Effect of *Lactobacillus Plantarum* on the Growth and Development and Gut Microbial Diversity of *Spodoptera Litura*

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7

Effect of *Lactobacillus plantarum* on the growth and development and gut microbial diversity of *Spodoptera litura*

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Abstract

Background: The main challenge for agricultural research is the determination of the best method for efficient and economical management of insects. Research on the possibility of regulating insect development by targeting the intestinal microbial community is in its early stages; however, the use of microorganisms to control the composition of the host's intestinal microbes and to affect its physiological functions has garnered considerable attention.

Results: This study evaluated the effects of probiotics (*Lactobacillus plantarum*) isolated from the intestine of *Spodoptera litura* (Fabricius) on the growth and development of the host and the diversity of its intestinal microbes. The results of the study showed that the larvae of *S. litura* fed with artificial diets with different feed units could proceed towards the development of generations normally. Compared with the control responses, after ingesting *L. plantarum*, larval gut sucrase and other digestive enzyme activities increased, growth and development accelerated, fecundity generally increased, and there was a significant change in the female-male ratio. Additionally, differences in

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1 microbial abundance and diversity were found in the gut of *S. litura* larvae fed with diets supplemented
2 with *L. plantarum* and without *L. plantarum*.

3 **Conclusions:** These findings indicate that supplementation of feed with *L. plantarum* can
4 effectively affect the growth and development of the host and the composition of the intestinal flora,
5 thereby providing useful applications in research regarding pest management.

6 **Keywords:** *Spodoptera litura*, *Lactobacillus plantarum*, enzyme activity, nutritional physiology, gut
7 microflora

8 **Background**

9 There are several types of insects with different morphologies, and insects are the most widely
10 distributed animal group in the world. Insect gut microbes are an important part of the symbiotic
11 system of insects and microbes formed during their long-term co-evolution (Paniagua Voirol 2018),
12 and they play an irreplaceable role in the evolution of insect diversity and the adaptability of the host
13 environment. Limited by the difficulty of separating and cultivating insect intestinal flora *in vitro*, the
14 diversity of insect intestinal microorganisms and related research continues to progress slowly;
15 however, in recent years, with the widespread application of 16S high-throughput sequencing in insect
16 research, a better understanding of the diversity of insect gut microbiota has been achieved. Multiple
17 studies have shown that insect gut microbes are crucial to the development, digestion, metabolism,
18 reproduction, immunity, and several physiological functions of their host (Li 2020). Studies have
19 shown that *Burkholderia* (Yabuuchi et al., 1993), a commensal bacterium that inhabits the intestinal
20 tract of stink bugs *Leptocoris chinensis* (Dall, 1852), confers resistance to the host against pyridoxine
21 (Kikuchi 2011, 2012), and the *Drosophila melanogaster* (Meigen, 1830) endosymbiont, *Wolbachia*

1 (Hertig, 1936) , can effectively inhibit the infection of the host by Drosophila C virus (DCV) (Hedges
 2 2008; Teixeira 2008). Moreover, symbiotic bacteria in the intestine of the desert migratory locust
 3 *Schistocerca gregaria* (Forskl, 1775), *Pantoea agglomerans* (Ewing and Fife, 1972), can synthesize
 4 pheromones such as guaiacol, thereby regulating population behavior (Dillon 2002). The fact that
 5 endogenous bacteria may affect the healthy development and nutrient absorption of the larvae of
 6 *Hepialus gonggaensis* (Fu et Huang, 1991) further hints at the importance of intestinal flora in the
 7 environmental adaptation of insect hosts (Yin 2011). Since insect gut microbes are major players in
 8 host nutrition and health, the potential manipulation of microbial communities has attracted the interest
 9 of several scholars, and studies have shown that the composition of insect intestinal flora is easily
 10 altered by the host environment and dietary composition (Hammer 2015). Therefore, this plasticity
 11 provides the possibility of using probiotics to manipulate insect intestinal flora for specific
 12 investigation. Evans manipulated the Italian bee diet with lactic acid bacteria to regulate the
 13 homeostasis of intestinal microbes, effectively promoting the host's internal immune response to resist
 14 the invasion of pathogenic bacteria (Evans 2006).

15 Currently, research regarding the possibility of regulating insect development by targeting the
 16 intestinal microbial community is in early stages; however, such an approach may be helpful in the
 17 development of an adequate method for the efficient and economical management of insects. Thus far,
 18 the widespread application of antibiotics and chemical pesticides in insect management has shown
 19 effectiveness in curbing the spread of pests and insect-borne diseases to a certain extent; however,
 20 issues such as insect resistance have compelled researchers to explore new solutions in insect
 21 management. Subsequently, the application of microorganisms to manage pests has garnered
 22 considerable attention from scholars at home and abroad. Among them, *Metarhizium anisopliae*

[Metschn.(1879) Sorokin, 1883] (Fite 2020; Mkiga 2020), *Bacillus thuringiensis* (Berliner, 1911) (Fite 2020; Heckel 2020), *Wolbachia* (Hertig, 1936) (Poinsot 2003; Crawford 2020), *Bacillus subtilis* [Ehrenberg.(1835) Cohn, 1872] (Fouda 2001), and other microorganisms have been the focus of extensive research regarding their application in insect management; however, research on endogenous probiotics and their regulation of host intestinal microbes has been less comprehensive. Additionally, although biological control and sterile insect technologies have contributed to certain achievements in insect management, they are insufficient; therefore, the use of probiotics to manipulate the composition of intestinal microbes to achieve ecological control of the host may be a potential supplement to these existing technologies and other insect management methods. Considering the manipulability of probiotics on the intestinal flora, we used the endogenous probiotic *L. plantarum* that is found in the intestine of *S. litura*, to regulate the composition of the host intestinal microbial community to varying degrees, and explored the influence of *L. plantarum* on the intestinal microbial diversity, growth, and development of *S. litura* under laboratory conditions. As a major pest, *S. litura* has been widely used in various studies; however, because large-scale breeding of *S. litura* is mainly conducted using approaches that include the provision of artificial diets, this study aimed to evaluate the influence of *L. plantarum* on the growth, development, and intestinal microbial diversity of *S. litura* fed with an artificial diet, to provide a theoretical reference for the subsequent screening of insect-specific microecological agents and biological controls.

Methods

Insect culture

The *S. litura* larvae were provided by the Key Laboratory of Natural Pesticide and Chemical

1 Biology of the Ministry of Education, P. R. China. The larvae were reared on an artificial diet as per
 2 methods recommended by Zhu et al. (2001) with slight modifications for subsequent generations,
 3 and under controlled temperature and humidity conditions of 26 ± 2 °C and $80\% \pm 5\%$, respectively.
 4 The main ingredients of the artificial diet were organic corn flour, cooked soy flour, nine vitamin
 5 pills, methyl-p-hydroxybenzoate, sorbic acid, and cholesterol. The rearing was performed in a
 6 transparent plastic box ($17 \times 11.5 \times 6$ cm³) with daily changes in diet. The mature larvae were
 7 placed in a ten-well plate for pupation. After the pupae were collected, they were placed in a
 8 transparent plastic box in which the bottom portion was moistened with wet filter paper. Freshly
 9 emerged adults were transferred to transparent plastic buckets ($22 \times 28 \times 26$ cm³) containing 10%
 10 honey water at the bottom to prepare the adults for laying eggs. The transparent plastic buckets were
 11 lined with butter paper to facilitate egg laying. Larvae from the fourth generation of laboratory
 12 cultures were used for experimental purposes.

13 **Bioassay studies**

14 The artificial diet of *S. litura* was supplemented with *L. plantarum* (GenBank accession number:
 15 MW174226), and suspensions were used to prepare diets with different unit feed bacterial loads,
 16 including 1.9×10^7 CFU/g (hereafter referred to as CD7), 1.9×10^6 CFU/g (hereafter referred to as CD6),
 17 1.9×10^5 CFU/g (hereafter referred to as CD5), and 1.9×10^4 CFU/g (hereafter referred to as CD4). A
 18 diet without *L. plantarum* served as a control (CK). Experiments were performed with 60 first-instar
 19 larvae with three replicates (20 larvae per replicate) for each treatment group. The experiments were
 20 conducted at 26 ± 2 °C temperature, $80\% \pm 5\%$ relative humidity, and a photoperiod of 16:8 h (L:D).
 21 Observations were performed daily on various biological parameters of *S. litura*, including single
 22 female egg production, egg hatching rate, sex ratio, percent pupation, and adult emergence.

Determination of nutritional index of larvae

The larvae of *S. litura* that underwent molting to transition into the fourth-instar stage and showed consistent healthy development during which they were fed with artificial diets with different bacterial loads, were subjected to starvation overnight.

1) Twenty *S. litura* larvae reared on artificial diets with different bacterial loads (including the control group) were selected, and their fresh weight was recorded; then, they were subjected to drying conditions in an oven at 80 °C to achieve a constant weight, and the dry-wet ratio was determined.

2) Twenty larvae were selected and their fresh weight was recorded. According to the dry-wet ratio of the tested larvae, the dry weight of the larvae before the test (C) was inferred, and then they were fed with artificial feed with different bacterial loads (including the control group). After 48 h, the residual feed and feces were removed, and the larvae and feces were subjected to drying conditions at 80 °C to achieve a constant weight to determine the dry weight of the feces (E) and the dry weight of the larvae (D).

3) Based on the same method as that mentioned above, the dry weight of feed with different bacterial loads was measured before (A) and after (B) execution of the tests.

Nutritional indices were calculated as per methods prescribed by Wheeler & Isman (2001) using the following formulae:

$$\text{Relative growth rate (RGR)} = \frac{D - C}{[(D + C)/2] \times T}$$

$$\text{Relative consumption rate (RCR)} = \frac{A - B}{[(C + D)/2] \times T}$$

$$\text{Approximate digestibility (AD)} = \frac{A - B - E}{A - B}$$

1 Efficiency of conversion of ingested food (ECI) = $\frac{D - C}{A - B}$

2 Efficiency of conversion of digested food (ECD) = $\frac{D - C}{A - B - E}$

3 In the formula, T represents the number of experimental days.

4 Each treatment contained 20 larvae, with 3 replicates.

5 **Determination of digestive enzyme activity in the larval midgut**

6 The intestinal crude enzyme solution was prepared according to the method described by Wang et
7 al. (2016). Additionally, amylase, trehalase, and sucrase activities were determined according to the
8 method described by Wang et al. (2016).

9 **16s high-throughput sequencing of the gut bacteria of *S. litura***

10 *Spodoptera litura* fed with a diet supplemented with *L. plantarum* and without *L. plantarum*, and
11 twenty healthy and well-developed fourth-instar larvae were randomly selected; they were subjected to
12 starvation for 24 h, and placed on a sterile ultra-clean workbench. The dissecting instrument was
13 sterilized in an autoclave before commencement of the experiments. The body surface of the *S. litura*
14 were submerged in 75% alcohol for 300 s. After rinsing with sterile distilled water, the intestines of the
15 *S. litura* larvae were dissected under aseptic conditions. The collected samples were mixed in distilled
16 water and sent to Beijing Kinco Biotechnology Co., Ltd. for sequencing.

17 **Statistical analysis**

18 The SPSS software (version 22.0; SPSS Inc., Chicago) was used for performing one-way analysis
19 of variance, and Duncan's new multiple range method was used for conducting multiple comparisons

1 (p ≤ 0.05).

2 **Results**

3 **Nutritional effect of artificial diets with different bacterial loads exerted on** 4 **fourth-instar larvae**

5 As shown in Table 1, the relative feeding rates of the different larval feeding groups were in the
6 order CD6 > CK ≥ CD4 > CD5 > CD7. There was a significant difference between rates of the CD6
7 group and the CD7 group, and there was no significant difference between the other groups. The
8 relative food conversion rates of the larval groups were in the order CD6 > CD5 > CD7 > CK > CD4.
9 The food conversion rate of the CD6 group was significantly higher than that of the other groups, and
10 the food conversion rates of the larvae in the other groups were not significantly different from those of
11 the CK group. The relative food utilization rates of the different larval groups were in the order CD6 >
12 CD7 ≥ CD5 ≥ CK > CD4. There was no significant difference between the food utilization rates of the
13 bacteria-fed larval groups and the control; however, the food utilization rate of the CD6 group was
14 significantly higher than that of the CD7 and CD4 groups. The relative growth rates of the larval
15 groups were in the order CD6 ≥ CK > CD5 > CD4 > CD7, and relative growth rates of the CD6 group
16 were significantly higher than those of the other bacteria-fed groups; however, none of the bacteria-fed
17 groups exhibited a significant difference compared with the control group. The relative approximate
18 digestibility of the larval groups were in the order CK > CD4 > CD5 > CD7 > CD6, and that of the
19 CD6 group was significantly lower than that of the control group.

20

21

1 **Table 1 Nutritional effects of artificial feeds with different bacterial loads exerted on fourth-instar larvae**

Nutrition index	CK	CD4	CD5	CD6	CD7	
(RCR)	2.77 ±	2.77 ±	2.24 ±	2.95 ± 0.22a	2.13 ± 0.30b	F=2.883,
Relative feeding rate of larvae	0.64ab	0.13ab	0.34ab			p≤0.05
(ECD)	0.51 ±	0.42 ± 0.05c	0.60 ± 0.15b	0.80 ± 0.06a	0.58 ± 0.06bc	F=7.393,
Efficiency of conversion of digested food	0.08bc					p≤0.05
(ECI)	0.18 ±	0.15 ± 0.04b	0.18 ±	0.24 ± 0.02a	0.18 ± 0.04b	F=3.447,
Efficiency of conversion of ingested food	0.01ab		0.03ab			p≤0.05
(RGR)	0.54 ±	0.41 ± 0.12b	0.42 ± 0.06b	0.62 ± 0.04a	0.38 ± 0.14b	F=3.751,
Relative growth rate	0.05ab					p≤0.05
(AD)	0.37 ± 0.04a	0.36 ±	0.32 ±	0.27 ± 0.02b	0.30 ± 0.04ab	F=2.146,
Approximate digestibility		0.08ab	0.02ab			p≤0.05

2 Note: The values in the table represent mean ± standard deviation. Different letters in the same row indicate significant differences
3 (P<0.05) as tested by using Duncan's new multiple range test. CD4-CD7: four treatment groups fed with an artificial diet with different
4 bacterial loads; CK: control group.

5
6 As shown in Table 2, the average fresh weights of the larvae fed with artificial diets containing
7 different bacterial loads were in the order CD6 > CD7 > CK > CD4 ≥ CD5, and those of the CD4
8 group and CD5 group were significantly lower than those of the CD6 group; however, none of
9 bacteria-fed groups presented a significant differences compared with the control group.

Table 2 Effect of artificial diets with different bacterial loads exerted on the average weight of fourth-instar larvae

The sample name	Average weight
CD7	$0.57 \pm 0.07ab$
CD6	$0.61 \pm 0.02a$
CD5	$0.52 \pm 0.02b$
CD4	$0.52 \pm 0.07b$
CK	$0.56 \pm 0.02ab$
	$F=1.750, p \leq 0.05$

Note: The values in the table represent mean $\pm$ standard deviation. Different letters in the same column indicate significant differences (P<0.05) as tested by using Duncan's new multiple range test. CD4-CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group.

Effect of artificial diets with different bacterial loads exerted on digestive enzyme activity in the larval midgut

As illustrated in Table 3, the effects of artificial diets with different bacterial loads exerted on the activity of digestive enzymes in the midgut of the fourth-instar larvae of *S. litura* were different. The relative amylase activity of the fourth-instar larvae fed with artificial diets with different bacterial loads was in the order CD5 > CD4 > CD7 > CD6 > CK, and all groups that were fed with a feed supplemented with bacteria exhibited significantly higher activity than the control group. The relative trehalase activity of the fourth-instar larvae fed with artificial diets with different bacterial loads was in the order CD4 > CD5 > CD7 > CD6 > CK; except for the CD6 group, the other bacteria-fed groups exhibited significantly higher activity than the control group. The relative sucrase activity of the

fourth-instar larvae fed with artificial diets with different bacterial loads was in the order $CD4 > CD5 > CD7 \geq CD6 > CK$, and the activities of the CD4 and CD5 groups were significantly different from those of the other groups.

Table 3 Effect of artificial feeds with different bacterial loads exerted on digestive enzyme activity of the larval midgut

The sample name	<i>S. litura</i> fourth-instar larvae		
	Amylase	Trehalase	Sucrase
	OD/(mg·min)	OD/(mg·min)	OD/(mg·min)
CD7	0.34 ± 0.02b	0.35 ± 0.02b	0.33 ± 0.02c
CD6	0.30 ± 0.00c	0.33 ± 0.02bc	0.33 ± 0.01c
CD5	0.39 ± 0.01a	0.41 ± 0.01a	0.41 ± 0.01b
CD4	0.35 ± 0.01b	0.43 ± 0.03a	0.46 ± 0.01a
CK	0.25 ± 0.01d	0.31 ± 0.02c	0.31 ± 0.00c
	F=46.716, p≤0.05	F=17.205, p≤0.05	F=81.289, p≤0.05

Note: The values in the table represent mean ± standard deviation. Different letters in the same column indicate significant differences (P<0.05) as tested by using Duncan's new multiple range test. CD4-CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group.

Effects of artificial diet with different bacterial loads exerted on pupae and eggs of *S. litura*

As shown in Table 4, regarding the effect of artificial feeds with different bacterial loads exerted on the pupae of *S. litura*, the relative pupation rates of *S. litura* larval groups were in the order $CD6 > CD7 > CD5 > CD4 > CK$; the relative pupation rate of the CD6 group was significantly higher than

that of the control group and there was no significant differences between the other groups. The relative ratios of female to male were in the order $CD5 > CK > CD7 > CD6 > CD4$, and both the CD5 and CD4 groups were significantly different from the control group. The relative weights of female pupae were in the order $CD6 > CD5 > CK > CD4 > CD7$, and there were no significant differences between the groups. The relative male pupae weights were in the order $CD6 > CD4 > CD5 > CK > CD7$, and those of the CD6 and CD4 groups were significantly different from those of the control group. The relative pupal emergence rates were in the order $CD6 > CK > CD5 > CD4 > CD7$, and all larvae fed with bacteria-supplemented diets exhibited significant differences compared with the control group. Regarding the effect of artificial feeds with different bacterial loads exerted on the eggs of *S. litura*, the relative number of eggs laid by a single female from each larval group was in the order $CD6 > CD4 > CD5 > CD7 > CK$, and values of the CD6 group were significantly higher than those of the control group. The relative egg hatching rates were in the order $CD5 > CD6 > CK > CD4 > CD7$, and, except for the CD6 group, the bacteria-fed groups were significantly different from the control group.

Table 4 The effect of artificial feeds with different bacterial loads exerted on the pupa and eggs of *S. litura*

The sample name	pupa				egg		
	Pupation rate	Female/male	Female pupa	Male pupal	Pupa	Single female	Egg hatching
	(%)	Sex ratio	weight (g)	weight (g)	emergence rate (%)	spawn	rate (%)
CD7	90.34 ± 1.46ab	0.87 ± 0.06bc	0.31 ± 0.03a	0.26 ± 0.01c	56.76 ± 2.71d	513.00 ± 6.93ab	69.15 ± 2.02c
CD6	92.74 ± 1.47a	0.85 ± 0.10bc	0.34 ± 0.01a	0.32 ± 0.02a	94.12 ± 2.94a	541.33 ± 33.83a	81.37 ± 1.01ab
CD5	90.28 ± 2.20ab	2.11 ± 0.29a	0.33 ± 0.03a	0.30 ± 0.01ab	62.50 ± 1.79c	523.33 ± 41.02ab	83.03 ± 1.02a
CD4	90.02 ± 1.51ab	0.69 ± 0.05c	0.32 ± 0.01a	0.32 ± 0.01a	61.11 ± 1.85c	524.33 ± 55.58ab	70.92 ± 1.88c
CK	89.34 ± 1.19b	1.13 ± 0.09b	0.33 ± 0.01a	0.28 ± 0.02bc	82.09 ± 1.49b	478.00 ± 38.20b	79.41 ± 0.79b
	F=1.949,	F=46.663,	F=0.944,	F=13.700,	F=155.694,	F=2.480,	F=58.054,
	p≤0.05	p≤0.05	p≤0.05	p≤0.05	p≤0.05	p≤0.05	p≤0.05

Note: The values in the table represent mean ± standard deviation. Different letters in the same column indicate significant differences (P<0.05) as tested by using Duncan's new multiple range test. CD4–CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group.

Dominant populations of gut bacteria

The dominant populations of CD4 comprised *Enterococcus* (Thiercelin and Jouhaud, 1984) (52.81%), *Ralstonia* (Yabuuchi et al., 1996) (38.05%), and *Rhodococcus* (Zopf, 1891) (2.79%). The

dominant populations of CD5 included *Enterococcus* (64.22%), *Ralstonia* (26.75%), and *Weissella* (Collins et al., 1994) (4.04%). The dominant populations of CD6 comprised *Enterococcus* (75.04%), *Ralstonia* (17.55%), and *Bacillus* (Cohn, 1872) (4.58%). The dominant populations of CD7 included *Enterococcus* (91.68%), *Bacillus* (3.97%), and *Rhodococcus* (1.90%). The dominant populations of CK comprised *Enterococcus* (79.45%), *Weissella* (15.99%), and *Rhodococcus* (1.76%). Compared with CK, the dominant populations of bacteria changed from *Weissella* (Firmicutes) and *Rhodococcus* (Firmicutes) to *Ralstonia* (Proteobacteria) and *Bacillus* (Firmicutes) (Fig. 1), and there was a negative correlation between *Enterococcus* (Firmicutes) and *Ralstonia* (Proteobacteria).

Fig. 1. Relative abundance of the detected gut bacteria at the genus level. The abscissa indicates the sample name; the ordinate indicates the relative abundance percentage. CD4–CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group. One color represents a species, and the length of the color block (bar graph) represents the relative abundance ratio of the species.

Population differences

According to the Venn diagram, there were 116 OTUs in the CK and four treatments of larvae in all, but only 65 OTUs were shared by all of them (Fig. 2). Of these, one OTU was unique to CK, CD4, or CD7, and no OTU was unique to CD5 or CD6. A multi-sample comparison tree was constructed and used to compare the community similarity of each sample at the OTU composition and phylogenetic level. As illustrated by Fig. 1, the bacterial communities of CD4, CD5, and CD6 showed increased similarity, and their phylogenetic distances were closer, compared with those of CK and CD7. Principal component analysis (PCA) (Dubois 2010) can reflect the differences and distances of samples by analyzing the OTU composition of different samples. The closer the distance between two samples, the more similar is the composition of the two; therefore, the bacterial communities of the three treatment groups (CD4, CD5, and CD6) were similar, as they were clustered in the same area of the PCA (right or lower right) (Fig. 3).

The relative abundance and diversity of gut bacteria in *S. litura* larvae fed with *L. plantarum* are shown in Fig. 2. The top ten genera were distributed into three phyla and eight families.

1

2 Fig. 2. Venn diagram of genus numbers among the control and four treatment groups in *S. litura*. CD4–CD7: four treatment groups fed
 3 with an artificial diet with different bacterial loads; CK: control group. Different colors represent different samples. The number of
 4 overlapping sections between multiple color graphs indicate the number of OTUs among multiple samples, and the non-overlapping
 5 sections indicate the number of OTUs unique to each sample. Blue: CD4 treatment group; orange: CD5 treatment group; pink: CD6
 6 treatment group; green: CD7 treatment group; purple: control group.

7

Fig. 3. Principal component analysis of gut bacteria diversity among the control and treatment groups. CD4–CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group. The dots represent each sample and different colors represent different groups. The abscissa represents the first principal component, and the percentage represents the contribution of the first principal component to the sample difference. The ordinate table indicates the second principal component, and the percentage indicates the contribution of the second principal component to the sample difference.

Discussion

In biological research, microorganisms and other agents are often added to insect feed to conduct study on the effect of the added components on insect growth and development. Nutritional indices, such as food conversion rate and food utilization rate, reflect the degree of insect development to a certain extent, and they are mostly used to express the degree of preference and absorption of food by insects(Zhu et al. 2005a). The results showed that the relative growth rate, food conversion rate, and food utilization rate of larvae of the CD6 group were higher than those of the other treatment groups, indicating that the larvae of *S. litura* had higher adaptability to CD6. Although the food conversion rate and food utilization rate of the larvae of the CD5 and CD7 groups were higher than those of the control group, their relative growth rates and relative feeding rates were lower than those of the control group. Additionally, the relative feeding rate, food conversion rate, and food utilization rate of the larvae of the CD4 group were lower than those of the control group. This indicates that the three treatments using bacteria-supplemented feed, CD4, CD5, and CD7, are less suitable for application as feed for *S. litura* larvae. Moreover, the high feeding rate, food utilization rate, food conversion rate, and insect weight, along with low approximate digestibility, of the larvae of the CD6 indicate that certain substances in the artificial diet of the CD6 group could not be digested well by the larvae; however, substances that were digested resulted in higher food conversion and utilization rates, which could be better transformed into insect tissues (Zhigang et al. 2005).

Changes in the activities of digestive enzymes, such as sucrase and amylase, in insect intestines usually reflect the effect of food on insect host feeding and fitness to a certain extent (Yuzhu et al. 2013). This study found that the larvae of the CD4 and CD5 groups had higher amylase, sucrase, and trehalase activities than the other groups, indicating the larvae in these two groups had relatively high

1 conversion utilization of sugars, even though their growth rate was relatively low. Additionally, it was
 2 also determined that there was a significant positive correlation between the trehalase and sucrase
 3 activities of the larvae and the larval period of each treatment group, and the larvae feeding on these
 4 two groups of food did not show a high pupation rate, suggesting that this might be related to an
 5 imbalanced diet under high-carbohydrate nutritional conditions (MERKX-JACQUES et al. 2008).

6 Foods ingested by insects are preferentially selected, and they also help to improve their
 7 development rate, fecundity, and other aspects of growth and development (Jia, Y et al. 2012). The
 8 larvae of the CD6 group had relatively short larval period, which might be directly related to the diet
 9 fed during the larval stage. Additionally, the relatively high pupa weight of an insect further reflects its
 10 good developmental quality during the larval stage (Zhu et al. 2005b); furthermore, the larvae of the
 11 CD6 group did not exhibit a higher male to female ratio, which might be related to changes in the gut
 12 microbes. The present study found that the larvae of the CD5 group had a relatively high egg hatching
 13 rate, which might be related to its relatively high amylase activity and sugar conversion utilization rate.
 14 Concurrently, the larvae of the CD7 group had relatively low pupal weights and egg hatching rates,
 15 which might be attributed to the poor development of the larvae and their relatively low utilization rate
 16 of sugar (Browne et al. 1993; Augner et al. 1995; Awmack et al. 2002).

17 The present study revealed that the addition of *L. plantarum* to the artificial diet of larvae altered
 18 their gut microbial flora composition as well as their abundance. After supplementing feed with
 19 *L. plantarum*, the growth of *Weissella* in the larval gut was inhibited to varying degrees, and to a
 20 certain extent, the growth of *Ralstonia* and *Bacillus* was promoted. *Enterococcus* (Lebreton et al. 2014;
 21 Vilanova et al. 2016), *Weissella* (Belda et al. 2011; Oh et al. 2013), and *Ralstonia* (Mohr et al. 2006;
 22 Anjum et al. 2018) have previously been reported as symbiotic bacteria in the gut. Similar to the

1 findings presented in our study, Wang et al. (2018) documented variation in the diversity of gut
 2 microflora within piglets due to provision of feed supplemented with *L. plantarum*. Diet may affect the
 3 physical and chemical environment of the gut (Flint et al. 2008; Ley et al. 2008; Clissold et al. 2010;
 4 Sorensen et al. 2010), thereby limiting the types of bacterial strains that can survive in the gut
 5 ecosystem (Thakur et al. 2016).

6 The difference in growth and development of *S. litura* may also be attributed to the changes in gut
 7 microbial diversity (Thakur et al. 2016). LEfSe (Linear discriminant analysis effect size) analysis
 8 showed that the abundance of *Ralstonia*, *Weissella*, *Bacillus*, *Enterococcus*, and the unclassified genus
 9 significantly influenced the gut microbial community in the larvae (Fig. 4). This suggests their
 10 sensitivity to dietary administration and may help elucidate important physiological functions in the
 11 larvae (Zeng et al. 2020). The symbiotic gut bacterial community provides advantages to host insects
 12 via various mechanisms, such as involvement in the secretion of digestive enzymes, aiding survival
 13 with the best available diet, vitamin synthesis, improvement in digestion efficiency, and pathogen
 14 prevention (Berenbaum et al. 1988; Douglas et al. 1992; Behar et al. 2008; Osborne et al. 2009; Koch
 15 & Schmid et al. 2011; Cheng et al. 2017; Al-Ghamdi et al. 2018); hence, they act as "microbial
 16 intermediaries" to compensate for the lack of certain biosynthetic and metabolic capabilities. Certain
 17 microorganisms may facilitate the production of digestive enzymes in certain insects (Terra et al. 1996).
 18 This study showed that after the larvae were fed with *L. plantarum*, the activity of digestive enzymes
 19 such as amylase also increased; however, the abundance of *L. plantarum* in the gut was less than 1%,
 20 which might be related to a shorter colonization time in the gut. As the number of bacteria per unit of
 21 feed increased, the abundance of *Ralstonia* decreased, which was consistent with the trends of the
 22 enzyme activities, such as those of sucrase. Therefore, it is speculated that *Ralstonia* may participate in

1 the induction of digestive enzymes.

2

3

4 Fig. 4. Linear discriminant analysis effect size cladogram (LEfSe). CD4–CD7: four treatment groups fed with an artificial diet with

5 different bacterial loads; CK: control group. The circles radiating from the inside to the outside of the cladogram represent the

6 classification level from phylum to species. Each small circle at different classification levels represents a classification at that level, and

7 the diameter of the small circle is proportional to the relative abundance. The coloring principle includes the uniform coloration of the

8 species name, with no significant differences highlighted in yellow, and other different species are colored according to the group with the

9 highest abundance of the species. Different colors indicate different groups, and nodes of different colors indicate the microbial groups

10 that play an important role in the group represented by the color.

11 However, certain gut microbes have also been reported as opportunistic pathogens, for example,

12 the *Enterococcus* and *Weissella* genera that include insect or animal pathogenic strains (Fisher et al.

13 2009; Fusco et al. 2015). They are usually considered opportunistic pathogens as they usually exert no

1 effect on insect health in the digestive tract; however, they enter the insect hemolymph after the gut
 2 periphagus is damaged, which adversely affects insect development (Bucher et al. 1963). After the *S.*
 3 *litura* larvae were fed on *L. plantarum*, the growth of both microbes in the gut was inhibited to varying
 4 degrees, but the abundance of *Enterococcus* increased with an increase in the abundance of
 5 *L. plantarum* in the diet, and exceeded the *Enterococcus* abundance in the CK group when the
 6 abundance of *L. plantarum* in the diet was at its maximum, which might be attributed to the dynamic
 7 nature of the gut and its physical and chemical properties (Thakur et al. 2016).

8 In summary, addition of *L. plantarum* to an artificial diet can regulate the abundance and diversity
 9 of the gut microbes in *S. litura* larvae and affect their physiological functions. Furthermore, *S. litura*
 10 larvae fed with an artificial diet with different bacterial loads can develop and reproduce normally;
 11 however, the effects on the physiological development and intestinal microbes of *S. litura* are varied.
 12 The larvae of the CD6 group showed the best performance, with the feed promoting their physiological
 13 development. Additionally, the larvae of the CD4 and CD5 groups were significantly different with
 14 regard to pupae or adult male-female ratio compared with the control (the female-male ratios
 15 (female/male) were 0.69 and 2.11, respectively.). And the research found that after fed with a diet
 16 supplemented with *L. plantarum*, *Faecalibaculum* (Chang et al., 2016) in the intestine was significantly
 17 and positively correlated with the host female-male ratio ($p < 0.01$). It is inferred that the change in host
 18 intestinal microbial abundance mediated by *L. plantarum* can induce a change in the host female-male
 19 ratio, which is similar to the results reported by Shan et al. (2019). Next, it is necessary to conduct
 20 related metabolomics analysis on the host female-male ratio changes and nutrient absorption mediated
 21 by probiotics based on existing judgments, in order to better explain this mediation mechanism, so as to
 22 achieve efficient management of economic pests; Therefore, the potential use of microorganisms to

1 regulate the reproduction and development of insects further promotes the application of
 2 microorganisms, especially probiotics, in laboratory insect breeding and the application of sterile insect
 3 technique (SIT) in pest control (Augustinos et al. 2015). As a lactic acid bacterium isolated from the
 4 gut cavity of *S. litura* larvae, *L. plantarum* presents with promising research and application prospects
 5 in terms of laboratory insect feeding and biological control of diseases and insects (Ptaszyńska et al.
 6 2016).

7 Conclusion

8 Feeding *S. litura* larvae with *L. plantarum* can effectively affect physiological and biochemical
 9 indicators, including host nutritional effect, digestive enzyme activity, and pupal weight, as well as
 10 exert a significant regulatory effect on the ratio of male to female adults. Additionally, changes in
 11 microbial abundance and diversity were found in the gut of *S. litura* larvae fed with diets supplemented
 12 with *L. plantarum* and without *L. plantarum*. Therefore, *L. plantarum* has potential applicability in pest
 13 management.

14 Abbreviations

15 *L. Plantarum*: *Lactobacillus plantarum*; *S. litura*: *Spodoptera litura* (Fabricius); CD4–CD7: four
 16 treatment groups fed with an artificial diet with different bacterial loads ; CK: control group ; PCA:
 17 Principal Component Analysis ; LEfSe : Linear discriminant analysis effect size .

18

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12

Figures

Figure 1

Relative abundance of the detected gut bacteria at the genus level. The abscissa indicates the sample name; the ordinate indicates the relative abundance percentage. CD4-CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group. One color represents a species, and the length of the color block (bar graph) represents the relative abundance ratio of the species.

Figure 2

Venn diagram of genus numbers among the control and four treatment groups in *S. litura*. CD4–CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group. Different colors represent different samples. The number of overlapping sections between multiple color graphs indicate the number of OTUs among multiple samples, and the non-overlapping sections indicate the number of OTUs unique to each sample. Blue: CD4 treatment group; orange: CD5 treatment group; pink: CD6 treatment group; green: CD7 treatment group; purple: control group.

Figure 3

Principal component analysis of gut bacteria diversity among the control and treatment groups. CD4–CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group. The dots represent each sample and different colors represent different groups. The abscissa represents the first principal component, and the percentage represents the contribution of the first principal component to the sample difference. The ordinate table indicates the second principal component, and the percentage indicates the contribution of the second principal component to the sample difference.

Cladogram

Figure 4

Linear discriminant analysis effect size cladogram (LEfSe). CD4–CD7: four treatment groups fed with an artificial diet with different bacterial loads; CK: control group. The circles radiating from the inside to the outside of the clade map represent the classification level from phylum to species. Each small circle at different classification levels represents a classification at that level, and the diameter of the small circle is proportional to the relative abundance. The coloring principle includes the uniform coloration of the species name, with no significant differences highlighted in yellow, and other different species are colored according to the group with the highest abundance of the species. Different colors indicate different groups, and nodes of different colors indicate the microbial groups that play an important role in the group represented by the color.