black soldier fly affected the growth and meat quality of growing and fattening pigs. In the nursery period (day 28–day 70), the black soldier flies were used to replace the fish meal partially

or completely; during the growth stage (day 70–day 141), the black soldier flies partially replaced the soybean meal. Digestible energy and crude protein content were equalized between the three groups by adjusting the contents of fish meal, soybean meal, corn and soybean oil in both feeding periods. Weaned piglets at 28 days after birth of equivalent weight were chosen. Twenty-four pigs were randomly assigned to one of three treatment groups, with each group divided into eight columns (repetition) and each pig assigned to one of the columns. The control group's basal food (C), the diet supplemented with 4% black soldier fly (T1), and the diet supplemented with 8% black soldier fly (T2) were fed to the three treatment groups, respectively. The two stages' diets met and exceeded the National Research Council (NRC) requirements. The diet plan for the fattening and growth stages is shown in Table 1. Piglets were kept in piglet beds (5 × 5 m) during the growth period, while pigs were housed in environmentally controlled confinement pens with partial concrete slatted flooring during the fattening stage. Throughout the trial, pigs were given unlimited access to water and food. The trial lasted 113 days in total; the specific test cycle map is shown in Figure 1. Pigs' weights were recorded at the start and end of the trial cycle to compute average daily gain (ADG).

Table 1. Pig diet formula.

Items	Weaned Piglet Feed Formula			Growing Piglet Feed Formula		
	C	T1	T2	C	T1	T2
Ingredient (%)						
Corn	52.98	51.53	50.08	57.02	57.16	57.29
Soybean meal	9.00	9.00	9.00	28.00	24.95	21.91
Puffed soybeans	9.00	9.34	9.68	—	—	—
Fish meal	4.00	2.00	0.00	—	—	—
Soy protein concentrate	6.00	6.00	6.00	—	—	—
Whey powder	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	2.00	2.00	2.00	—	—	—
Black soldier fly	0.00	4.00	8.00	0.00	4.00	8.00
Soybean oil	2.00	1.18	0.36	2.00	1.18	0.35
DL-Methionine	0.36	0.38	0.40	0.08	0.09	0.10
Lys-HCl (78%)	0.80	0.82	0.84	0.18	0.18	0.19
Threonine (98%)	0.38	0.38	0.38	0.03	0.03	0.03
Tryptophan (99%)	0.08	0.08	0.08	0.00	0.01	0.02
Limestone	0.65	0.44	0.22	0.78	0.54	0.30
Dicalcium phosphate	1.10	1.20	1.30	0.53	0.48	0.43
Choline chloride	0.20	0.20	0.20	0.08	0.08	0.08
Salt	0.45	0.45	0.45	0.30	0.30	0.30
Premix [1]	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100	100	100
Calculated content						
Crude protein (%)	19.15	19.15	19.15	18.09	18.09	18.09
Digestible energy (MJ/kg)	14.59	14.59	14.59	14.36	14.36	14.36
Ether extract	6.65	7.07	7.55	4.54	5.39	6.15
Ca	0.80	0.80	0.80	0.60	0.60	0.60
P	0.65	0.65	0.65	0.52	0.52	0.52
AP	0.44	0.41	0.39	0.25	0.23	0.21
Lysine	1.35	1.35	1.35	0.99	0.99	0.99
Methionine + cysteine	0.76	0.75	0.73	0.58	0.58	0.58
Threonine	0.80	0.81	0.80	0.62	0.62	0.62
Tryptophan	0.22	0.22	0.22	0.19	0.19	0.19

[1] Provided per kilogram of complete diet: vitamin A, 15,000 IU; vitamin D3, 3000 IU; vitamin E, 150 mg; vitamin K3, 3 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 0.03 mg; niacin, 45 mg; vitamin C, 250 mg; calcium pantothenate, 9 mg; folic acid, 1 mg; biotin, 0.3 mg; choline chloride, 500 mg; Fe ($FeSO_4 \cdot H_2O$), 170 mg; Cu ($CuSO_4 \cdot 5H_2O$), 150 mg; I (KI),0.90 mg; Se (Na_2SeO_3), 0.2 mg; Zn ($ZnSO_4 \cdot H_2O$), 150 mg; Mg (MgO), 68 mg; Mn($MnSO_4 \cdot H_2O$), 80 mg; Co ($CoCl_2$), 0.3 mg. Abbreviations: C, control group; T1, supplement 4% black soldier fly; T2, supplement 8% black soldier fly.

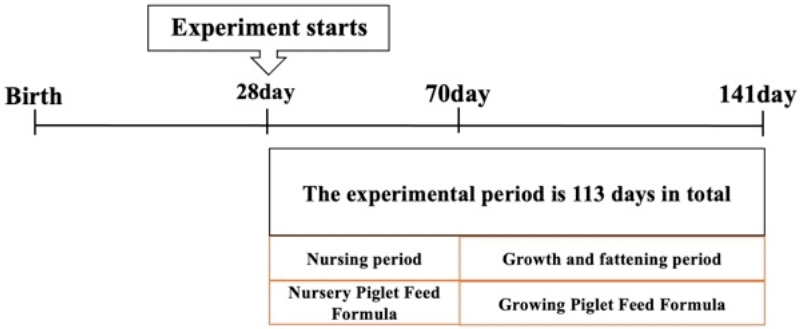

Figure 1. Schematic diagram of the experimental cycle.

2.2. Sample Collection

The pigs were slaughtered at the slaughter site using carbon dioxide anesthesia at the end of the experiment. We separated the left and right sides of the carcass and measured three spots with a Vernier caliper: first rib, the last rib and the waist backfat thickness, according to Chen et al. [23]. For histology and gene expression analysis, sections of white adipose tissue were removed and kept in formalin solution and at −80 °C, respectively. The right loin muscle was completely peeled, and the marble pattern score was determined using Goldberg [24].The area eye muscle was determined using Photoshop software (Adobe Systems Software Ireland Ltd., San Jose, CA, USA) after a complete cross-section of the longissimus dorsi at 10th intercostals was accurately represented. The 15 cm loin muscle of the left 13th rib was stored at 4 °C to measure pH, color, shear force, drip loss and cooking loss. The 150 g of loin muscle was taken from the right side of the carcass before the 10th rib and was measured immediately for IMF, sensory evaluation and metabolite detection. After the 10th rib of the right loin muscle, 1 cm thick loin muscle samples were obtained and stored at −80 °C for RNA extraction.

2.3. Measurement of Meat Quality

To test the pH and color of meat, the meat was cut into small pieces. A portable pH meter was used to test the pH of the meat samples after 45 min, 24 h and 48 h (Matthaeus pH Star, Germany). At 45 min after slaughter, the color attributes of the meat, including brightness (L*), redness (a*) and yellowness (b*), were assessed with a handheld colorimeter (CR-300 Osaka, Tokyo, Japan). Following the approach of Li et al. [25], 2 × 2 × 2 cm pieces of meat were extracted from the left loin muscle (approximately 6–8 g in weight) after 45 min of slaughter, and four pieces of meat were selected from each sample. The meat was hung on the hook and wrapped in a sealed bag after weighing (*m1*). The meat was chilled in a refrigerator at 4 °C for 24 h. The meat block was taken from the sealed bag after 24 h, and the water on the top was dried with absorbent paper and weighed as *m2.*

$$Drip\ loss = \frac{m1 - m2}{m1} \times 100\%$$

To pre-treat the samples, the meat pieces were weighed and heated in an 80 °C water bath until the core temperature reached 70 degrees Celsius. The shear force and cooking loss were measured after weighing (*m4*). The meat core was inserted into the shear force tester, and its shear force was measured at a speed of 3 mm/s.

$$Cook\ loss = \frac{m3 - m4}{m3} \times 100\%$$

An IMF test kit was used to determine the amount of intramuscular fat (IMF) (RF-Z702 Rui Pan, Shanghai, China).

2.4. Tissue Histology

To replace part of the soybean meal and soybean oil or fish meal, 4% and 8% black soldier fly larvae meal were added. We performed a histological study of backfat and evaluated fat cell density and volume to see if fat cell volume growth causes backfat

thickening. The diameter and density of adipocytes were assessed during a histological examination of subcutaneous adipose tissue in the back, using the procedure described previously [26]. Briefly, fresh white adipose tissue was placed in 4% paraformaldehyde, allowed to stand for 24 h, embedded in paraffin, sliced with a thickness of 5 μm, and fixed, dehydrated, and stained. Image analysis was performed using Image J (NIH Image J system, Bethesda, MD, New York, NY, USA) [27].

2.5. Meat Metabolomics

On the Xplore MET platform, untargeted metabolomic profiling was carried out (Metabo-Profile, Shanghai, China). The frozen samples were combined with 25 mg of pre-chilled zirconium oxide beads and 10 μL of the internal standard after being harvested and kept in an Eppendorf Safe Lock microcentrifuge tube. For automatic homogenization, to each aliquot of 50 μL, 50% pre-chilled methanol was added (BB24, Next Advance, Inc., Averill Park, NY, USA). A time-of-flight mass spectrometry (GC-TOF/MS) system (Pegasus HT, Leco Corp., St. Joseph, MO, USA) with an Agilent 7890B gas chromatography system and a Gerstel multipurpose sample MPS2 with dual heads were used (Gerstel, Muehlheim, Germany).

2.6. RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from loin muscle samples and subcutaneous adipose tissue using the Trizol reagent (Takara Biotechnology, Dalian, China). By spectrophotometry, Nano DropND2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA) was used to measure RNA concentration, and the purity of RNA was assessed at 260:280 nm (1.8–2.0). The cDNA was synthesized from about 1–2 μg RNA using a reverse transcription kit. The kit was used to perform real-time PCR (TaKaRa, Dalian, China). The primer information used in the experiment is shown in Table 2. The housekeeping gene β-actin was used to normalize the expression level of the target gene.

Table 2. Primer sequences used in quantitative real-time polymerase chain reaction.

Gene	Primer Sequences (5′–3′)	Size (bp)	NCBI Gene ID
MyHC 1	F: GAGGAAGCGGAGGAACAATCCA R: GACCTGGGACTCAGCAATGTCA	105	NM_001104951.2
MyHC 2A	F: GATGGAGATCGACGACCTTGCT R: CTGCTGCTCTTCCTCCTTGGAT	127	XM_021066217.1
MyHC 2B	F: CGCCAAGCTACTGAGGCAATAA R: GTTCCACCATGGCCAGTTGTTC	127	XM_021066036.1
PGC-1α	F: CGCAAGCTTCTCTGAGCTTCTTT R: GGATACACTTTGCGCAGGTCGAA	188	XM_021100444.1
PPAR-γ	F: CCAGCATTTCCACTCCACACTA R: GACACAGGCTCCACTTTGATG	124	XM_005669788.3
FAS	F: AGCCTAACTCCTCGCTGCAAT R: TCCTTGGAACCGTCTGTGTTC	196	NM_001099930.1
ACCα	F: AGCAAGGTCGAGACCGAAAG R: TAAGACCACCGGCGGATAGA	169	XM_021066238.1
HSL	F: GCAGCATCTTCTTCCGCACA R: AGCCCTTGCGTAGAGTGACA	195	NM_214315.3
DAGT1	F: GACAAGGACGGACACGACGATG R: AATTCAGGATGCCACGGTAGTTGC	118	XM_005655311.3

MyHC, myosin heavy chain; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PPAR-γ, peroxisome proliferator-activated receptor γ; FAS, fatty acid synthase; ACCα, acetyl coenzyme A carboxylase α; HSL, hormone-sensitive lipase; DAGT1, diacylglycerol acyltransferase 1.

2.7. Statistical Analysis

All data between groups were tested for normal distribution by the Shapiro–Wilk test before being tested for significance. All normally distributed data were tested using one-way

ANOVA. Differences among treatments were compared using Tukey's post hoc test, which was conducted in GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA). Data were expressed as the mean ± SEM. Differences were identified as significant at $p < 0.05$.

Partial least square discriminant analysis (PLS-DA) is a generalized multiple regression method that can deal with multiple collinear X (herein mass spectral data) and Y (classes) variables. Models and statistical algorithms used in experiments are adapted from the widely used statistical analysis software packages in R studio (http://cran.r-project.org/), accessed on 18 June 2021. The optimal choice of statistical methods is driven by the data and the project goals.

3. Results

3.1. Growth Performance, Carcass Traits and Analysis of Tissue Histology

The average daily gain (ADG) of the T2 group was significantly higher than that of the C and T1 groups (Table 3) ($p < 0.05$). In terms of carcass features, the initial intercostal backfat thickness of the T2 group was significantly higher than that of the C and T1 groups ($p < 0.05$), whereas the final intercostal and waist backfat thickness was not statistically different. The C group had a significantly larger tenth intercostal muscle area than the T1 and T2 groups ($p < 0.05$). Because the thickness of back adipose tissue increased significantly after adding black soldier fly to the diet of growing pigs, the adipose tissue was analyzed histologically, and the diameter of adipocytes was counted. Adipocyte diameter was substantially smaller in the C group than in the T1 and T2 groups (Figure 2) ($p < 0.05$).

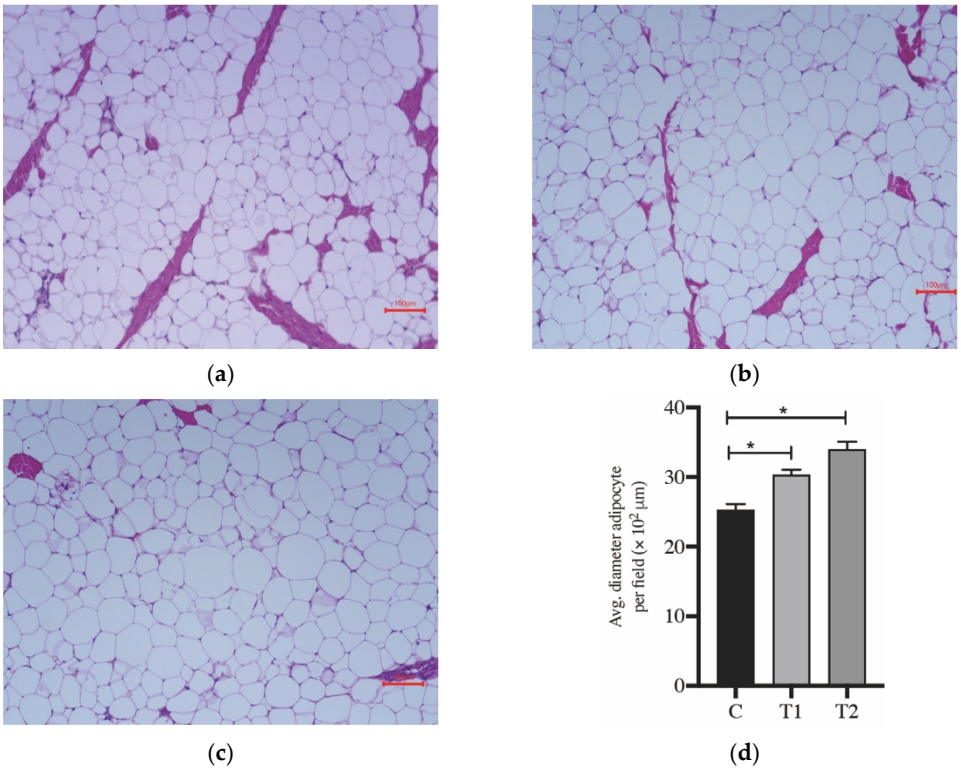

Figure 2. Representative H&E staining of white adipose tissue. The results of hematoxylin-eosin staining of pig dorsal white adipose tissue in groups C (**a**), T1 (**b**) and T2 (**c**) are shown in the figure; the adipocyte diameter was the largest in group T2, followed by group T1, and was the smallest in group C. The findings of the statistical analysis revealed that there were significant differences between the groups (**d**) ($p < 0.05$). Statistical data were given as mean ± standard error, $n = 8$. Significance was calculated using one-way ANOVA and Tukey's post hoc test. Diets with black soldier fly treatments have distinct bars with various letters ($p < 0.05$). Each scale bar is 100 µm long. * Indicates that there is a significant difference between the different treatment groups ($p < 0.05$).

Table 3. Growth performance and carcass traits during the experiment.

Items	C	T1	T2	*p*-Value
Initial body weight (kg)	7.82 ± 0.23	7.43 ± 0.37	7.62 ± 0.29	0.597
Final body weight (kg)	63.98 ± 2.53	60.94 ± 2.70	66.38 ± 3.51	0.427
Average daily gain (kg/d)	0.47 ± 0.01 ^{b}	0.46 ± 0.02 ^{b}	0.56 ± 0.03 ^{a}	0.029
First intercostal backfat, (cm)	16.13 ± 1.48 ^{b}	17.79 ± 2.05 ^{b}	24.04 ± 1.82 ^{a}	0.024
Last intercostal backfat (cm)	12.76 ± 0.51	11.87 ± 0.65	13.97 ± 0.72	0.318
Lumbar backfat, (cm)	8.83 ± 0.48	8.23 ± 0.43	10.82 ± 0.99	0.364
Loin eye area, (cm)	52.85 ± 2.62 ^{a}	43.30 ± 0.95 ^{b}	45.69 ± 1.59 ^{b}	0.012

Data are means for 8 replicates of 1 pig/replicate. Abbreviation: C, control group; T1, supplement 4% black soldier fly; T2, supplement 8% black soldier fly. All data are expressed as mean ± standard error. All data were tested for significance using one-way ANOVA and Tukey's post hoc test. Differences were identified as significant at $p < 0.05$. ^{a,b} Bars with different letters are different among diets with black soldier fly treatments.

3.2. Meat Quality

Meat quality is reflected in many aspects, including physical and sensory properties. However, due to the low age of pigs selected in this experiment, they do not reach the age of listing, and the focus is on the meat quality changes in the long term, so only physical properties are detected, including dripping loss of meat, intragranular fat, meat color and pH changes. Intramuscular fat (IMF) is one of the important traits of pork quality. Properly increasing intramuscular fat content is beneficial to improve meat quality. The intramuscular fat content (IMF) of the T1 group and T2 group increased in comparison to the C group ($p < 0.05$) after the addition of black soldier fly larvae (Table 4). In terms of drip loss, the T1 group and T2 group were much less than the C group ($p < 0.05$). On the other hand, adding black soldier fly larvae to the diet had no effect on meat pH at 45 min, 24 h and 48 h, color L*, a*, b* at 45 min, marbling score, or shear force and cooking loss.

Table 4. Longest back muscle quality in growing pigs.

Items	C	T1	T2	*p*-Value
IMF%	1.49 ± 0.01 ^{b}	1.74 ± 0.05 ^{a}	1.65 ± 0.03 ^{a}	0.013
$pH_{45\ min}$	6.62 ± 0.06	6.52 ± 0.07	6.52 ± 0.11	0.961
$pH_{24\ h}$	5.57 ± 0.02	5.56 ± 0.02	5.53 ± 0.02	0.834
$pH_{48\ h}$	5.52 ± 0.03	5.47 ± 0.04	5.52 ± 0.03	0.748
$L^{*45\ min}$	39.74 ± 1.23	39.24 ± 0.79	39.60 ± 1.00	0.931
$a^{*45\ min}$	5.59 ± 0.63	6.02 ± 0.87	5.62 ± 0.82	0.747
$b^{*45\ min}$	3.65 ± 0.39	3.16 ± 0.31	3.43 ± 0.37	0.712
Marbling scores	2.43 ± 0.22	2.75 ± 0.16	2.60 ± 0.40	0.234
Shear force, (N)	70.27 ± 4.35	68.20 ± 4.26	73.03 ± 12.46	0.546
Cooking loss, %	0.30 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.638
Drip loss, %	0.17 ± 0.01 ^{a}	0.12 ± 0.01 ^{b}	0.13 ± 0.02 ^{b}	0.026

Data are means for 8 replicates of 1 pig/replicate. Abbreviation: C, control group; T1, supplement 4% black soldier fly; T2, supplement 8% black soldier fly. IMF, intramuscular fat content. All data are expressed as mean ± standard error. Data were processed using one-way ANOVA and Tukey's post hoc test. Differences were identified as significant at $p < 0.05$. ^{a,b} Bars with different letters are different among diets with black soldier fly treatments.

3.3. Detection of Loin Muscle Metabolomics

A total of 149 metabolites were examined in the loin muscle of growing pigs in the three dietary treatments, including fatty acids, amino acids, nucleotides, lipids, hydrocarbons, alkyl amines and organic acids. The metabolites of the three dietary treatments were subjected to principal component analysis (PLS-DA), and the results revealed a significant separation between the metabolites of groups C, T1 and T2 (Figure 3a). Non-targeted metabolomics was used to analyze the loin muscle tissue of growing pigs in three dietary treatment groups, and a total of 15 different metabolites were screened (Figure 3b). These differential metabolites are mainly concentrated in six kinds of substances, including: alcohols, alkylamines, amino acids, carbohydrates, fatty acids, inorganic oxide, lipids and vitamins. It is worth noting that some substances in these metabolites show regular changes with the addition of black soldier fly, such as sarcosine and glutamic acid in amino acids; elaidic acid and oleic acid in fatty acids; fructose 6-phosphate in carbohydrates. The content

of sarcosine ($p = 1.1 \times 10^{-2}$), glutamic acid ($p = 3.2 \times 10^{-2}$), elaidic acid ($p = 3.8 \times 10^{-2}$) and oleic acid ($p = 8.3 \times 10^{-3}$) was significantly increased and the content of fructose 6-phosphate ($p = 4.5 \times 10^{-2}$) was decreased in the diet of growing pigs (Figure 3c). In Table 5, all detected free amino acids are sorted in the table according to their taste.

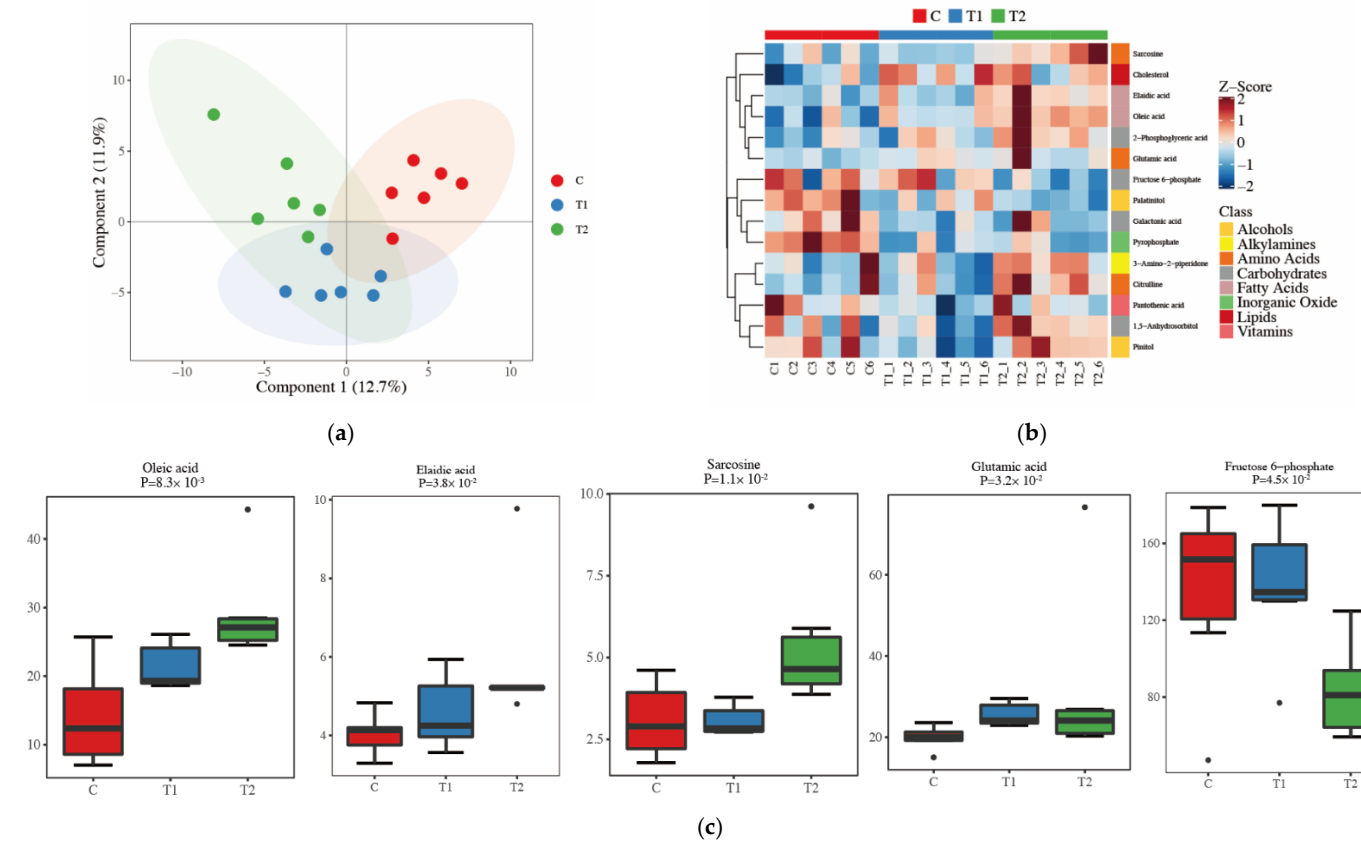

Figure 3. PLS-DA plot of the loin muscle metabolite in three groups (**a**). A total of 15 different metabolites were screened by metabonomics analysis (**b**). Carbohydrates and inorganic oxides were reduced in the screened differential metabolites when black soldier flies were given lipids and free amino acids, different colors represent different treatment groups: red represents group C, blue represents group T1, and green represents group T2. (**c**); $n = 6$.

Table 5. Levels of major free amino acids in loin muscle of growing pigs in each group.

Amino Acids	Taste Attribute	C	T1	T2	*p*-Value
Sarcosine	Swt (+)	3.08 ± 0.47 ^{b}	3.07 ± 0.19 ^{b}	5.46 ± 0.88 ^{a}	0.011
Glutamic acid	Uma (+)	19.90 ± 1.18 ^{b}	25.50 ± 1.21 ^{a}	32.06 ± 8.98 ^{a}	0.032
Asparagine	Uma (+)	8.38 ± 0.52	8.31 ± 0.53	9.56 ± 0.48	0.186
Threonine	Swt (+)	30.6 ± 3.17	26.5 ± 3.10	31.39 ± 4.42	0.631
Serine	Swt (+)	38.43 ± 4.20	39.76 ± 3.17	48.27 ± 5.62	0.331
Glycine	Swt (+)	305.99 ± 24.88	301.98 ± 27.98	297.93 ± 22.44	0.688
Alanine	Swt (+)	804.37 ± 45.79	791.35 ± 35.87	860.86 ± 40.10	0.455
Citrulline	Swt (+)/Bit (−)	15.93 ± 1.67 ^{b}	14.41 ± 1.25 ^{b}	19.64 ± 1.25 ^{a}	0.028
Cysteine	Bit (−)	2.10 ± 0.40	1.41 ± 0.13	2.28 ± 0.49	0.347
Valine	Bit (−)	96.09 ± 13.12	87.79 ± 12.97	108.62 ± 13.81	0.550
Isoleucine	Bit (−)	40.69 ± 9.14	29.73 ± 5.57	40.27 ± 8.37	0.547
Leucine	Bit (−)	91.73 ± 7.83	94.39 ± 15.39	106.66 ± 8.81	0.385
Tyrosine	Bit (−)	62.45 ± 3.36	55.32 ± 5.81	57.18 ± 5.65	0.590
Phenylalanine	Bit (−)	32.47 ± 1.43	29.56 ± 2.71	35.01 ± 2.30	0.250
Lysine	Bit (−)	41.67 ± 11.15	32.20 ± 6.47	44.72 ± 8.70	0.599
Histidine	Bit (−)	16.04 ± 2.20	16.22 ± 2.12	19.34 ± 1.65	0.446

Data are means for 8 replicates of 1 pig/replicate. Abbreviation: C, control group; T1, supplement 4% black soldier fly; T2, supplement 8% black soldier fly. Swt, sweet. Bit, bitterness. Uma, umami. All data are expressed as mean ± standard error. Data were processed using one-way ANOVA and Tukey's post hoc test. Differences were identified as significant at $p < 0.05$. ^{a,b} Bars with different letters are different among diets with black soldier fly treatments.

3.4. Expression of Genes Related to Fatty Acid Metabolism and Muscle Fiber Type

As the metabolite types and levels of loin muscle changed significantly (Figure 3), it was observed that the backfat thickness of T1 group and T2 group increased significantly (Table 3), and the expression of related genes was detected (Figure 4). In loin muscle tis-sue, the expression of MyHC gene in T1 group was significantly higher than that in C group and T2 group ($p < 0.001$), and the expression of PGC-1α gene in T1 group and T2 group was significantly higher than that in C group ($p < 0.05$). The expression of PPAR γ in white adipose tissue in group C was significantly lower than that in T1 ($p < 0.01$) and T2 groups ($p < 0.05$). The expression levels of MyHC I in the T1 group were higher than in the C and T2 groups. It should be noted that these results are different from those of Yu, Li, Chen, Rong, Wang, Li and Ma [15]. However, the pigs used in this experiment have a shorter growth cycle and fewer days of age than those used in the experiment of Yu et al. Thus, the results may be different.

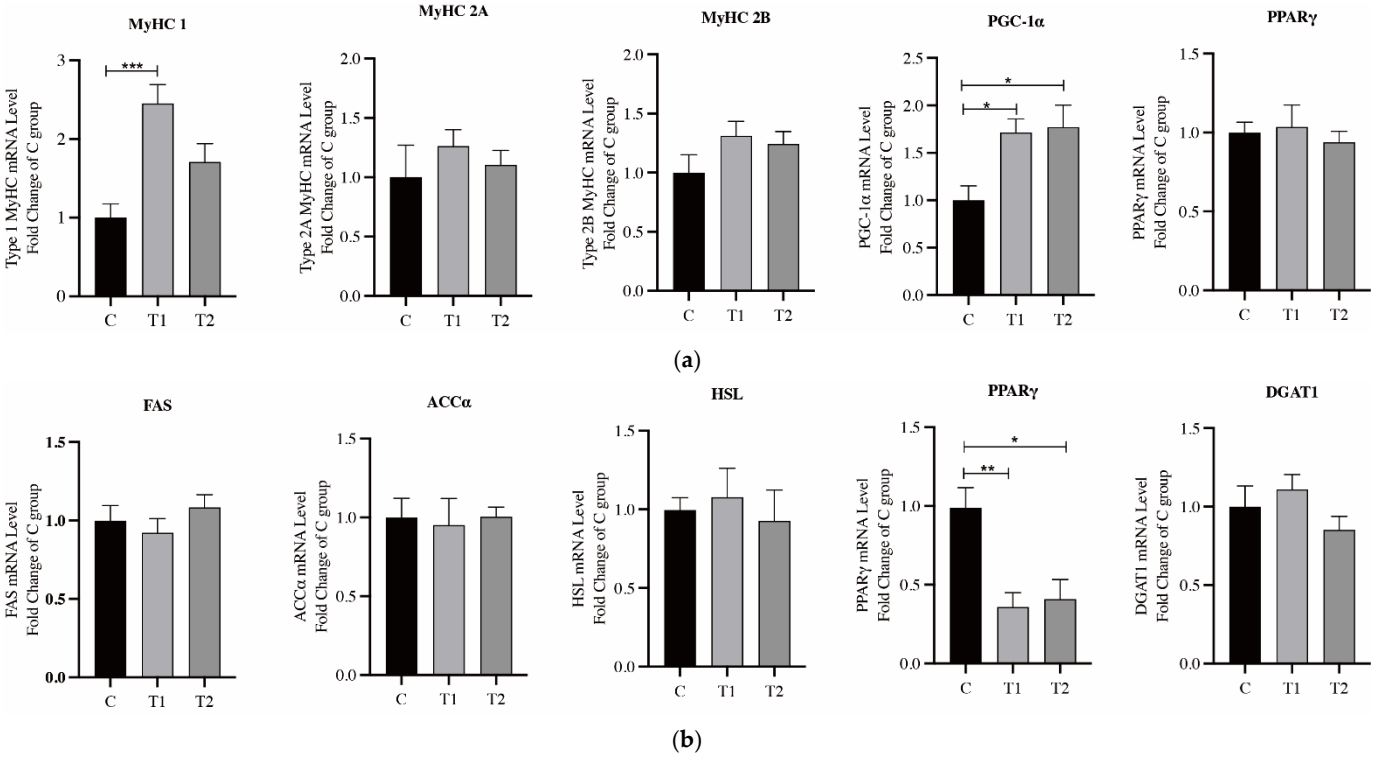

Figure 4. T-PCR was used to detect the expression of myosin heavy chain (MyHC) I, IIa, IIb, peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), peroxisome proliferator-activated receptor γ (PPAR-γ) in muscle (**a**) and fatty acid synthase (FAS), acetyl coenzyme A carboxylase α (ACCα), hormone-sensitive lipase (HSL), PPAR-γ, diacylglycerol acyltransferase 1 (DGAT1) in adipose tissue (**b**). Diets with black soldier fly treatments ($p < 0.05$) had different bars with various letters. Statistical data were given as mean ± standard error, $n = 8$. One-way ANOVA and Tukey's post hoc test were used. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$.

4. Discussion

In recent years, black soldier fly larvae have been widely employed as an appropriate single white source for insect species in the feed of fish [28,29], poultry [30] and pigs [31]. It can not only be utilized as a raw material to meet the fundamental energy needs of cattle, but it also contains lauric acid [32], antimicrobial peptides and chitin [33] that can help animals grow and produce more efficiently. The effects of black soldier fly larvae on growth performance, carcass traits and meat quality in growing pigs were investigated in this study. According to our findings, feeding with black soldier fly larvae increased the volume of subcutaneous fat cells, increased backfat thickness and reduced eye muscle area. Meat quality was significantly improved by increasing intramuscular fat content and myosin

heavy chain subtype. The meat quality of growing pigs was also increased by feeding with 4% black soldier fly larvae.

The average daily gain of the T2 group was significantly higher than that of the T1 and C groups. In order to meet the nutritional requirements of NRC, the diets of growing pigs were formulated using equal digestible energy (DEKJ/kg) and ileal digestible amino acids among the three groups. Simultaneously, the expression of the PPAR-γ pathway and genes involved in fat synthesis and metabolism in adipose tissue was analyzed, and it was discovered that PPAR-γ was downregulated. These alterations may cause lipid buildup in the rear adipose tissue, resulting in a thicker phenotype of backfat. As a result of the high levels of fatty acids in the diet, the expression of the FAS gene and the ACCα is reduced [34].

Meat quality is the most important factor influencing consumer perceptions of meat. Physical qualities of meat, such as shear force, drip loss, PH45 min, PH24 h and color L* a*, b*, as well as pH and intramuscular fat content, are most commonly used to assess meat quality. Previous research has shown that the composition of muscle fiber types is closely related to the physical properties of meat [35]. The expression of type 1 muscle fibers representing slow muscle fibers is positively correlated with the myoglobin content and pH45 min and pH24 h value of meat quality [6,36]. The sensory indexes of meat, such as juiciness, tenderness and flavor, are affected by the physical qualities of the meat. For instance, meat juiciness and tenderness are positively connected with intramuscular fat concentration. Intramuscular fat concentration was also found to be linked to distinct subtypes of myosin heavy chain. The muscle with a higher fraction of type 1 muscle fibers has a higher intramuscular fat content than type 2 muscle fibers [37]. The intramuscular fat level of the T1 group and T2 group showed an increasing trend when compared to the C group. The changes in intramuscular fat content and muscle fiber types are consistent with previous explanations.

Furthermore, a higher glutamate level in T2 muscle tissue compared to C muscle tissue may trigger the expression of some fatty acid synthesis genes, such as FAS and PPAR-γ [38]. Many factors influence the amount of meat that drips out. The length of sarcomere shortening and the rate of pH drop are two of them (whether PSE or DFD meat is formed). Furthermore, the drip loss of pork is linked to the metabolic properties of animal muscle, muscle fiber characteristics and the degree of cytochrome P450c21 (CYP21) protein expression level [39]. According to the study, the drip loss in the T2 group was much lower than in the C group. Muscle is employed for metabolomics detection and related gene expression abundance detection to further establish if the drip loss of meat and change in intramuscular fat are related to the metabolic state of muscle fiber characteristics.

Myosin heavy chains have different subtypes including types 1, 2a and 2b, which are associated with metabolic profiles [40]. Muscles with a larger concentration of type 1 muscle fibers are more oxidized and have higher mitochondrial density [41], which is good for reduced fatigue during continuous contraction and is more conducive to the oxidation of fatty acids [42]. Slow oxidation muscle fibers are the usual name for this type of muscle fiber [40]. Type 2 muscle fibers, also known as fast glycolysis muscle fibers, are primarily produced by glycolysis as the primary source of ATP, have lower mitochondrial density, and experience more fatigue [43]. Different metabolic characteristics lead to the appearance of different types of metabolites, resulting in differences in meat quality. For a slow oxidizing muscle with a high proportion of type 1 muscle fibers, because its ATP is mainly provided by fatty acid oxidation rather than glycolysis, the level of lactic acid in the muscle is low, and the decline rate of pH value after slaughter is slower than that of fast glycolytic muscle, so it is more difficult to form pale soft exudative (PSE) meat. Therefore, increasing the proportion of slow-twitch muscle fibers is of great significance for improving meat quality.

A variety of endogenous or exogenous substances regulate the expression of the myosin gene in muscle fibers during myofibers. Some of these fatty acids can also influence myosin gene expression: in C2C12 cells, oleic acid increased myoblast globulin heavy chain

1, mitochondrial mass and respiration. Lauric acid, as a short-chain fatty acid, can activate the TLR4 pathway, causing genes involved in glycolysis to overexpress, promoting the formation of glycolytic muscle fibers [44]. On the other hand, several investigations have found that overexpressing some essential genes involved in muscle fiber development promotes the formation of slow oxidized muscle fibers. The peroxisome proliferator-activated receptor-δ (PPAR-δ) and its coactivator play a key role in the transformation and differentiation of various types of muscle fibers. Overexpression of PGC-1α in skeletal muscle can increase mitochondrial respiration and β-oxidation of fatty acids, causing muscle fibers from pigs and mice to convert into slow oxidative muscle fibers [45]. The experimental results revealed that the expression of PGC-1α in the muscles of the T2 group and T1 group was higher than that of the C group. This demonstrates that the addition of black soldier fly larvae has a positive impact on pork quality.

The results of metabolomics suggest that the metabolic energy supply mode of loin muscle is mainly fatty acid oxidation rather than glycolysis after adding black water gadfly to the diet of growing pigs. Among the differential metabolites, fatty acids increased and carbohydrates decreased with the addition of black water gadfly. These results were also verified in the gene expression of loin muscle, PGC-1α. The increase in gene expression indicates an increase in the number and quality of mitochondria. However, these are only indirect results, which may need more accurate results to be confirmed in the future. At the same time, it also provides a certain direction for future research. At the same time, metabonomics analysis also showed that black soldier fly has the potential to directly improve the meat quality of growing pigs, such as significantly increasing the level of some free amino acids [46].

Overall, these results indicate that the addition of black soldier flies to growing pig diets can significantly improve meat quality, which is partly caused by changes in muscle metabolic status and metabolites and changes in muscle fiber composition.

5. Conclusions

In conclusion, in this study, the meat quality phenotype of growing pigs was examined. At the same time, this work attempts to analyze the changes of animal meat quality from the perspective of metabolism and incorporate the analysis of gene expression to explain the reasons for the changes in meat quality. The results showed that adding black soldier flies to growing pig feed could significantly improve the quality of pork, increase the amount of individual amino acids in muscles, and increase gene expression of slow-twitch muscle fibers.

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Data Availability Statement: The data used to generate the results in the paper will be shared upon reasonable request to the corresponding author.

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