Effects of Dietary Straw Content on Growth and Development of *Locusta migratoria* and Its Egg Hatching

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Abstract [Objectives] This study was conducted to investigate the effects of dietary straw content on the growth and development of oriental migratory locusts (*Locusta migratoria*), as well as the effects of temperature and substrate on their egg hatching. [Methods] The eggs of oriental migratory locusts were hatched at 15, 20, 25, 30, 35, and 40 °C in sawdust, soil and sawdust + soil conditions, respectively, to determine suitable environmental conditions for artificial hatching of oriental migratory locusts. Rice straw was added at 0%, 20%, 40%, 60%, 80% and 100% to the basic diet, and the obtained diets was feed to oriental migratory locusts at the 4th instar, to investigate the effects on their growth and development. [Results] The eggs of oriental migratory locusts had a good hatching rate at 25 − 30 °C, and sawdust was the best choice for their hatching substrate. Adding 40% of rice straw to the diet could ensure the normal growth and development of oriental migratory locusts while consuming as much rice straw as possible. [Conclusions] This study explored a technical solution for efficiently producing insect protein from discarded crop straw (treating waste with insects, and turning waste into treasure), providing support for breeding of oriental migratory locusts and locust disaster prevention.

Key words Straw feed; Substrate; Oriental migratory locust; Hatching rate; Temperature

Straw is the remaining part of crops after maturity and harvest, usually referring to the general term for the remaining stem and leaf parts of crops such as corn, rice, wheat, soybean, and sweet potato after harvesting the fruits^[1]. The recycling and utilization of straw as agricultural waste carbon has always been a global problem. In order to prevent environmental problems caused by burning straw, the resource utilization of straw is still a research hotspot today^[2]. In recent years, locusts have attracted much attention in the field of special insect breeding. Oriental migratory locusts (Locusta migratoria) with the largest breeding scale belong to the family Locustinae, and they prefer to eat plants such as the Poaceae and Cyperaceae families. They grow rapidly and have a short generation cycle. The body of the insects contains abundant protein and energy substances^[3], and the crude protein content of adult insects is about $50\% - 70\%^{[4-5]}$, which is higher than all plant-based and numerous animal-based materials commonly found in various human food sources. Moreover, research has shown that oriental migratory locusts are a nutrient source low in fat and cholesterol and rich in vitamin contents^[6-7]. Waste straw is mainly composed of structural carbohydrates such as lignin and cellulose, and its nutrient composition cannot fully meet the growth needs of locusts even under suitable environmental conditions [8-9].

The developmental status of insects is most influenced by temperature among various environmental factors $^{[10]}$, and the nor-

mal hatching of locusts needs to be within an appropriate temperature range. At present, research on the hatching conditions of locusts mostly focuses on the prediction and management of grassland locust disasters^[11]. There is little research on the hatching conditions of eggs of oriental migratory locusts under artifical breeding, and the successful hatching of locust eggs is a key factor in culturing oriental migratory locusts.

In this study, in order to explore the optimal hatching temperature and medium for the eggs of oriental migratory locusts under artificial breeding conditions, as well as the effects of adding different amounts of rice straw to the diet on the growth and development of oriental migratory locusts, the hatching of oriental migratory locust eggs under different temperature and substrate conditions was investigated to determine suitable environmental conditions for artificial hatching of oriental migratory locusts. In this study, the effects of rice straw content on the growth and development of oriental migratory locusts were investigated by measuring the feed intake, digestibility, survival rate, eclosion rate and other indexes of oriental migratory locusts feeding on diets with different contents of rice straw, in order to determine the appropriate addition amount of straw to the diet with which oriental migratory locusts could consume as much waste straw as possible while ensuring their survival. This study explored strategies and technical solutions for "carbon consumption" and "opening sources of protein", achieved "treating waste with insects, nourishing insects with waste, and turning waste into treasure", and rapidly improved the effectiveness of carbon conversion and utilization, exploring the "Locust-Straw" technology route for rational utilization of straw and providing reference ideas for locust growth and development control or field prevention.

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Materials and Methods

Experimental materials

Tested materials The eggs of oriental migratory locusts were purchased from the locust breeding farm in Cangzhou, Hebei Province. The eggs and the soil outside the ootheca were sieved through a sieve, and complete and undamaged full egg sheaths were taken and separated with tweezers for later use. In the substrate for the hatching of locust eggs, the soil was taken from the surface soil (0 - 10 cm) of the grassland at Xiushui Road of Daging City, and determined for its properties as follows: pH 8. 96, and electrical conductivity 1.17 mS/cm. And the sawdust was poplar sawdust. Both substrates were sterilized under high pressure and dried in an oven at 105 °C for use. The basic diet for oriental migratory locust culture is usually white sugar and wheat bran at 1: 10^[8]. The wheat bran used in this study was purchased from local farmers, and the rice straw powder added to the diet was taken from local rice growers. The materials were crushed for 5 min using an Aizela 800A crusher and sieved through a 200 mesh sieve (Table 1).

Table 1 Nutrient content of feed materials

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Materials	CP	EE	CF	Ca	P
Fresh A. sativa	9.95	2.30	9.72	0.12	0.23
Wheat bran	15.72	3.98	7.24	0.11	0.92
Rice straw	4.84	1.42	40.63	0.35	0.08

Experimental design The experiment was carried out at the Grassland Science Laboratory of College of Animal Science and Veterinary Medicine. Water was added to the sawdust until its moisture content was 80%. After stirring evenly, the sawdust was added in 1.75 L plastic circular net boxes with a cover, about 5 cm thick, and 30 eggs were spread evenly on its surface and covered with 1 cm of sawdust. Each net box served as a test unit. The self-made constant-temperature breeding boxes were set to corresponding temperatures of 15, 20, 25, 30, 35, and 40 °C (Fig. 1), respectively, and under L: D = 12 h: 12 h, each treatment was done in 3 replicates. The hatching situation was recorded daily. When there were no new hatched larvae within 3 d, it was deemed that hatching had ended, and the hatching rate was calculated.



Fig. 1 Original constant-temperature breeding box

The soil and sawdust were mixed in a volume ratio of 1:1 evenly to obtain a mixture, which was added with water and stirred until the moisture content was 80%. The pure soil and sawdust were treated in the same way and added in breeding boxes to about 5 cm thick. Thirty eggs were spread evenly on the surface and covered with 1 cm of substrate. After the hatching temperature test was completed, the temperature was set according to its optimum temperature, and photoperiod was set as $L:D=12\ h:12\ h$. Each substrate was repeated three times, and the hatching situation was recorded daily. When there were no new hatched larvae within 3 d, it was deemed that hatching had ended, and the hatching rate was calculated.

At the same time as the above test, the oriental migratory locusts were hatched according to the method provided by Cangzhou Farm for straw diet experiment. Rice straw powder was mixed evenly with the basic diet according to the mass fraction of 0, 20%, 40%, 60%, 80%, and 100%, and water was added to a moisture content of 70%. Thirty locusts that had just molted and entered the 4th instar with good and consistent growth status were selected, weighed for the initial weight and put in a breeding box. Each kind of diet was repeated three times, and Avena sativa L. was fed as the control group. At 9:00 am every day, corresponding diet exceeding twice the weight of locusts in the cage was fed and the feed weight was recorded. At 19:00, the remaining feed weight was recorded to calculate the feed intake. Before feeding in the morning, the frass was collected as the fecal amount of the previous day^[9]. The breeding temperature of locusts was 25 – 30 $^{\circ}\mathrm{C}$, and the relative humidity was 60% . The photoperiod was set to L: D = 12 h: 12 h using a full-spectrum lamp. When there were no newly emerged locusts in a cage within 3 d, the weight of all locusts in the cage was measured to calculate the average weight gain. If oriental migratory locusts died in the net cage, oriental migratory locusts raised by corresponding treatment and feeding method in the same growth state were immediately supplemented, and the number of oriental migratory locust deaths and the number of emerged locusts were recorded. The mortality rate and eclosion rate of oriental migratory locusts were calculated.

Feed intake $[g/(locust \cdot d)] = \sum (Daily feed input - Daily feed surplus)/30/Breeding days;$

Amount of feces $[g/(locust \cdot d)] = \sum Daily fecal amount/30/ Breeding days;$

Feed conversion ratio (%) = (Feed intake – Fecal amount)/ Feed intake $\times 100\%$;

Weight gain = Weight of locusts after feeding - Initial weight of locusts;

Survival rate (%) = 1 - (Number of dead locusts/Initial number of locusts) $\times 100\%$:

Eclosion rate (%) = Number of emerged insects/Initial number of locusts $\times\,100\%$.

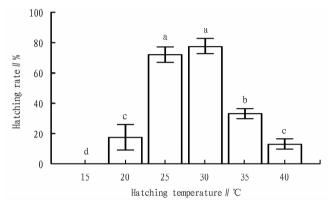
Data processing

The experimental data were plotted using GraphPad Prism 8, and ANOVA analysis of variance was performed using SPSS 22.0.

Result and Analysis

Effects of hatching temperature on the hatching rate of oriental migratory locusts

The hatching temperature had a very significant impact on the hatching rate of oriental migratory locust eggs (Fig. 2). As the temperature increased, the hatching rate of locust eggs showed an inverted bell-shaped trend, with the highest hatching rate of 77.78% at 30 °C, followed by 72.22% at 25 °C, which had a significant difference from the highest hatching rate (P > 0.05). At 15 °C, the eggs of oriental migratory locusts did not hatch within 15 d, and the hatching rate was 0, significantly lower than other temperatures (P < 0.05). The eggs of oriental migratory locusts had a higher hatching rate in the range of 25 – 30 °C, which was significantly increased by 116.67% – 483.33% compared with the three temperatures except for 15 °C (P < 0.05).



Different lowercase letters indicate significant differences in the same index between different treatments (P < 0.05). The same below.

Fig. 2 Effects of different temperatures on the hatching rate of oriental migratory locust eggs

Effects of hatching substrate on the hatching rate of oriental migratory locusts

Different hatching substrates had different effects on the hatching rate of oriental migratory locust eggs (Table 2). The hatching rate of locust eggs hatched with sawdust was as high as 71.1%, significantly higher than those with soil and mixed substrates (P < 0.05). The hatching rate of locust eggs hatched in soil substrate was the lowest, only 17.78%, while adding equal volume of sawdust to the soil significantly increased the hatching rate by 2.12 times (P < 0.05).

Table 2 Effects of different substrates on the hatching rate of oriental migratory locust eggs %

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Substrate	Substrate moisture	Hatching rate	
Soil	80	17.78 ±5.09 c	
Sawdust	80	71.11 ±5.09 a	
Soil + Sawdust	80	$37.78 \pm 6.94 \text{ b}$	

Effects of rice straw content by weight in the diet on the feed intake of oriental migratory locusts

As the content of rice straw in the diet increased, the daily intake of oriental migratory locusts showed a downward trend (Fig. 3). The oriental migratory locusts fed the most on A. sativa, at a

rate of 4.551 9 g/(locust \cdot d). The intake of oriental migratory locusts fed with diets added with 0%, 20%, and 40% of straw was 4.416 7, 4.355 6, and 4.300 0 g/(locust \cdot d), respectively, and there were no significant differences compared with the A. sativa control (P > 0.05). When the content of rice straw was 60%, the feed intake of oriental migratory locusts significantly decreased (P < 0.05). When the amount of rice straw added to the daily diet was 80%, the feed intake of oriental migratory locusts significantly decreased by 38.92% compared with the control group (P < 0.05). When fed with pure rice straw powder, its feed intake was the lowest, only 2.288 6 g/(locust \cdot d).

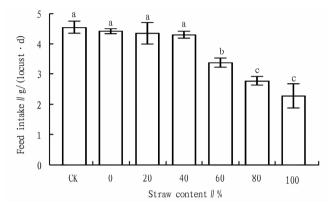


Fig. 3 Effects of rice straw added in the diet on the feed intake of oriental migratory locusts

Effects of rice straw content in the diet on the digestibility of oriental migratory locusts

The changes in rice straw content in the diet had a significant impact on the digestibility of oriental migratory locusts. As shown in Fig. 4, the digestibility of oriental migratory locusts on A. sativa was the highest, at 70.59%. The digestibility of the diet with 40% or less rice straw addition ranged from 52.27% to 61.33%, which decreased compared with the control group but did not show a significant difference (P > 0.05). When the content of rice straw was 60%, the digestibility of oriental migratory locusts in the diet significantly decreased (P < 0.05). When fed with pure rice straw powder, the digestibility was the lowest, at only 14.48%, significantly reduced by 79.49% and 76.39% compared with the control group and the basic diet, respectively (P < 0.05).

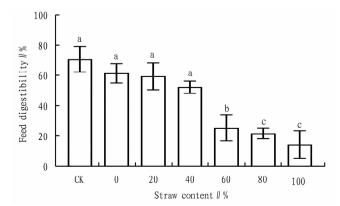


Fig. 4 Effects of rice straw content in the diet on the digestibility of oriental migratory locusts

Effects of rice straw content by weight in the diet on the weight gain of oriental migratory locusts

From Fig. 5, it can be seen that when the amount of rice straw added to the diet was less than or equal to 40%, there was no significant difference in the weight gain of individual oriental migratory locust from the control group (P > 0.05). When the content of rice straw reached 60%, the weight gain effect significantly deteriorated, with a reduction of more than 50% compared with the control group and the basic diet. When the rice straw content in the daily diet reached 100%, the weight gain of the insect body was only one-third of that of the control group (P < 0.05).

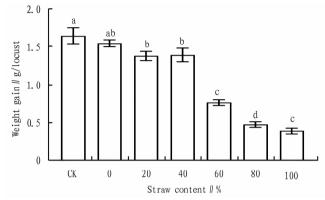


Fig. 5 Effects of rice straw content in the diet on the weight gain of oriental migratory locusts

Effects of rice straw content by weight in the diet on the survival rate of oriental migratory locusts

As shown in Table 3, with the increase of rice straw content in the diet, the survival rate of oriental migratory locusts showed a downward trend. When the rice straw content in the diet was below 20%, there was no significant difference in the survival rate of oriental migratory locusts compared with the control group (P > 0.05). When the rice straw content was 40%, the survival rate of oriental migratory locusts was 81.13%, which showed a significant difference compared with the control group (P < 0.05), but there were no significant differences compared with the basic diet and the treatment group with rice straw addition of 20% (P > 0.05). Among all treatment groups, the survival rate of oriental migratory locusts with 100% rice straw was the lowest, only 16.85%, significantly lower than the control group and the treatment groups with rice straw addition below 60% (P < 0.05).

Table 3 Effects of rice straw added in the diet on the survival rate of oriental migratory locusts

Straw content	Survival rate//%	
CK	90.01 ±1.90 a	
0%	86.07 ± 1.87 ab	
20%	83.80 ± 4.51 ab	
40%	$81.13 \pm 2.97 \text{ b}$	
60%	$61.47 \pm 1.00 \text{ c}$	
80%	$48.81 \pm 5.42 \text{ d}$	
100%	$16.85 \pm 5.90 \text{ d}$	

Effects of rice straw content in the diet on the eclosion rate of oriental migratory locusts

From Table 4, it can be seen that compared with the control group, the rice straw addition within 0-40% did not have a significant impact on the adult eclosion rate of oriental migratory locusts (P>0.05). When the addition of rice straw reached 60%, the eclosion rate significantly decreased (P<0.05), which was 57.28% and 55.61% lower than the control group and the basic diet, respectively. Feeding pure rice straw powder to oriental migratory locusts did not result in eclosion of adult insects.

Table 4 Effects of rice straw content in the diet on the eclosion rate of oriental migratory locusts

Straw content	Eclosion rate // %
CK	90.38 ± 2.52 a
0%	86.97 ± 2.37 a
20%	82.60 ± 1.45 a
40%	78.99 ± 1.24 a
60%	$38.61 \pm 17.02 \text{ b}$
80%	$6.33 \pm 4.73 \text{ c}$
100%	$0.00 \pm 0.00 \text{ c}$

Discussion

This study investigated two hatching conditions for oriental migratory locust eggs: temperature and substrate. The results showed that the eggs of oriental migratory locusts had a good hatching rate at 25-30 °C, up to 77. 22%-77.78%. When the temperature was below 20 °C, the hatching rate of locust eggs sharply decreased, and even at 15 °C, the eggs were not hatched, which might be due to the fact that 15 °C has not yet reached the hatching threshold temperature of oriental migratory locust eggs^[11]. The developmental threshold temperature of different insects varies [12-14]. The research on the hatching temperature of oriental migratory locusts can not only provide guidance for their breeding, but also predict their occurrence period and generation in a certain region using their developmental threshold temperature, so as to achieve timely prevention and control and avoid causing losses due to disasters [15-17]. The research results indicated that under 80% water content conditions, using sawdust as the hatching substrate for oriental migratory locust eggs was the best, while the effect of soil substrate was the worst. The reason might be that the local soil has high salinity, heavy viscosity and poor air permeability, and there are no oriental migratory locusts in this area. The soil conditions are not suitable for the survival and development of oriental migratory locust eggs. After the addition of sawdust, the soil structure was improved, significantly increasing the hatching rate of oriental migratory locust eggs (P < 0.05). Wheat bran is an important feed material in the cultivation of oriental migratory locusts, so wheat bran and white sugar were used to prepare a basic diet in this study. Studies have shown that sucrose can stimulate oriental migratory locusts to increase their feed intake^[18]. Wu et al. ^[19] have shown that carbohydrates could provide the energy required for insect life activities. Meanwhile, carbohydrates not only promote the feeding of many insects, but also have important effects on insect growth and reproduction.

In this study, the feed intake, digestibility, individual weight

gain, survival rate, and eclosion rate of oriental migratory locusts with 40% of rice straw addition had no significant differences from those with the basic diet (P > 0.05), and also, there were no significant differences in feed intake, digestibility and eclosion rate from the control group (P > 0.05). However, when the rice straw content in the diet reached 60%, all five indexes significantly decreased, significantly lower than the control group and the treatments with 0-40% of rice straw addition. Zhao [20] found through research that with the increase of straw content, the weight gain of the insect body generally presented a normal distribution, and the mortality rate increased, and the development duration gradually extended. Such phenomenon is related to the decrease in effective nutrients such as protein and fat that can be used by oriental migratory locusts in feed to accumulate dry matter.

Meanwhile, with the increase of rice straw content, oriental migratory locusts had difficulty in eclosion. The oriental migratory locusts, which fed on a diet with high rice straw content (>60%), died during molting or eclosion. The oriental migratory locusts, which fed on the diet with a rice straw content of 100%, had a mortality rate of up to 100% and an eclosion rate of 0. Single corn straw provides fewer nutrients such as energy, protein, and fat. The molting and eclosion processes of locusts require high energy reserves before their eclosion, and 100% of corn straw could not provide sufficient energy for the insect to complete the molting and eclosion processes, which is basically consistent with the conclusion of Delvi^[10]. When the amount of rice straw added was 40%, the diet was more suitable for locusts, and the growth and development status of locusts at this time was very similar to the basic diet and A. sativa. However, there was still a significant difference between artificial feed and green feed, so in the process of artificially raising oriental migratory locusts, it is not advisable to use only straw diets. At the 1st-3rd instar, the mouthparts of oriental migratory locusts are relatively underdeveloped and can only feed on some green feed, and they should feed on corn seedlings, wheat seedlings, ryegrass, oat grass, etc. After reaching the 4th instar, straw diet can be mainly fed, and green forage should also be regularly fed to supplement vitamins and nutrients.

This study was conducted in indoor breeding boxes from July to September, and there are still significant differences between the experimental environment and actual greenhouse breeding. Further experiments will be conducted in breeding greenhouses built outdoor in the future.

Conclusions

In this study, the effects of temperature and substrate on the hatching rate of oriental migratory locust eggs were investigated through indoor box culture, and different levels of rice straw were added to the diet to investigate their effects on the growth and development of oriental migratory locusts. The results showed that the eggs of oriental migratory locusts had a good hatching rate at $25-30~^{\circ}\mathrm{C}$, and sawdust was the best choice of hatching substrate. Adding 40% of rice straw into the diet could ensure the normal growth and development status of oriental migratory locusts while consuming as much rice straw as possible.

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