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Effects of Inhibiting the Expression of Chitin Synthase Gene *SfCHSB* on the Metabolism of Trehalose and Chitin in *Spodoptera frugiperda* Larvae

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Abstract: *Spodoptera frugiperda* is one of the world's major agricultural pests and it occurs in many countries around the world. In Lepidoptera, the peritrophic membrane in the midgut protects the midgut epithelial cells and facilitates the digestion and absorption of food. Its main components are chitin and protein. Chitin synthase (*CHS*), as the last enzyme in the chitin biosynthesis pathway, is very important. Here, the expression of chitin synthase B (*CHSB*) gene identified from midgut was inhibited by RNA interference to explore its function in the metabolism of trehalose and chitin of *S. frugiperda* larvae. The results were as follows: *SfCHSB* was highly expressed in actively feeding larvae. Second, knockdown of *SfCHSB* resulted in decreased expression of most genes involved in chitin metabolism, including chitinase, and abnormal phenotypes of *S. frugiperda* were observed. In addition, membrane-bound trehalase activity and glucose content increased, and trehalose content decreased at 24 h after ds*SfCHSB* injection. Trehalase activities increased significantly and the glycogen content decreased at 48 h after ds*SfCHSB* injection. Finally, the activity of chitinase decreased and the content of chitin increased significantly. Our results indicate that *SfCHSB* expression is specific, *SfCHSB* has a large role in regulating chitin metabolism, and it has broad application prospects in the biocontrol of *S. frugiperda*.

Keywords: fall armyworm; chitin synthase; chitin metabolism; peritrophic membrane; RNAi

Citation: Liu, X.; Wang, S.; Yu, Y.; Cheng, Y.; Hu, C.; Zhou, M.; Li, C.; Tang, B.; Wu, Y. Effects of Inhibiting the Expression of Chitin Synthase Gene *SfCHSB* on the Metabolism of Trehalose and Chitin in *Spodoptera frugiperda* Larvae. *Agriculture* **2022**, *12*, 2019. https://doi.org/10.3390/agriculture12122019

Academic Editor: Geraldo Andrade Carvalho

Received: 9 September 2022
Accepted: 23 November 2022
Published: 26 November 2022

1. Introduction

Spodoptera frugiperda (Lepidoptera: Noctuidae) is a native crop pest in South America and North America [1]. Due to the ability of adults to migrate over long distances [2,3], this pest invaded Africa in 2016 [1,4–6] and many Asian countries in 2018 [1,7–9]. The larvae of *S. frugiperda* feed notably on gramineous plants, developing and reproducing rapidly, causing damages to plants [1,9,10]. Corn is its main host, and the larvae can feed on the stem, leaves, tassel, ear, and other parts of corn, which greatly affects the yield and quality of corn [1]. Adults feed on the nectar of various plants and have strong fecundity—a female moth can lay more than 1000 eggs. At present, spraying chemical agents is still the main means to control *S. frugiperda*, although various alternative methods are currently under development [1]. However, agricultural pests are prone to develop pesticide resistance, which makes management less effective [11–13]. Residues of chemical pesticides can also cause environmental pollution and side effects to human health and nontarget organisms [14,15], so we urgently need to develop new technologies and new strategies for pest biocontrol.

It is well known that insects undergo molting for growth and reproduction. After hatching from the egg, the larvae stop growing as the outer epidermis forms. At this point, the larvae need to molt, that is, the old epidermis needs to be removed and a larger, new epidermis needs to be formed. The major chemical components of insect epidermis are chitin, carbohydrate, and protein. Among them, chitin is the main scaffold component of insect stratum corneum, accounting for 20–50% of the stratum corneum weight [16]. Chemically, chitin is a linear polymer of N-acetyl-D-glucosamine (GlcNAc) [17]. Trehalase (TRE) is also participating in initiating the chitin synthesis pathway, catalyzing the hydrolysis of one molecule of trehalose to produce two molecules of glucose. A series of reactions are followed by hexokinase (HK), glucose-6-phosphate isomerase (G6PI), glutamine: fructose-6-phosphate amidotransferase (GFAT), glucosamine-6-phosphate-N-acetyltransferase (GNPNA), phosphoacetylglucosamine mutase (PAGM), UDP-N-acetylglucosamine pyrophosphorylases (UAP). The final step is completed by the participation of chitin synthase (CHS), which synthesizes chitin chains using the direct precursor of chitin, UDP-N-acetylglucosamine (UDP-GlcNAc), as a substrate by removing UDP through transfer (Figure S1) [18,19]. Transcriptomic analysis revealed that the expression levels of genes involved in chitin degradation and synthesis pathways changed during the development of *Bombyx mori* [20]. Chitin is not only a structural element of extracellular matrix, but also may be hidden in other organs and tissues during development, such as trachea and peritrophic membrane (PM) [17]. CHS belongs to the glycosyltransferase family, and there are two types of CHS in insects, which are encoded by the *CHS1* (*CHSA*) gene and the *CHS2* (*CHSB*) gene, respectively. The *CHS1*/*CHSA* gene is usually expressed in the epidermis and trachea of insects, and the encoded enzyme can catalyze the formation of epidermal chitin and trachea chitin. The *CHS2*/*CHSB* gene is expressed in the midgut peritrophic membrane, and the encoded enzyme catalyzes the formation of the PM [19].

Several studies have found that blocked *CHS* gene expression can cause changes in chitin content, and even lethal phenotypes, in a variety of insects [21–24]. In *Culex pipiens pallens*, it was demonstrated that *CHSA* knockdown affected chitin metabolism, resulting in the abnormal development of larvae and the appearance of mosquito's molting-deficient phenotype [25]. In *Henosepilachna vigintioctopunctata*, it was found that ingestion of ds*CHS1* would have negative effects on ladybird, including damaging the larva–pupa–adult transition, affecting the gut structure and cuticle formation, inhibiting the formation of trachea, and thinning the taenidia of trachea [26]. Kelkenberg et al. found that knocking down the expression of the *CHS2* gene in *Tribolium castaneum* decreased the chitin content in the midgut and disrupted PM function [27]. Therefore, the regulation of chitin metabolism may be a potential pest control method [26,28].

Bolognesi et al. identified chitin synthase (*SfCHSB*) and chitinase (*SfCHI*) genes from the midgut of *S. frugiperda* [29]. SfCHSB has a high degree of amino acid sequence identity with CHSB sequences of other insects and is more closely related to Lepidopteran insects [29–32]. RNA interference (RNAi) experiments showed that inhibiting the expression of *SfCHI* and *SfCHSB* at the pupal and adult stages would have a negative effect on *S. frugiperda* [32]. Because *SfCHSB* is highly expressed in the third and fifth instar larval stages [29], this study took the third instar larvae as the research object and used RNAi to explore the changes of trehalose and chitin metabolism in the larvae after injection of ds*SfCHSB*. Our results enriched the research on the function of target gene *SfCHSB* based on previous studies. Moreover, since *SfCHSA* in the epidermis of *S. frugiperda* has not been reported, our work will be conducive to explore the potential use of *SfCHSB* in the control of *S. frugiperda* larvae.

2. Material and Methods

2.1. Test Insect

Eggs of *S. frugiperda* were originally obtained from Zhejiang Academy of Agricultural Sciences. Larvae and adults were reared in a lighted incubator under conditions of

25 ± 1 °C, 60 ± 10% relative humidity, and a 16: 8 h light: dark photoperiod. Larvae were fed with an artificial diet [33] and adults were fed with a 10% honey solution.

2.2. Total RNA Extraction and cDNA First Strand Synthesis

Total RNA was extracted from *S. frugiperda* according to the instructions of RNAiso Plus (Invitrogen, Carlsbad, CA, USA) reagent. Subsequently, RNA integrity was detected with 1% agarose, and the concentration and purity of extracted RNA were determined with NanoDrop™ 2000. The PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, Kyoto, Japan) was used for reverse transcription experiment to synthesize cDNA.

2.3. Synthesis of Double-Stranded RNA (dsRNA)

The specific amplification primers of ds*SfCHSB* were designed by using the online website Primer 3 (Table S1). After the polymerase chain reaction (PCR) experiment, the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit was used to recover the specific size of PCR products. First, the products were ligated to the vector (pMD®18-T), and then the vectors were transformed into competent cells (DH5α). The candidate bacterial solutions were sent to the company (Zhejiang Shangya Biotechnology Co., Ltd., Hangzhou, China) for sequencing to verify whether the gene fragment was correct. For the right bacterial solutions, the plasmids were returned for subsequent experiments. The T7 RiboMAXTM Express RNAi System kit (Promega, Madison, WI, USA) was used to synthesize dsRNA. In this study, dsRNA of green fluorescent protein (GFP) was used as control.

2.4. dsRNA Injection

The dsRNA was injected into *S. frugiperda* larvae using a microinjection system (Eppendorf TransferMan® 4r). The injection instar was one-day-old 3rd instar larvae. The injection site was between the second and third pairs of thoracic feet, and 300 nL of dsRNA (about 2500 ng·μL^{-1}) was injected into each larva. Referring to the research of Wan et al. [32], the larvae at 24 and 48 h after injection were selected to study the effects on the larvae before and after molting.

2.5. Collection and Observation of Spodoptera Frugiperda

To analyze the expression pattern, individuals at different instar (egg, 1st–6th instar larvae, prepupae, pupae, one-day-old female adult, one-day-old male adult) were used to detect the expression of *SfCHSB*. Larval development was observed persistently after injection of ds*SfCHSB* and ds*GFP*, respectively. When abnormal phenotypes were present, they were recorded using a stereomicroscope (Leica EZ4 HD) with LAS EZ version 2.0.0.292 software.

2.6. Real-Time Fluorescence Quantitative PCR (qRT-PCR)

qRT-PCR reaction system (TaKaRa TB Green® Premix Ex Taq™): one μL of cDNA, ten μL of TB Green, one μL of forward primer, one μL of reverse primer, seven μL of ddH_2O. qRT-PCR reaction procedure: predenaturation at 95 °C for 30 s, forty cycles of 95 °C for 5 s, 55–60 °C for 30 s. Ribosomal protein L10 (*RPL10*) was an internal control [34], and the primer sequences are shown in Table S1. The data of qRT-PCR were analyzed using the $2^{-\Delta\Delta CT}$ method [35].

2.7. Carbohydrates Content and Trehalase Activity Tests

This part of the experiment was carried out with reference to the research of Zhang et al. [36]. Four or five larvae were taken as one sample, and three samples were collected for each treatment. The larvae were ground in 200 μL phosphate-buffered saline (PBS) and treated with sonication. After that, 800 μL PBS was added to each sample. After centrifugation at 1000× *g* for 20 min at 4 °C, 350 μL supernatant was used to determinate the contents of trehalose, protein, and glycogen. Another 350 μL of supernatant were centrifuged (4 °C, 20,800× *g*, 1 h). Then, 300 μL of the supernatant was taken for the detection of glucose content, protein content, and soluble trehalase activity, and 300 μL of PBS was added to

the original centrifuge tube and mixed to make a suspension for the detection of glucose content, protein content, and membrane-bound trehalase activity. Trehalose content was determined by anthrone method; the principle is that trehalose dehydrates under strong acidic conditions to produce 5-hydroxymethylfurfural, which can react with anthrone to produce a blue–green furfural derivative. The glucose content, glycogen content, and trehalase activities were measured using the Glucose Assay Kit (Sigma, St. Louis, MO, USA), and the protein content was measured using the BCA Protein Assay Kit (Beyotime, Shanghai, China).

2.8. Chitin Content and Chitinase Activity Tests

Four or five larvae were taken as one sample, and three samples were collected for each treatment. The chitin content of larvae was determined with reference to Arakane et al. [37], and the principle is that chitinase hydrolyzes chitin to produce N-acetylglucosamine, and the intermediate compound produced by the coheating of N-acetylglucosamine and alkali can further react with p-dimethylaminobenzaldehyde to produce chromogenic substances. The chitinase activity in the larvae was determined with the chitinase kit (Comin, Suzhou, China), and the specific experimental steps were in accordance with the instructions. At the same time, the protein concentration in the sample was determined to calculate the chitinase activity.

2.9. Statistical Analysis

Significant difference tests for the data were carried out by using IBM SPSS Statistics 20 software. Different lowercase letters in the figures indicate significant differences between treatments ($p < 0.05$). t-test analysis results were indicated by *, "***" means $p < 0.001$, "**" means $p < 0.01$, "*" means $p < 0.05$, and "ns" means $p > 0.05$. Finally, the GraphPad Prism 8.4.0 software was used to draw the result figures, and the data in the figures were represented by the mean ± standard error.

3. Results

3.1. Developmental Expression Pattern of SfCHSB

The expression of the *SfCHSB* gene in *S. frugiperda* changed during the whole development process, and the overall trend was that it first increased and then decreased (Figure 1). At the beginning, the expression level of *SfCHSB* in egg stage was relatively low (Figure 1). However, when the larvae hatched, the expression level of *SfCHSB* increased significantly, and the expression level remained high throughout the larval stage (Figure 1). As the moths developed, the expression level of *SfCHSB* reduced significantly and was very low at the prepupa–pupa–the first day of adult stage (Figure 1).

3.2. Expression of SfCHSB after RNAi

Compared with injection of ds*GFP*, the expression level of the target gene *SfCHSB* decreased significantly at 24 h after injection of ds*SfCHSB* (Figure 2). However, the expression level increased at 48 h after injection and was not significantly different compared with the control group (Figure 2).

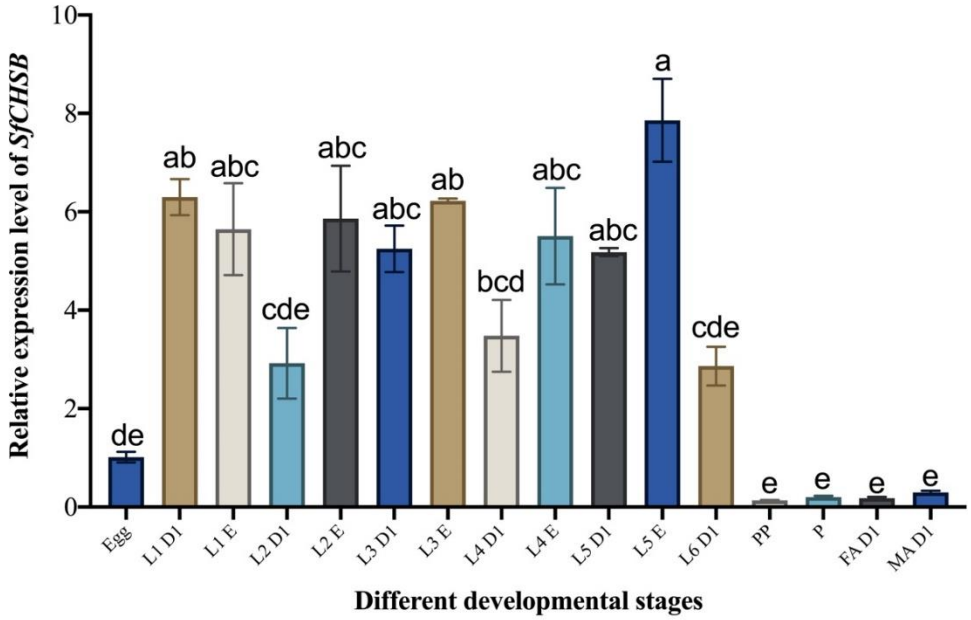

Figure 1. Expression level of *SfCHSB* at different developmental stages in *Spodoptera frugiperda.* L1 D1: one-day-old 1st instar larvae, L1 E: larvae at the end of the 1st instar, L2 D1: one-day-old 2nd instar larvae, L2 E: larvae at the end of the 2nd instar, L3 D1: one-day-old 3rd instar larvae, L3 E: larvae at the end of the 3rd instar, L4 D1: one-day-old 4th instar larvae, L4 E: larvae at the end of the 4th instar, L5 D1: one-day-old 5th instar larvae, L5 E: larvae at the end of the 5th instar, L6 D1: one-day-old 6th instar larvae. PP: prepupae, P: pupae, FA D1: one-day-old female adults, MA D1: one-day-old male adults (mean ± SE; Tukey's test; different lowercase letters in the figure indicate significant differences between treatments ($p < 0.05$)).

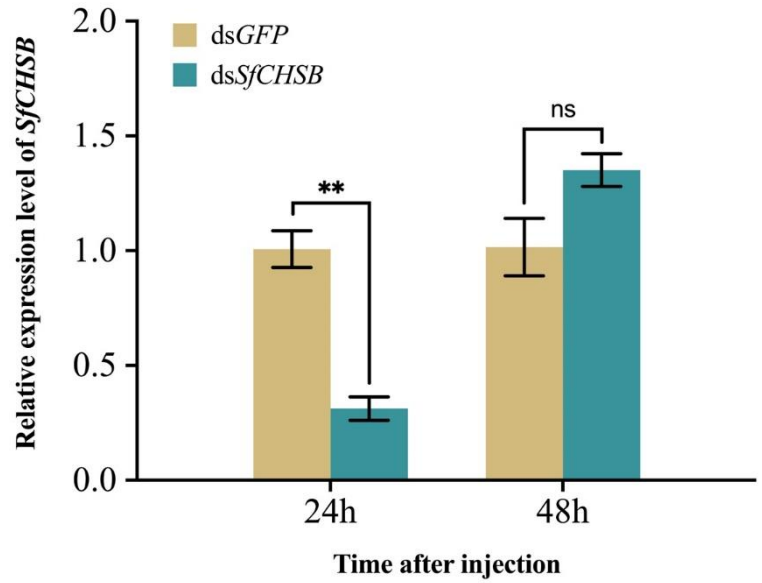

Figure 2. The expression level of *SfCHSB* gene after RNAi. *SfCHSB*: chitin synthase B in *Spodoptera frugiperda* (mean ± SE; *t*-test; ns $p > 0.05$, ** $p < 0.01$).

3.3. Expression of Trehalose and Chitin Metabolism-Related Genes after RNAi

Compared with injection of ds*GFP*, in addition to the *SfGNPNA* gene, the expression on mRNA level of the remaining nine genes (membrane-bound trehalase, soluble trehalase, trehalose-6-phosphate synthase (*SfTPS*), *SfGFAT*, *SfUAP*, *SfHK*, *SfG6PI*, *SfPAGM*, and *SfCHI*) reduced significantly at 24 h in ds*SfCHSB* treatment (Figure 3). However, only the mRNA expression level of the *SfHK* gene reduced significantly at 48 h after ds*SfCHSB* injection

(Figure 3G), and the expression levels of *SfGNPNA*, *SfG6PI*, *SfPAGM*, and *SfCHI* increased significantly (Figure 3E,H–J).

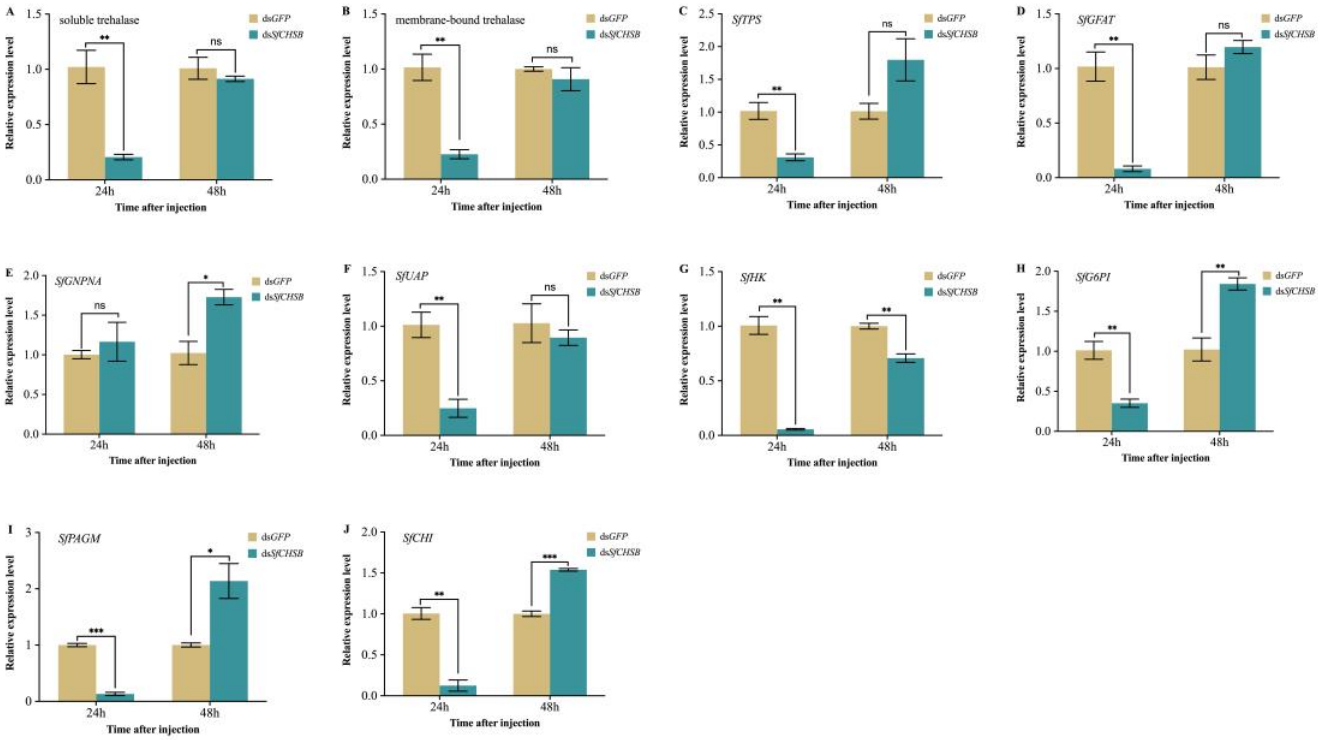

Figure 3. The expression levels of genes involved in trehalose and chitin metabolism after RNAi. (**A**): soluble trehalase; (**B**): membrane-bound trehalase; (**C**): *SfTPS*, trehalose-6-phosphate synthase; (**D**): *SfGFAT*, glutamine: fructose-6-phosphate amidotransferase; (**E**): *SfGNPNA*, glucosamine-6-phosphate-N-acetyltransferase; (**F**): *SfUAP*, UDP-N-acetylglucosamine pyrophosphorylases; (**G**): *SfHK*, hexokinase; (**H**): *SfG6PI*, glucose-6-phosphate isomerase; (**I**): *SfPAGM*, phosphoacetylglucosamine mutase; (**J**): *SfCHI*, chitinase (mean ± SE; *t*-test; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.4. Trehalase and Chitinase Activities after RNAi

Although the soluble trehalase activity was not significantly different from the control group at 24 h after injection of ds*SfCHSB*, the soluble trehalase activity measured at 48 h was significantly higher than that of the ds*GFP* injection (Figure 4A). At 24 and 48 h after injection of ds*SfCHSB*, the activity of membrane-bound trehalase increased significantly (Figure 4B), and chitinase activity decreased significantly (Figure 4C).

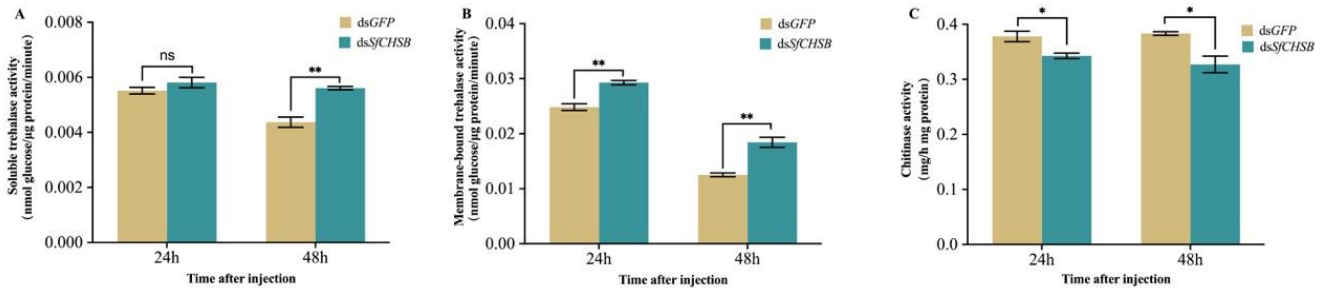

Figure 4. Changes in the trehalase and chitinase activities after RNAi. (**A**): Soluble trehalase activity, (**B**): membrane-bound trehalase activity, (**C**): chitinase activity (mean ± SE; *t*-test; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$).

3.5. Carbohydrate and Chitin Contents after RNAi

The glucose content increased significantly compared with the control group ds*GFP* (Figure 5B), while the trehalose content decreased significantly (Figure 5C) and the glycogen content was not significantly changed at 24 h after injection of ds*SfCHSB* (Figure 5A). At 48 h after the injection of ds*SfCHSB*, the content of glycogen was significantly lower than that of the control group (Figure 5A), and there was no difference in the content of glucose and trehalose compared with the control group (Figure 5B,C). Furthermore, the data showed that chitin content increased significantly both 24 and 48 h after injection of ds*SfCHSB* (Figure 5D).

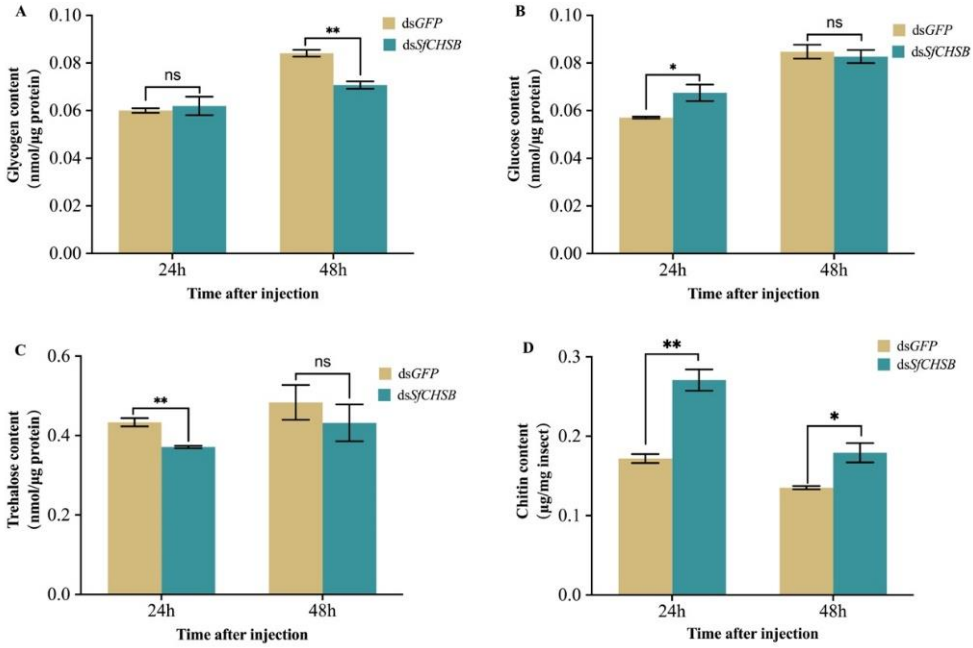

Figure 5. Carbohydrate and chitin contents after RNAi. (**A**): Glycogen content, (**B**): glucose content, (**C**): trehalose content, (**D**): chitin content (mean ± SE; *t*-test; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$).

3.6. Survival and Abnormal Phenotype

The survival rates at 24 and 48 h after injection of ds*SfCHSB* were 93.4% and 88.5%, respectively. The survival rates at 24 and 48 h after injection of ds*GFP* were 93.9% and 89.4%, respectively (Figure 6A), and there are two abnormal phenotypes of the third instar larvae; the first is that the moth is full of liquid contents, and the second is that it cannot molt normally (Figure 6B).

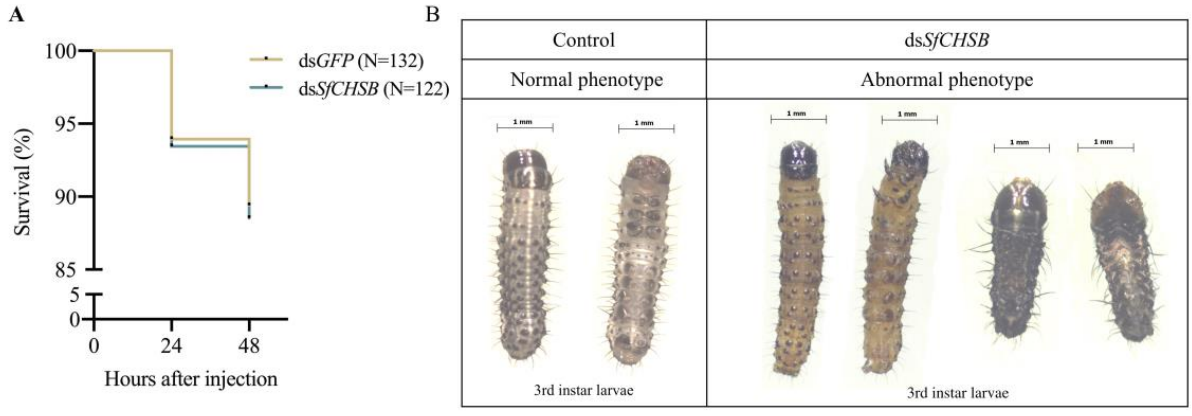

Figure 6. Survival (**A**) and abnormal phenotype (**B**) of *Spodoptera frugiperda* after RNAi.

4. Discussion

SfCHSB contains an open reading frame (ORF) of 4572 bp encoding a 1523 amino acid protein with a molecular weight of 174 kDa and a pI of 5.8, incorporating a central chitin synthase domain, N-terminal domain with seven TM helices, and C-terminal domain with an additional seven TM helices [29,32]. The experimental results of Bolognesi et al. showed that *SfCHSB* expressed highly in the midgut of the third and fifth instar larvae, and trace amounts of *SfCHSB* transcripts were detected in the wandering, prepupal, and pupal stages [29]. This is similar to the results of our study; we also found that the expression level of *SfCHSB* was higher in feeding larval stages (first instar–early sixth instar) (Figure 1). This result was also verified in other insects. *CHSB* was identified in the midgut of *Manduca sexta*, *Spodoptera exigua, and T. castaneum*, and was highly expressed during insect feeding [30,31,38]. In *Aedes aegypti*, *CHSB* was also expressed in the midgut and the expression level increased after blood feeding in females [39]. The *B. mori* transcriptome data showed that *CHSB* downregulated significantly from the third day of fifth instar larvae to the pupal stage [20]. Further study found that the mRNA expression level of *CHS2* in the anterior midgut of *M. sexta* and *Locusta migratoria* was significantly higher than that in the middle and posterior parts [31,40]. It can be seen that the mRNA expression of *CHSA* and *CHSB* in insects has strict tissue specificity, and *CHSB* is gut-specific and specializes in the production of PM chitin.

Since its discovery, RNAi has rapidly emerged as a powerful reverse genetics tool for studying gene function [41,42], regulation, and interactions at the cellular and organismal levels [43–45]. In this study, after ds*SfCHSB* injection, the expression on mRNA level of *SfCHSB* was significantly downregulated at 24 h, and the inhibition rate was about 60% (Figure 2). However, the expression of the target gene increased at 48 h, and the expression level was almost the same as that of the control group (Figure 2). This result is related to the large variation in RNAi efficiency among insects. RNAi is efficient and systemic in Coleoptera, but variable in Lepidoptera, Diptera, Hymenoptera, and Hemiptera [46]. The reason is that dsRNase that can degrade dsRNA was found in some insects [47,48], and insects belonging to Lepidoptera and Hemiptera generally showed higher nuclease activity [49,50]. Wan et al. performed RNA interference by feeding *S. frugiperda* larvae with bacteria expressing ds*SfCHSB* or injecting synthetic ds*SfCHSB* into pupae and adults, and collected individuals after 8 days of feeding and 24 h after injection to detect the inhibition efficiency [32]. Compared with the injection of ds*EGFP*, the reduction rate of *SfCHSB* was 36.89% in larvae, 58.72% in pupae, and 29.09% in adults [32]. Therefore, it is necessary to select or develop an efficient, economical, simple, and noninvasive dsRNA delivery method for the study of *S. frugiperda* by RNAi technology.

In this study, most genes on the chitin synthesis and degradation pathways detected at 24 h were affected by downregulation of *SfCHSB*, and their expression levels also showed significant reductions (Figure 3). At 48 h after injection, nearly half of the detected genes on the trehalose and chitin metabolism pathway had no significant difference from the control group (Figure 3). We speculate that this result is related to the increased expression level of *SfCHSB* and the weakened inhibition effect (Figure 2). Similarly, *HK* and *GFAT* knockdown were found to lead to downregulation of chitin metabolism-related genes in *Nilaparvata lugens* [51,52]. GNPNA, PAGM, and UAP are all essential enzymes of the chitin biosynthesis pathway. Studies of *A. aegypti* [53], *T. castaneum* [54], *Sogatella furcifera* [55], and *Cnaphalocrocis medinalis* [56] showed that silencing of these genes would negatively affect insect development or reproduction. Trehalose is an important substrate for the de novo synthesis of chitin in insects. Trehalase is a key enzyme involved in the hydrolysis of trehalose and regulated insect trehalose balance to cope with the stress of environment [57–59]. In *Diaphorina citri*, the expression levels of genes involved in chitin metabolism significantly reduced at 24 and 48 h after treatment with trehalase inhibitor validamycin [60]. In addition, silencing of *TRE1-1*, *TRE1-2*, and *TRE2* of *D. citri* decreased the expression on mRNA level of chitin metabolism-related genes and led to a malformed phenotype of *D. citri* [60]. It shows that trehalase has a large role in regu-

lating chitin metabolism [36,60–62]. On the other hand, trehalose-6-phosphate synthase (TPS) is involved in trehalose biosynthesis, and *TPS* knockdown changed the expression of genes related to chitin metabolism [62,63]; therefore, the relationship between trehalose metabolism and chitin synthesis is very close. The mechanism regulation in organisms is very complex. According to the genetic central dogma, genes direct protein synthesis, and biological traits are directly reflected by proteins. Although the expression of soluble trehalase and membrane-bound trehalase genes of *S. frugiperda* were downregulated, the activity of trehalase was enhanced (Figures 3A,B and 4A,B). Furthermore, the expression of *SfCHI* increased at 48 h, while the activity of chitinase decreased (Figures 3J and 4C). It is speculated that there are two reasons for this: there may be a certain time difference between the expression at the gene level and the enzyme activity level; there are multiple trehalase gene and chitinase gene sequences in some insects [52,60], and whether there are other sequences in *S. frugiperda* needs further study. At 24 h after RNAi, an increase in trehalase activity resulted in a decrease in trehalose and an increase in glucose (Figures 4A,B and 5B,C). In addition, the increase of chitin content may be the joint action of *SfCHSB* expression and chitinase activity (Figures 2, 4C and 5D). After the injection of dsRNA, results indicated that the chitin degradation pathway of larvae was also affected. It is speculated that the biosynthesis of chitin in the epidermis, which SfCHSA participates in, and the disruption of chitin degradation together led to the increase of chitin content. After *CHS2* of *T. castaneum* was RNAi, the larvae stopped feeding, became smaller, and the content of chitin in the midgut decreased. In *Acyrthosiphon pisum*, RNAi of *TRE* and *TPS* reduced chitin content [62]. One study showed that the chitin content in the dry matter of insect molts was as high as 40%, while the chitin content in the PM is typically between 3–13% [64,65]. This study only measured the chitin content of the whole larvae and could not judge the changes of chitin content in the epidermis and midgut. Since the detection of chitin synthase activity is difficult [66], and *SfCHSA* in the epidermis of *S. frugiperda* has not been reported, the change of chitin content can be explored through experiments to explore its response molecular mechanism.

As mentioned earlier, CHS2/CHSB is very important in midgut development and formation of the PM. The PM is a fibrillar network composed of chitin and proteins, and its function is to surround food particles and protect the apical membrane microvilli of intestinal parietal cells from damage by food particles and microorganisms. In *T. castaneum* [27], *A. aegypti* [67], and *Anthonomus grandis* [68], inhibition of *CHS2* expression by RNAi affected the formation, integrity, and permeability of PM. Defective PM disrupts the cycle of digestive enzymes in the insect midgut, which in turn increases larval mortality [69,70]. Although $p > 0.05$, inhibition of *SfCHSB* resulted in an increased number of larval deaths and an abnormal phenotype (Figure 6). The above results indicate that *SfCHSB* has a large role in regulating chitin metabolism and provides a possibility and theoretical basis for the control of *S. frugiperda*.

Since plants and vertebrates do not contain chitin, the regulation of enzymes related to chitin synthesis and degradation provides potential excellent targets for the design and development of novel and highly effective insecticides. Studies by other scholars and the authors have shown that *CHS2/CHSB* has broad application prospects in the biocontrol of pest [28,32,40,67,68]. Future research needs to focus on two aspects. One is to explore more effective ways of gene silencing, such as dsRNA nanoformulations and inhibitors [59,71], gene editing [72], and feeding [73]. The other is to clone the sequence of *CHSA* of *S. frugiperda* and conduct in-depth research on the chitin synthase gene in combination with *CHSB*, which will promote the development of insect growth regulators and biopesticides targeting chitin metabolism.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12122019/s1, Table S1: Primers for dsRNA synthesis and real-time fluorescence quantitative PCR; Figure S1: Chitin metabolism pathway of insects [18,19].

Author Contributions: Conceptualization, C.H., C.L., B.T. and Y.W.; data curation, Y.C. and M.Z.; investigation, X.L., S.W., Y.Y., Y.C. and M.Z.; methodology, X.L., S.W., Y.Y. and Y.C.; supervision, C.H., C.L., B.T. and Y.W.; writing—original draft, X.L. and S.W.; writing—review and editing, X.L., S.W., B.T. and Y.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Guizhou Provincial Science and Technology Foundation (ZK [2019] 1012), the Special Funding of Guiyang Science and Technology Bureau and Guiyang University (GYU-KY-2021), the local projects guided by the central government [2022] 4013, and the Guiyang Science and Technology Personnel Training Project [2022] 43-16.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data produced from this study are included in this published paper. The data are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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