

NOTE

Open Access



# Effects of dietary variation on lignocellulose degradation and physiological properties of *Nicobium hirtum* larvae

Ni Putu Ratna Ayu Krishanti<sup>1,2\*</sup>, Yuki Tobimatsu<sup>1\*</sup>, Osama Ahmed Afifi<sup>1,3</sup>, Didi Tarmadi<sup>2</sup>, Setiawan Khoirul Himmi<sup>2</sup>, Toshiaki Umezawa<sup>1</sup>, Wakako Ohmura<sup>1</sup> and Tsuyoshi Yoshimura<sup>1^</sup>

## Abstract

We investigated the feeding behavior of larvae of the wood-destroying beetle *Nicobium hirtum* (Coleoptera: Anobiidae), an important wood pest in Japan, to determine the effects of dietary variation on lignocellulose degradation and larval growth and survival. Cultured colonies of *N. hirtum* larvae were fed artificial diets containing various amounts of starch (20, 50, and 80 wt%) mixed with hardwood (*Shorea*) lignocellulose. The polysaccharide degradation by *N. hirtum* was determined by chemical analyses of the initial artificial diets and fecal residues collected during the feeding experiment. Starch was preferentially decomposed when the larvae were fed the high-starch diet, whereas the decompositions of cellulose and hemicelluloses were more prominent when the larvae were fed medium- or low-starch diets. The larvae's size and survival were recorded periodically to determine the diets' effects on larval development. The survival rates ranged from 60 to 87% and were highest for the larvae fed the medium-starch diet and lowest for those fed the high-starch diet. Body size was highest in the larvae fed the high-starch diet. Fecal size increased along with the larval size increase. Overall, these results suggest that although starch is an essential carbon source for *N. hirtum* larval growth, lignocellulose also plays a key role as a nutrient that maintains the physiological activities of *N. hirtum* larvae and enhances their survival.

**Keywords** Dietary effect, Larval growth, *Nicobium hirtum*, Polysaccharide digestion, Wood-boring beetle

## Introduction

Wood-destroying insects such as wood borers and termites cause great economic damage in wood industries [1]. Coleopterans are known to exploit live, dead, and

nearly dead trees and new and old timber with varying degrees of decay [2]. With globalization and rapid urbanization, the increased use and movement of wood products worldwide ensure that drywood beetles will maintain their status as invasive and economically important pests [3, 4]. An infestation of wood by drywood beetles can be recognized by the presence of numerous "shot-holes" on the surface. This damage is primarily the result of the beetle larvae's feeding, and it varies based on the species and their feeding preferences [5]. Among the drywood beetles, anobiid beetles have a broad range of diet substrates and have attacked many historic wooden structures and artifacts in Japan [6, 7]. The larvae of anobiid beetles have strong-toothed mandibles that can seriously damage beams, joists, and other structural components of buildings. Unlike powder-post beetles, anobiid beetles

<sup>^</sup>Tsuyoshi Yoshimura—Deceased 18 May 2021

\*Correspondence:

Ni Putu Ratna Ayu Krishanti  
ratna.a.krishanti@gmail.com;  
Yuki Tobimatsu

ytohimatsu@rishi.kyoto-u.ac.jp

<sup>1</sup> Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan

<sup>2</sup> Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Jl. Raya Bogor Km.46 Cibinong, Bogor 16911, Indonesia

<sup>3</sup> Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo 11884, Egypt

are capable of attacking (by oviposition and larval feeding) both hardwood and softwood trees and digesting the cellulose of wood while being less dependent on starch [8–10]. Anobiid beetles can attack and infest many types of wood, regardless of the age of the tree [11, 12].

The nutritional challenges within the beetle digestive system are an essential factor affecting the insect's performance and survival. The major nutrients present in wood are starch, hemicelluloses, and cellulose. Larvae of some wood-boring beetles may be restricted to using only starch, but the larvae of other species can digest cellulose with the help of intestinal microbial symbionts [13, 14]. These symbiotic microorganisms support cellulose digestion by supplying the plant cell wall degradation enzymes (PCWDEs) needed to decompose the biopolymers that constitute lignocellulose, i.e., cellulose, hemicelluloses, and lignin, in woody plant cell walls [15–18].

Insects' consumption of a nutrient-rich diet can result in a higher body mass and a shorter life cycle [19, 20]. Anobiid beetles, which chew and eat timber, most likely evolved the ability to degrade wood lignocellulose as the source of nutrients. However, since these beetles typically prefer digesting starch to cellulose, cellulose itself may not be the ideal dietary component for the growth of anobiid beetle larvae [5, 13]. Anobiid beetles have a broad range of substrates for their diet. The majority of anobiid larvae feed on dry hardwood and softwood, and some prefer softwood to hardwood [8–10]. Variations in the levels of nutrients in the diet may affect the biology and digestive physiology of anobiid beetles [21–24].

Several varieties of wood-boring beetles have been successfully mass-reared after the examples provided by Kartika and Yoshimura [19, 25]. A culture of the anobiid beetle *Nicobium hirtum* (Coleoptera: Anobiidae), one of the most important wood product pests in Japan [6, 7], was successfully mass-bred in the laboratory [25]. Using this culture system, we recently showed that *N. hirtum* larvae can degrade starch, cellulose, and hemicelluloses in softwood and hardwood lignocellulose diets [25]. Such polysaccharide degradation could be facilitated by structural modifications of recalcitrant lignin polymers in the larval digestive system [26]. It was also observed that different types of lignocellulose diets (i.e., softwood vs. hardwood) have significant impacts on the survival and growth of *N. hirtum* larvae [26]. However, the details of how starch and lignocellulose content in the diet affects the physiological responses of *N. hirtum* larvae have not been known. In the present study, we compared the effects of artificial diets with different contents of starch and hardwood lignocellulose compounds on the survival and growth

of *N. hirtum* larvae. We also analyzed the chemical composition of both the original undigested diets and digested fecal residues to obtain information about the *N. hirtum* digestive system's degradation of the various diets.

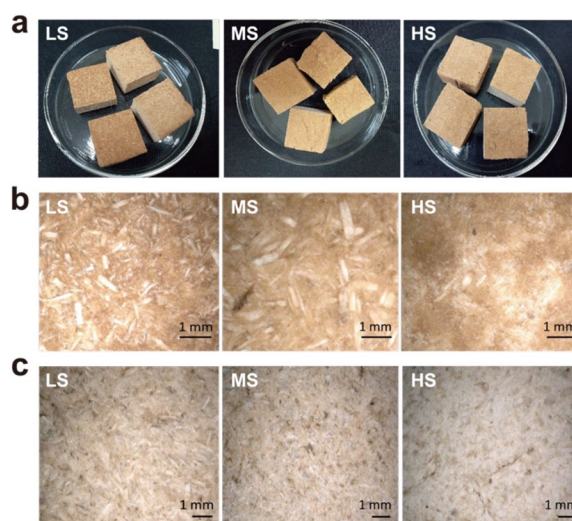
## Materials and methods

### Insects

*N. hirtum* larvae were raised at the Deterioration Organisms Laboratory (DOL), Research Institute for Sustainable Humansphere (RISH), Kyoto University, Japan [25, 26]. The larvae were raised in a controlled-growth chamber maintained at 26 °C and 65% relative humidity (RH) and a photoperiod of 12/12 h (light/dark) with an artificial diet consisting of 50% (w/w) starch, 24% (w/w) yeast extract, and 26% (w/w) *Shorea* sp. sawdust [19, 25–28]. Fifteen middle-stage (i.e., third- to fourth-instar) larvae were randomly collected for each feeding experiment and allocated for the subsequent feeding experiments.

### Artificial diet preparations

The artificial diets used in this study had three components: soluble starch (Nacalai Tesque, Kyoto, Japan), proteins from dried brewer's yeast powder (Asahi Food and Health Care, Tokyo, Japan), and sapwood lignocellulose (*Shorea* sp., 20–40 mesh size) used as filler. Low-starch (LS), medium-starch (MS), and high-starch (HS) diets (Fig. 1) were prepared with the starch and lignocellulose composition listed in Table 1. The three diet components were mixed with distilled water, compacted into blocks [20 (length) × 20 (width) × 20 (height) mm], oven-dried



**Fig. 1** Whole view (a) and magnified horizontal (b) and vertical (c) surfaces of artificial diets used in this study. LS, low-starch content diet. MS, medium-starch content diet. HS, high-starch content diet

**Table 1** Composition of artificial diets used in this study

Diet	Low-starch diet (LS)	Medium-starch diet (MS)	High-starch diet (LS)
Composition (% w/w)			
Starch	20	50	80
<i>Shorea</i> wood powder	70	40	10
Dry yeast	10	10	10

at 60 °C for 3 days, and kept in a chamber maintained at 26 °C and 65% RH until they were used for the feeding experiment.

### Feeding experiments

Fifteen larvae were subjected to each feeding experiment using the three (LS, MS, and HS) diets. Each larva was placed into a hole (3.5 mm diameter, 5 mm depth) in the center of each diet block and allowed to grow in an incubator chamber maintained at 25 °C and 65% RH for 6 months [25, 26]. The number of living larvae was recorded each month. At the end of the feeding experiments (after 6 months of feeding), the body weights of the larvae were recorded with an electronic balance (series GH-252, A&D, Tokyo, Japan), and the head width and body length of the larvae were measured with a digital microscope (VHX-5000, Keyence, Osaka, Japan). The cylindrical fecal residues were collected by the removal of non-fecal particles by passing the fecal samples through a 20–40 mesh screen. The collected fecal residues were subjected to chemical analyses (see below) and size measurements using the digital microscope as described by Zega et al. [29]. The remaining diet blocks were oven-dried at 60 °C for 3 days and weighed to determine the mass loss during the feeding period.

### Chemical analysis of the fecal residues and original diets

The collected fecal residues and original (undigested) diet samples were pulverized into fine powder with a Tissue-Lyzer II (Qiagen, Tokyo, Japan). First, the starch contents of the digested fecal residues and original diet samples were determined by measuring the glucose released by treatment with  $\alpha$ -amylase and amyloglucosidase (Megazyme, Wicklow, Ireland) [30]. The contents of other sugar units in crystalline cellulose and non-crystalline hemicellulosic glycans were subsequently determined by the two-step acid hydrolysis method using trifluoroacetic acid and sulfuric acid [31, 32]. The lignin contents were determined by a thioglycolic acid lignin assay [33]. For the analysis of the lignin composition, analytical thioacidolysis [31, 34] was performed on cell wall residue (CWR) samples prepared by a sequential extraction of the fecal

residues and original undigested diet samples with water and 80% ethanol in water [26, 32]. All chemical analyses were performed with three replicates.

### Statistical analysis

Student's *t*-test ( $p < 0.05$ ) and a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test ( $p < 0.05$ ) were performed using IBM SPSS Statistics ver. 27 (IBM Corp., Armonk, NY, USA).

## Results

### Starch and lignocellulose degradation in the different diets

To investigate the effect of lignocellulose diets on the digestive system of *N. hirtum*, we first characterized the carbohydrate contents, including starch, cellulose, and hemicelluloses in the artificial diets before digestion (i.e., the original undigested diets) and after digestion (the fecal residues). As shown in Table 2, the starch content of the digested fecal residues was significantly depleted compared to the corresponding undigested original diets ( $p < 0.05$ ). The depletion was greatest (ca. 50%) for the LS and MS diets and only 17% for the HS diet. The cellulosic glucan decreased by 17% in the LS diet and increased in the HS (by 48%) and MS (34%) diets. These results indicated that although the larvae digest starch in preference to cellulose, they utilize cellulose for nutrition if there is a shortage of starch in the diet. Like cellulose-derived glucose, some hemicellulosic sugars, such as glucose and xylose, were clearly and relatively increased in the digested fecal residues from the MS and HS diets but not in the digested fecal residues from the LS diet (Table 2). This result suggested that similar to cellulosic glucose, hemicellulosic glucose and xylose were at least partially digested by the larvae when there was a shortage of starch in the diet. On the other hand, the mannose content decreased significantly by 40%–48% compared to the original undigested diets in all of the fecal residue samples (Table 2). Therefore, the larvae may digest mannose in preference to other cellulosic and hemicellulosic sugars, even in the presence of abundant starch in the diet. Conversely, the arabinose content increased in all of the fecal residue samples (Table 2), suggesting that the larvae tend to avoid arabinose, even when there is a shortage of starch in the diet.

The lignin content was relatively increased by more than 50% in the digested fecal residues from the LS and MS diets (Table 2), indicating that the majority of lignin polymers remained in the fecal residue. Nevertheless, the increase in lignin was relatively low (19%), with no significant differences between the original LS diet and

**Table 2** Starch and lignocellulose compositional analyses of original artificial diets and fecal residues of *N. hirtum* larvae

Content (mg/g diet or fecal residues)	Low-starch diet (LS)			Medium-starch diet (MS)			High-starch diet (HS)		
	Original	Digested	% Change	Original	Digested	% Change	Original	Digested	% Change
Starch <sup>a</sup>	137.7 ± 4.7	64.6 ± 1.2**	− 53	343.2 ± 20.6	182.6 ± 13.4**	− 47	562.9 ± 13.3	467.5 ± 19.6**	− 17
Cellulose <sup>b</sup>	291.7 ± 8.0	242.1 ± 14.8*	− 17	167.2 ± 1.5	224.6 ± 12.8**	34	85.1 ± 1.1	125.9 ± 3.6**	48
Hemicelluloses <sup>c</sup>									
Glucose	18.8 ± 0.7	20.0 ± 1.0	6	11.4 ± 0.2	15.1 ± 1.0**	32	9.1 ± 0.3	10.0 ± 0.5*	10
Xylose	33.6 ± 1.6	33.9 ± 1.3	1	19.6 ± 0.7	24.0 ± 1.0**	22	11.9 ± 0.3	14.9 ± 0.7**	25
Mannose	7.5 ± 0.2	3.9 ± 0.2**	− 48	4.7 ± 0.0	2.8 ± 0.0**	− 40	5.3 ± 0.2	2.8 ± 0.1**	− 47
Galactose	3.9 ± 0.1	3.5 ± 0.1*	− 10	2.2 ± 0.1	2.6 ± 0.1**	18	1.6 ± 0.1	1.4 ± 0.1*	− 13
Arabinose	1.5 ± 0.1	1.8 ± 0.1**	20	1.0 ± 0.1	1.4 ± 0.1**	40	0.5 ± 0.0	0.8 ± 0.1**	60
Total	65.2 ± 2.5	63.1 ± 2.6	− 3	38.9 ± 0.6	45.9 ± 1.9**	18	28.4 ± 0.8	29.9 ± 1.4	5
Lignin <sup>d</sup>	206.5 ± 10.4	246.3 ± 7.5	19	136.3 ± 2.6	215.6 ± 10.3**	58	57.1 ± 3.1	87.0 ± 4.2**	52

Values are mean ± standard deviation from three independent runs (n = 3). Asterisks indicate significant differences between fecal residues and original diets [Student's t-test, p < 0.05 (\*), p < 0.01 (\*\*)]. Bold values of %Change indicate significant decrease or increase in fecal residues compared with original diets

<sup>a</sup> Glucose released via treatment with α-amylase and amyloglucosidase

<sup>b</sup> Glucose released from trifluoroacetic acid-insoluble fractions

<sup>c</sup> Monosaccharides released from trifluoroacetic acid-soluble fractions

<sup>d</sup> Determined by thioglycolic acid lignin assay

**Table 3** Thioacidolysis-based lignin compositional analysis of original artificial diets and fecal residues of *N. hirtum* larvae

	Low-starch diet (LS)		Medium-starch diet (MS)		High-starch diet (HS)	
	Original	Digested	Original	Digested	Original	Digested
Lignin monomer composition (% per S + G + H)						
Syringyl, S	55.1 ± 0.4	55.7 ± 0.1	54.8 ± 0.3	55.2 ± 0.3	55.2 ± 0.7	55.3 ± 0.5
Guaiacyl, G	44.8 ± 0.4	44.1 ± 0.1	45.1 ± 0.3	44.6 ± 0.3	44.6 ± 0.7	44.5 ± 0.5
p-Hydroxyphenyl, H	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
S/G ratio	1.23 ± 0.2	1.26 ± 0.1	1.22 ± 0.2	1.24 ± 0.2	1.24 ± 0.4	1.24 ± 0.3

Thioacidolysis was performed on cell wall residue (CWR) samples prepared by sequential extraction of the original diet and fecal residue samples collected after 6-month feeding. Values are mean ± standard deviation from three independent runs (n = 3). No statistical significance (Student's t-test, p < 0.05) was detected between the lignin-derived monomers released from the fecal residues and original diets

S syringyl-type monomer, G guaiacyl-type monomer, H p-hydroxyphenyl-type monomer, LS low-starch diet, MS medium-starch diet, HS high-starch diet

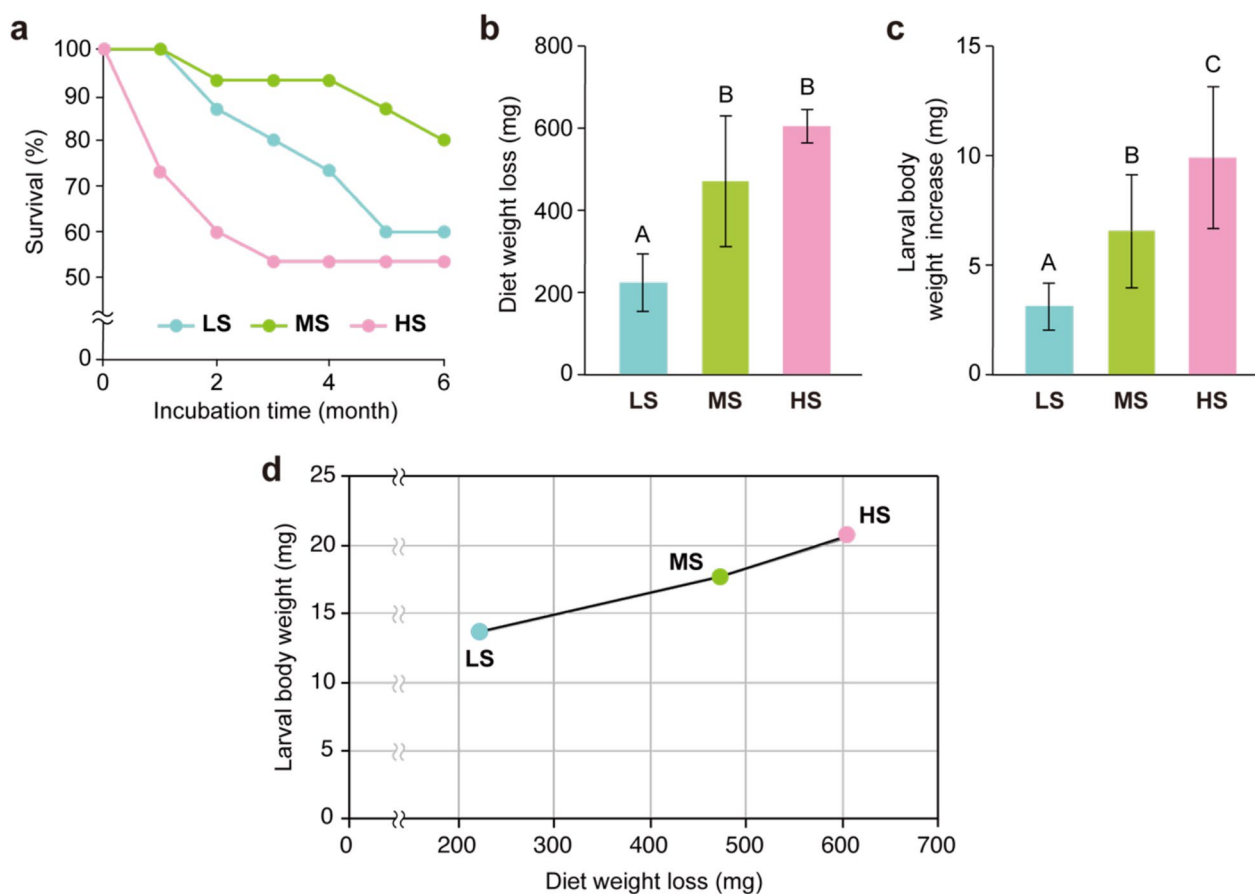
the digested fecal residue samples from the LS diet. This result suggested that, as we have observed in the digestion of wood lignocellulose by *N. hirtum* larvae [26], lignin could be at least partially degraded along with starch and lignocellulosic polysaccharides in the LS diet samples. However, the lignin compositional analysis by analytical thioacidolysis did not detect any significant differences between the original and digested samples in this study (Table 3).

**Larval survival and growth characteristics**

We next investigated the effects of dietary variation on the growth characteristics of *N. hirtum* larvae. The larval survival during 6 months of feeding is depicted in Fig. 2a. The larvae fed the HS diet showed a rapid decline in survival from the beginning of feeding, reaching a plateau at 53% survival after 3 months of feeding. In contrast, the

larvae fed the MS or LS diet maintained a 100% survival rate at 1 month of feeding; the survival rates started to decline after 2 months of feeding, more slowly than the decline observed in the larvae fed the HS diet. The reduction in the survival rate was more remarkable for the larvae fed the HS diet than for those fed the MS diet. Consequently, the final survival rates of the larvae after 6 months of feeding were MS (80%) > LS (60%) > HS (53%) (Fig. 2a, Additional file 1: Table S1). These results indicate the importance of the diet composition, i.e., the balance of starch versus lignocellulose, for the survival of *N. hirtum* larvae.

Both the diet weight loss (Fig. 2b, Additional file 1: Table S1) and the increase in the body weight of the larvae that survived after 6 months of feeding (Fig. 2c, Additional file 1: Table S1) were positively correlated with the starch content of the diet (HS ≥ MS > LS). It was



**Fig. 2** Physiological characterizations of *N. hirtum* larvae fed with artificial diets. Survival rate (a) during the feeding period, and diet weight loss (b), larvae body weight increase (c) and the relationship between diet weight loss and larval body weight (d) after 6 months of feeding period are shown. Value refers to mean ± standard deviation. Different letters (A, B, and C) indicate significant differences among the diet treatments (Tukey HSD test;  $p < 0.05$ ). Individual diet weight and larval body weight data are listed in Additional file 1: Table S1. LS, low-starch content diet. MS, medium-starch content diet. HS, high-starch content diet

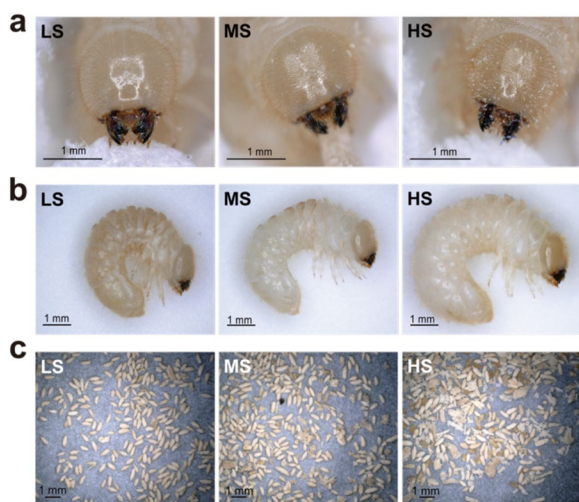
also apparent that the larval body weight was linearly correlated with the diet weight loss (Fig. 2d, Additional file 1: Table S1). The effects of dietary variation on the physical characteristics of the larvae were further investigated (Fig. 3, Table 4). Body length (Fig. 3b) and total body weight were also positively correlated with the starch content of the diet (HS>MS>LS), whereas head width (Fig. 3a) was not affected by the dietary variation (Table 4). Collectively, these results emphasize the importance of dietary starch for *N. hirtum* larval growth, although, as demonstrated above (Fig. 2a), appropriate amounts of lignocellulose in the diet are important for the survival of *N. hirtum* larvae.

**Morphology of digested fecal residues**

We also investigated the morphology (width, length, maximum diameter, minimum diameter, and perimeter)

of the cylindrical fecal residue pellets for each dietary treatment (Fig. 3c). A total of 1,140 fecal residue pellets from larvae fed the three different diets (421, 422, and 297 pellets from larvae fed the LS, MS, and HS diets, respectively) were collected and measured by an imaging analysis [29]. As shown in Table 4, the mean values of the maximum diameter (length) and minimum diameter (widest width) differed among the diets. Because the larval body length and weight were greatest for the larvae fed the HS diet (Fig. 2, Table 4), the size of their fecal residue pellets was also greatest, followed by the larvae fed the MS diet and those fed the LS diet (Table 4).

The shape ratio (SR) score indicates the general outline of fecal residue samples, and the enclosure ratio (ER) score is a further classification of the SR. Interestingly, the differences in diet did not affect the SR or the ER; there were no significant differences in the SR or ER among the diets. Notably, differences in the diet affected



**Fig. 3** Morphology of *N. hirtum* larvae after feeding with artificial diets for 6 months. Facial (a), and whole body (b), of the larvae and cylindrical fecal residue pellets (c). LS, low-starch content diet. MS, medium-starch content diet. HS, high-starch content diet

the size of the fecal residue samples of the larvae but did not affect the shape or outline of the fecal residue pellets (Table 4).

**Discussion**

Like many other xylophagous insects [35, 36], the larvae of the anobiid beetle *N. hirtum* prefer to utilize starch rather than lignocellulose components as a major source of nutrients (Table 2). However, we observed that *N. hirtum* larvae seemed to adjust their feeding preference to lignocellulose over starch depending on the availability of starch in the diet (Table 2). With the high availability of starch, the larvae digested primarily starch for assimilation, and when the availability of starch was low, the larvae degraded lignocellulosic polysaccharides such as cellulose and a part of hemicelluloses along with starch (Table 2). These results suggest that *N. hirtum* larvae tend to utilize lignocellulosic polysaccharides as an alternative

carbon source when there is a shortage of starch, which is a more preferential carbon source.

Anobiid beetles can attack both softwood and hardwood indiscriminately. When fed on softwood (pine) lignocellulose, the larvae of *N. hirtum* are highly capable of degrading hemicellulosic polysaccharides, especially glucomannan, which is the major hemicellulosic component in softwood lignocellulose [26]. In the present study, a preferential digestion of hemicellulosic mannan was also observed when a hardwood (*Shorea* sp.) was used as the primary source of lignocellulose (Table 2). Even though xylan (glucuronoxylan) is the major hemicellulosic sugar in hardwood, *N. hirtum* larvae consumed mannan in preference to other hemicellulosic sugars (such as xylan), even with the HS diet. Xylophagous insects must overcome the lignin barrier to efficiently degrade plant cell wall polysaccharides. Our present experiment revealed that the lignin content largely increased after digestion, particularly with the MS and HS diets, due to the preferential degradation of starch and lignocellulose polysaccharides (Table 2). The increase in the lignin content was less prominent with the LS diet (Table 2). Thus, as our previous analysis of lignocellulose degradation by *N. hirtum* larvae showed [26], with the present LS diet treatment, lignin may have been at least partially degraded to further enhance the polysaccharide degradation. Such structural modifications and degradation of lignin by other xylophagous insects, especially termites, have been documented [32, 37–41].

There were clear positive correlations between the starch content in the diet and the body size and weight of the larvae, as well as the fecal size (Fig. 2, Table 4). These results affirmed that starch is an essential nutrient in the growth of *N. hirtum* larvae. However, interestingly, the larvae fed the starch-rich HS diet displayed a more prominent decline in the survival rate compared to the larvae fed the lignocellulose-rich LS and MS diets (Fig. 2a). Therefore, although the larvae need

**Table 4** Larval and fecal size measurements of *N. hirtum* fed on different artificial diets

Diet	Larval size			Fecal size				
	Head width (mm)	Body length (mm)	Body weight (mg)	Width (µm)	Length (µm)	Perimeter (µm)	Shape ratio (SR)	Enclosure ratio (ER)
Low-starch diet (LS)	1.6 ± 0.1 <sup>A</sup>	4.3 ± 0.2 <sup>A</sup>	13.7 ± 1.7 <sup>A</sup>	267.0 ± 9.9 <sup>A</sup>	636.8 ± 19.8 <sup>A</sup>	1708.6 ± 61.7 <sup>A</sup>	0.4 <sup>A</sup>	0.9 <sup>A</sup>
Medium-starch diet (MS)	1.6 ± 0.1 <sup>A</sup>	4.7 ± 0.2 <sup>B</sup>	17.7 ± 1.9 <sup>B</sup>	287.8 ± 4.9 <sup>A</sup>	710.7 ± 10.9 <sup>B</sup>	1945.4 ± 43.5 <sup>B</sup>	0.4 <sup>A</sup>	1.0 <sup>A</sup>
High-starch diet (HS)	1.6 ± 0.0 <sup>A</sup>	5.5 ± 0.3 <sup>C</sup>	21.0 ± 2.6 <sup>C</sup>	309.5 ± 7.1 <sup>B</sup>	750.9 ± 7.7 <sup>C</sup>	2076.5 ± 64.8 <sup>B</sup>	0.4 <sup>A</sup>	1.0 <sup>A</sup>

Different letters (A, B and C) indicate significant differences among the diet treatments (Tukey HSD test, *p* < 0.05)

starch as a continuous source of carbon and energy, lignocellulose also provides vital nutrition to support the physiology of *N. hirtum* larvae for survival.

The quantity and quality of food are significant determinants of body size and other characteristics of adult insects, affecting their growth rate, development time, hormones related to metamorphosis, and combinations of these factors during the larval stages. Differences in body size resulting from differences in the larval feeding period have been reported by Shafiei et al. [42] and Emlen [43], who observed that a variation of the food provided during larval development can trigger metamorphosis pupae. A positive correlation between food quantity and quality was reported for rhinoceros, dung, and stag beetles [42, 44, 45]. In the dung beetle *Onthophagus taurus*, only larvae that reach a critical weight threshold before pupation become horned (major morph) males [42]. The main cause of intraspecific differences in body size among wild stag beetles is thought to be the availability of food (decayed wood) in their natural habitat [45–47]. Larvae of the stag beetle *Cyclommatus metallifer* reared on greater amounts of food had greater mandibular lengths, longer larval periods, and higher pupal weights than those reared on lower amounts of food [45]. Nutrient levels and protein deficiencies in the diet often affect diet suitability and larval survival [48]. Intriguingly, study of the termite *Coptotermes formosanus* reported that lignin in lignocellulose-based diets was hardly utilized as a major nutrient source but had marked positive effects on the survival rate of the termite workers as well as the maintenance of the lignocellulose-degrading protistan gut symbionts when fed with polysaccharides [49].

Overall, our present results emphasize the importance of lignocellulose components as nutrients that maintain the physiological activities of *N. hirtum* larvae and enhance their survival, although starch is an essential nutrient promoting the growth of the larval body. The survival of xylophagous insects may depend on physiological mechanisms such as insect responses to physicochemical changes in host plants and food, morphological characteristics of digestive organs, and biochemical characteristics of the principal digestive enzymes, as well as on the gut symbiotic fauna. Analyses of the gut symbiotic microbial community of *N. hirtum* fed different diets would provide further insights into the nutritional effects of lignocellulose and the mechanisms that underlie the biodegradation of lignocellulose in the gut digestive system.

## Conclusions

The lignocellulose decomposition and physiological responses of *N. hirtum* larvae fed artificial diets with varied starch and lignocellulose composition were investigated. Chemical analyses of the fecal residues and original undigested diets demonstrated that the *N. hirtum* larvae digested primarily starch when they were fed the high-starch diet, whereas the decomposition of cellulose and hemicelluloses (especially mannan) became more prominent when the larvae were fed the medium- and low-starch diets. The larvae fed the high-starch diet grew faster and developed larger bodies, albeit with a prominently declined survival rate, compared to the larvae fed the medium- and low-starch diets. Thus, although starch is important for larval growth, lignocellulose is also important for maintaining the physiological activities of *N. hirtum* larvae and enhancing their survival rate. Further studies of the gut symbiotic microbial community of *N. hirtum* may help to elucidate the mechanisms of lignocellulose biodegradation by xylophagous insects and contribute to the development of innovative strategies for the biomass conversion industry.

## Abbreviations

ANOVA	Analysis of variance
CWR	Cell wall residue
ER	Enclosure ratio
G	Guaiacyl
HS	High-starch
HSD	Honestly significant difference
LS	Low-starch
MS	Medium-starch
PCWDE	Plant cell wall-degrading enzyme
RH	Relative humidity
S	Syringyl
SR	Shape ratio

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s10086-022-02074-x>.

**Additional file 1: Table S1.** Diet weight loss and larva weight increase of *N. hirtum* larvae.

## Acknowledgements

The authors thank Dr. Izumi Fujimoto and Mr. Akio Adachi (RISH, Kyoto University) for preparing the insects and technical assistance during the experiment. This study was partly supported by the Deterioration Organisms Laboratory/LSF Cooperative Program and used the facilities in the DASH/FBAS at RISH, Kyoto University, Japan.

## Author contributions

NPRAK, YT, TU, WO, and TY conceived the research and designed experiments. NPRAK, YT, and OAA performed experiments and analyzed data with DT, SKH, and TU. NPRAK, WO, and YT wrote the manuscript with help from all other authors. All authors read and approved the final manuscript.

### Funding

This study was supported in part by the Japan Society for the Promotion of Science KAKENHI grants (JP20H03044) and Japan-ASEAN Science Technology Innovation Platform (JASTIP) Program, a collaborative project of National Research and Innovation Agency (BRIN) and Kyoto University, Japan under Work-Package (WP) 3: Bioresources and Biodiversity. NPRAK acknowledges a Ph.D. Scholarship from MEXT of Japan. OAA acknowledges a scholarship (PD36) from the Ministry of Higher Education of the Arab Republic of Egypt.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

### Competing interests

The authors declare that they have no competing interests.

Received: 2 September 2022 Accepted: 26 December 2022

Published online: 06 January 2023

### References

- Creffield JW (1996) Wood destroying insect: wood borers and termites. CSIRO Publishing, Melbourne
- Ewart D (2014) Urban timber pest beetles: risks and management. In: Dhang P (ed) Urban insect pests: sustainable management strategies. CABI Publishing, Oxfordshire
- Mito T, Uesugi T (2004) Invasive alien species in Japan: the status quo and the new regulation for prevention of their adverse effects. *Glob Environ Res* 8:171–193
- Lewis V, Forschler B (2014) Management of drywood termites: past practices, present situation, and future prospects. In: Dhang P (ed) Urban insect pests: sustainable management strategies. CABI Publishing, Oxfordshire
- Pournou A (2020) Wood deterioration by insects. In: Anastasia P (ed) Biodeterioration of wooden cultural heritage. Springer, Cham
- Yamano K (2003) Insect damage to wooden cultural properties and countermeasures. *Wood Ind* 58:576–581 (in Japanese)
- Komine Y (2019) The deathwatch beetles as a pest to cultural properties. *Tobunkennews* 69:45–47 (in Japanese)
- Parkin EA (1940) The digestive enzymes of some wood-boring beetle larvae. *J Exp Biol* 17:364–377. <https://doi.org/10.1242/jeb.17.4.364>
- Robinson WH (2005) Handbook of urban insects and arachnids. Cambridge University Press, Cambridge
- Resh VH, Carde RT (2009) Insecta overview. In *Encyclopedia of insects*, 5th edn. Academic Press, London
- Potter MF (1993) Diagnosis, management of powder-post beetles. *Pest Control Technol* 21(4):10
- Jackman JA. Structure-infesting wood-boring beetles. Texas A&M Agrilife Extension. <https://counties.agrilife.org/liberty/files/2020/05/Structure-Infesting-Wood-Boring-Beetles-Publ.-E-394.pdf>. Accessed 30 Nov 2022.
- Chiappini E, Aldini RN (2011) Morphological and physiological adaptations of wood-boring beetle larvae in timber. *J Ent Acarol Res Ser II* 43:47–59. <https://doi.org/10.4081/jear.2011.47>
- Douglas AE (2009) Nutritional ecology: the microbial dimension in insect nutritional ecology. *Func Ecol* 23:38–47. <https://doi.org/10.1111/j.1365-2435.2008.01442.x>
- Dohet L, Gregoire JC, Berasategui A, Kaltenpoth M, Biedermann HW (2016) Bacterial and fungal symbionts of parasitic *Dendroctonus* bark beetles. *FEMS Microbiol Ecol* 92:1–12. <https://doi.org/10.1093/femsec/fiw129>
- Garcia-Fraile P (2017) Roles of bacteria in the bark beetle halobiont - how do they shape this forest pest? *Ann Appl Biol* 172:111–125. <https://doi.org/10.1111/aab.12406>
- McKenna DD, Shin S, Ahrens D, Balke M, Beza-Beza C, Clarke DJ, Beutel RG (2019) The evolution and genomic basis of beetle diversity. *Proc Natl Acad Sci* 116:24729–24737. <https://doi.org/10.1073/pnas.1909655116>
- Goodell B, Winandy JE, Morrell JJ (2020) Fungal degradation of wood: emerging data, new insights, and changing perceptions. *Coatings* 10:1210. <https://doi.org/10.3390/coatings10121210>
- Kartika T, Yoshimura T (2015) Evaluation of wood and cellulosic materials as fillers in artificial diets for *Lyctus africanus* Lesne (Coleoptera: Bostrichidae). *Insects* 6:696–703. <https://doi.org/10.3390/insects6030696>
- Tremmel M, Muller C (2013) Insect personality depends on environmental conditions. *Behav Ecol* 24:386–392. <https://doi.org/10.1093/beheco/ars175>
- Tremmel M, Muller C (2014) Diet dependent experience and physiological state shape the behavior of a generalist herbivore. *Physiol Behav* 129:95–103. <https://doi.org/10.1016/j.physbeh.2014.02.030>
- Naya DE, Lardies MA, Bozinovic F (2007) The effect of diet quality on physiological and life-history traits in the harvestman *Pachylus paessleri*. *J Insect Physiol* 53:132–138. <https://doi.org/10.1016/j.jinsphys.2006.11.004>
- Borzoui E, Naseri B, Namin FR (2015) Different diets affecting biology and digestive physiology of the kapra beetle, *Trogoderma granarium* everts (coleoptera: dermestidae). *J Stored Prod Res* 62:1–7. <https://doi.org/10.1016/j.jspr.2015.03.003>
- Borzoui E, Naseri B (2016) Wheat cultivars affecting life history and digestive amylolytic activity of *Sitotroga cerealella* olivier (Lepidoptera: gelechiidae). *Bull Entomol Res* 106:464–473. <https://doi.org/10.1017/s000748531600016x>
- Fujimoto I, Yoshimura T (2018) Establishment of mass cultures of wood-attacking beetles. *Sustain Humanosp* 14:13
- Krishanti NPRA, Tobimatsu Y, Miyamoto T, Fujimoto I, Kartika T, Umezawa T, Hata T, Yoshimura T (2022) Structural basis of lignocellulose deconstruction by the wood-feeding anobiid beetle *Nicobium hirtum*. *J Wood Sci* 68:10. <https://doi.org/10.1186/s10086-022-02017-6>
- Kartika T, Nobuhiro S, Yoshimura T (2015) Identification of esters as novel aggregation pheromone components produced by the male powder-post beetle, *Lyctus africanus* Lesne (coleoptera: lyctinae). *PLoS ONE* 10:e0141799. <https://doi.org/10.1371/journal.pone.0141799>
- Kartika T, Shimizu N, Himmi SK, Guswenriwo I, Tarmadi D, Yusuf S, Yoshimura T (2021) Influence of age and mating status on pheromone production in a powder-post beetle *Lyctus africanus* (coleoptera: lyctinae). *Insects* 12:8. <https://doi.org/10.3390/insects12010008>
- Zega SLD, Fajar A, Himmi SK, Adi DS, Tarmadi D, Nandika D, Yusuf S (2020) Examination of fecal pellet physical characteristics of an invasive drywood termite, *Cryptotermes dudleyi* (isoptera: kalotermitidae): a potential approach for species marker and non-destructive monitoring method. *IOP Conf Ser Mater Sci Eng* 935:012050
- Hattori T, Murakami S, Mukai M, Yamada T, Hirochika H, Ike M, Tokuyasu K, Suzuki S, Sakamoto M, Umezawa T (2012) Rapid analysis of transgenic rice straw using near-infrared spectroscopy. *Plant Biotechnol* 29:359–366. <https://doi.org/10.5511/plantbiotechnology.12.0501a>
- Lam PY, Tobimatsu Y, Takeda Y, Suzuki S, Yamamura M, Umezawa T, Lo C (2017) Disrupting flavone synthase II alters lignin and improves biomass digestibility. *Plant Physiol* 174:972–985. <https://doi.org/10.1104/pp.16.01973>
- Tarmadi D, Tobimatsu Y, Yamamura M, Miyamoto T, Miyagawa Y, Umezawa T, Yoshimura T (2018) NMR studies on lignocellulose deconstructions in the digestive system of the lower termite *Coptotermes formosanus* Shiraki. *Sci Rep* 8:1290. <https://doi.org/10.1038/s41598-018-19562-0>
- Suzuki S, Suzuki Y, Yamamoto N, Hattori T, Sakamoto M, Umezawa T (2009) High-throughput determination of thioglycolic acid lignin from rice. *Plant Biotechnol* 26:337–340. <https://doi.org/10.5511/plantbiotechnology.26.337>
- Yamamura M, Hattori T, Suzuki S, Shibata D, Umezawa T (2012) Microscale thioacidolysis method for the rapid analysis of substructures in lignin. *Plant Biotechnol* 29:419–423. <https://doi.org/10.5511/plantbiotechnology.12.0627a>
- Tokuda G (2019) Plant cell wall degradation in insects: recent progress on endogenous enzymes revealed by multi-omics technologist. *Adv Insect Physiol* 57:97–136. <https://doi.org/10.1146/annurev-ento-112408-085319>
- Watanabe H, Tokuda G (2010) Cellulolytic systems in insects. *Annu Rev Entomol* 55:609–632. <https://doi.org/10.1146/annurev-ento-112408-085319>
- Katsumata KS, Jin Z, Hori K, Iiyama K (2007) Structural changes in lignin of tropical woods during digestion by termite *Cryptotermes brevis*. *J Wood Sci* 53:419–426. <https://doi.org/10.1007/s10086-007-0882-z>



38. Geib SM, Filley TR, Hatcher PG, Hoover K, Carlson JE, Jimenez-Gasco MdM, Nakagawa-Izumi A, Sleighter RL, Tien M (2008) Lignin degradation in wood-feeding insects. *Proc Natl Acad Sci USA* 105:12932–12937. <https://doi.org/10.1073/pnas.0805257105>
39. Ke J, Laskar DD, Singh D, Chen S (2011) *In situ* lignocellulosic unlocking mechanisms for carbohydrate hydrolysis in termites: crucial lignin modification. *Biotechnol Biofuel* 4:1–12. <https://doi.org/10.1186/1754-6834-4-17>
40. Ceja-Navarro JA, Karaoz U, Bill M, Hao Z, White RA III, Ramanculova L et al (2019) Gut anatomical properties and microbial functional assembly promote lignocellulose deconstruction and colony subsistence of a wood-feeding beetle. *Nat Microbiol* 4:864–875. <https://doi.org/10.1038/s41564-019-0384-y>
41. Dumond L, Lam P, G-van E, Kabel M, Mounet F, Grima-Pettenati G, Tobimatsu Y, Hernandez-Raquet G (2021) Termite gut microbiota contribution to wheat straw delignification in anaerobic bioreactors. *ACS Sustain Chem Eng* 9:2191–2202. <https://doi.org/10.1021/acssuschemeng.0c07817>
42. Shafiei M, Moczek AP, Nijhout HF (2001) Food availability controls the onset of metamorphosis in the dung beetle, *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Physiol Entomol* 26:173–180. <https://doi.org/10.1046/j.1365-3032.2001.00231.x>
43. Emlen DJ (1997) Diet alters male horn allometry in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc R Soc Lond Series Biol Sci* 264:567–574. <https://doi.org/10.1098/rspb.1997.0081>
44. Karino K, Seki N, Chiba M (2004) Larval nutritional environment determines adult size in Japanese horned beetles, *Allomyrina dichotoma*. *Ecol Res* 19:663–668. <https://doi.org/10.1111/j.1440-1703.2004.00681.x>
45. Gotoh H, Fukaya K, Miura T (2012) Heritability of male mandible length in the stag beetle *Cyclommatus metallifer*. *Entomol Sci* 15:430–433. <https://doi.org/10.1111/j.1479-8298.2012.00527.x>
46. Harvey DJ, Gange AC (2006) Size variation and mating success in the stag beetle, *Lucanus cervus*. *Physiol Entomol* 31:218–226. <https://doi.org/10.1111/j.1365-3032.2006.00509.x>
47. Gotoh H, Cornette R, Koshikawa S, Okada Y, Lavine LC, Emlen DJ, Miura T (2011) Juvenile hormone regulates extreme mandible growth in male stag beetles. *PLoS ONE* 6:e21139. <https://doi.org/10.1371/journal.pone.0021139>
48. Chen Y, Ni X, Buntin GD (2008) Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. *Entomol Exp Appl* 126:244–255. <https://doi.org/10.1111/j.1570-7458.2007.00662.x>
49. Tarmadi D, Yoshimura T, Tobimatsu Y, Yamamura M, Umezawa T (2017) Effects of lignin as diet components on the physiological activities of a lower termite, *Coptotermes formosanus* Shiraki. *J Insect Physiol* 103:57–63. <https://doi.org/10.1016/j.jinsphys.2017.10.006>

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

---

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)

---