

# The Influence of the Size of BN NSs on Silkworm Development and Tissue Microstructure

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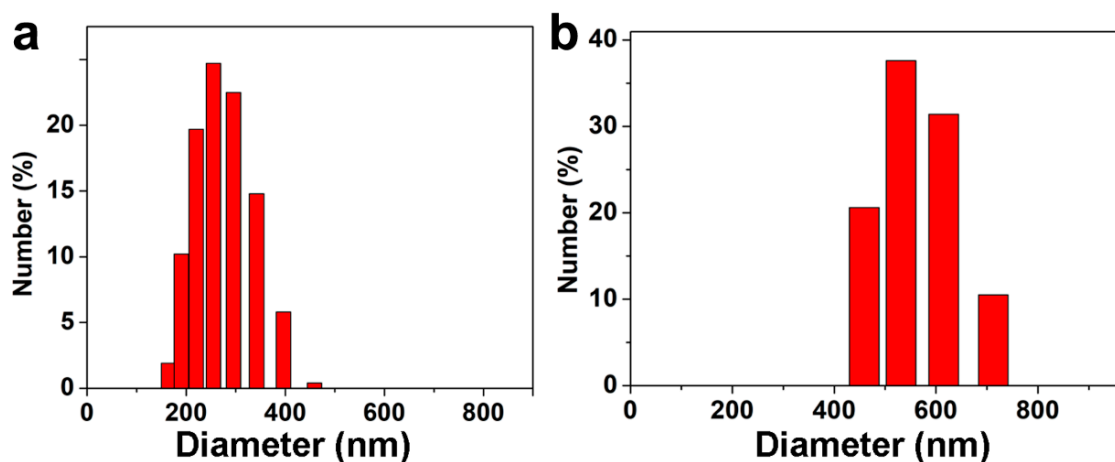
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## Results and Discussions

### Size distribution of BN NSs



**Figure S1.** Size distribution of BN NSs-1 a) and BN NSs-2 b).

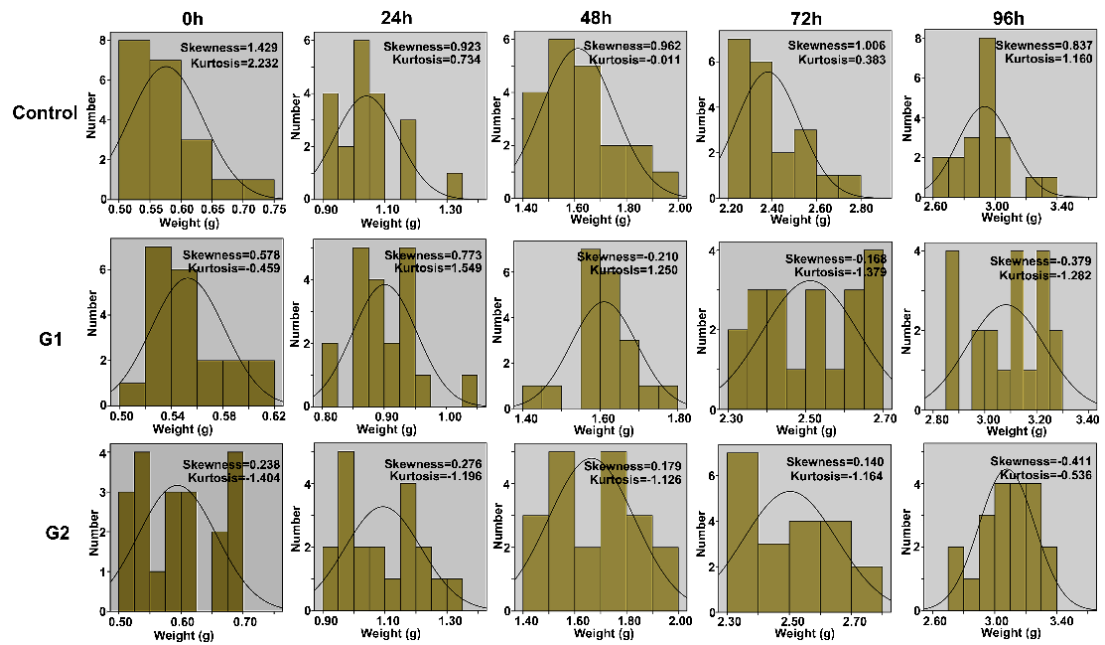
DLS data (Figure S1) show that the diameter of BN NSs-1 (Figure S1a) ranges from 164.2 nm to 458.7 nm, with an average diameter of 270.7 nm, and BN NSs-2 ranges from 458.7 nm to 712.4 nm, with an average diameter of 562.2 nm. BN NSs-1 and BN NSs-2 have PDI of 0.295 and 0.863, respectively.

### Statistical Analysis

**Table S1.** The average weight and length of silkworm larvae (qiufeng × baiyu).

Sample	Average Weight (g)	Average Length (cm)
Control (0 h)	0.576	4.1
G1 (0 h)	0.553	4.0
G2 (0 h)	0.594	4.1
Control (24 h)	1.040	5.0
G1 (24 h)	0.901	4.7
G2 (24 h)	1.094	5.1
Control (48 h)	1.613	5.7
G1 (48 h)	1.610	5.6

G2 (48 h)	1.665	5.8
Control (72 h)	2.381	6.4
G1 (72 h)	2.511	6.5
G2 (72 h)	2.503	6.5
Control (96 h)	2.932	6.8
G1 (96 h)	3.082	6.9
G2 (96 h)	3.078	6.9



**Figure S2.** One sample Kolmogorov–Smirnov test used to assess the data of silkworm larvae (qiufeng × baiyu) weight.

**Table S2.** Kruskal–Wallis test used to assess the data of silkworm larvae (qiufeng × baiyu) weight from different groups (g) by changing time.

		Number	Average Grade	$\chi^2$	df	Progressive Significance
0h	Control	20	31.25	3.568	2	0.168
	G1	20	24.95			
	G2	20	35.30			

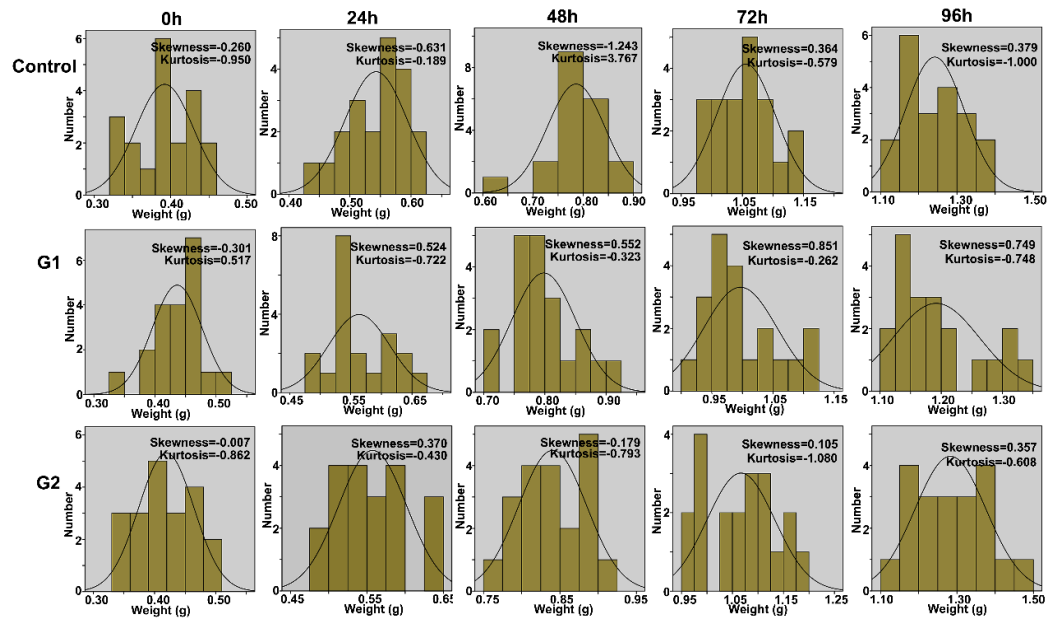
	<b>Total</b>	60				
<b>24h</b>	<b>Control</b>	20	36.28	31.191	2	0
	<b>G1</b>	20	13.03			
	<b>G2</b>	20	42.20			
	<b>Total</b>	60				
<b>48h</b>	<b>Control</b>	20	27.35	1.555	2	0.46
	<b>G1</b>	20	29.98			
	<b>G2</b>	20	34.18			
	<b>Total</b>	60				
<b>72h</b>	<b>Control</b>	20	20.65	9.639	2	0.008
	<b>G1</b>	20	36.28			
	<b>G2</b>	20	34.58			
	<b>Total</b>	60				
<b>96h</b>	<b>Control</b>	20	20.63	9.615	2	0.008
	<b>G1</b>	20	35.85			
	<b>G2</b>	20	35.03			
	<b>Total</b>	60				

Note: When the degree of freedom (df) is 2, and the confidence level ( $\alpha$ ) is 0.05, the critical value of  $\chi^2$  is 5.991 by referring to the  $\chi^2$  distribution critical value table.  $\chi^2$  value is less than 5.991, and the progressive significance value is larger than 0.05, indicating that the conclusion is to accept the null hypothesis, and there is no significant difference among the three groups.  $\chi^2$  value is larger than 5.991, and the progressive significance value is less than 0.05, indicating that the conclusion is to reject the null hypothesis, and there is a significant difference among the three groups.

**Table S3.** The average weight and length of silkworm larvae (Nistari 7019).

<b>Sample</b>	<b>Average Weight (g)</b>	<b>Average Length (cm)</b>
Control (0 h)	0.407	3.8
G1 (0 h)	0.436	3.8
G2 (0 h)	0.419	3.9
Control (24 h)	0.543	4.2
G1 (24 h)	0.564	4.4
G2 (24 h)	0.557	4.4

Control (48 h)	0.786	5.0
G1 (48 h)	0.798	5.0
G2 (48 h)	0.841	5.0
Control (72 h)	1.056	5.4
G1 (72 h)	0.997	5.3
G2 (72 h)	1.067	5.4
Control (96 h)	1.242	5.7
G1 (96 h)	1.192	5.7
G2 (96 h)	1.283	5.8



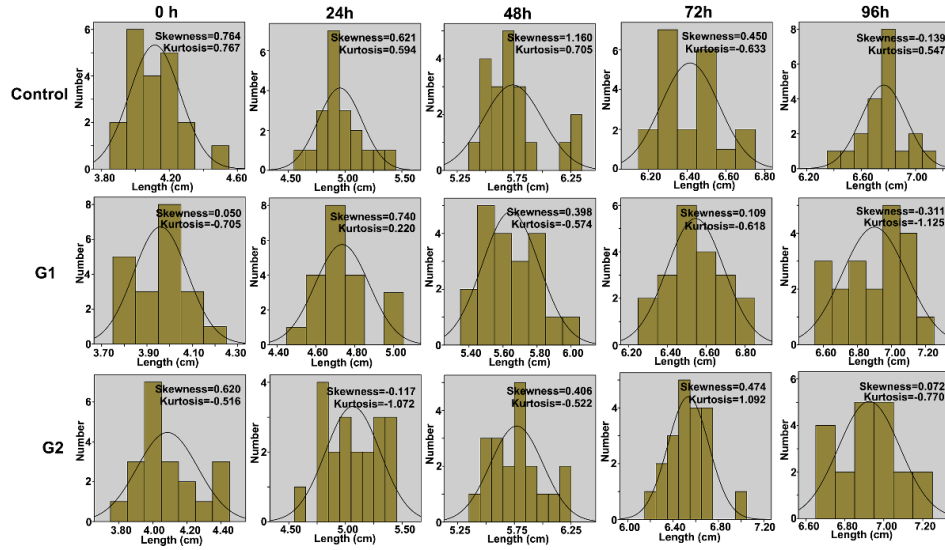
**Figure S3.** One sample Kolmogorov–Smirnov test used to assess the data of silkworm larvae (Nistari 7019) weight.

**Table S4.** Kruskal–Wallis test used to assess the data of silkworm larvae (Nistari

7019) weight from different groups (g) by changing time.

		Number	Average Grade	$X^2$	df	Progressive Significance
<b>0h</b>	<b>Control</b>	20	24.60	5.032	2	0.081
	<b>G1</b>	20	36.95			
	<b>G2</b>	20	29.95			
	<b>Total</b>	60	-			
<b>24h</b>	<b>Control</b>	20	27.93	0.739	2	0.691
	<b>G1</b>	20	32.60			
	<b>G2</b>	20	30.98			
	<b>Total</b>	60	-			
<b>48h</b>	<b>Control</b>	20	24.33	10.434	2	0.005
	<b>G1</b>	20	26.45			
	<b>G2</b>	20	40.73			
	<b>Total</b>	60	-			
<b>72h</b>	<b>Control</b>	20	35.43	14.07	2	0.001
	<b>G1</b>	20	18.60			
	<b>G2</b>	20	37.48			
	<b>Total</b>	60	-			
<b>96h</b>	<b>Control</b>	20	32.20	11.568	2	0.003
	<b>G1</b>	20	20.38			
	<b>G2</b>	20	38.93			
	<b>Total</b>	60	-			

Note: When the degree of freedom (df) is 2, and the confidence level ( $\alpha$ ) is 0.05, the critical value of  $X^2$  is 5.991 by referring to the  $X^2$  distribution critical value table.  $X^2$  value is less than 5.991, and the progressive significance value is larger than 0.05, indicating that the conclusion is to accept the null hypothesis, and there is no significant difference among the three groups.  $X^2$  value is larger than 5.991, and the progressive significance value is less than 0.05, indicating that the conclusion is to reject the null hypothesis, and there is a significant difference among the three groups.



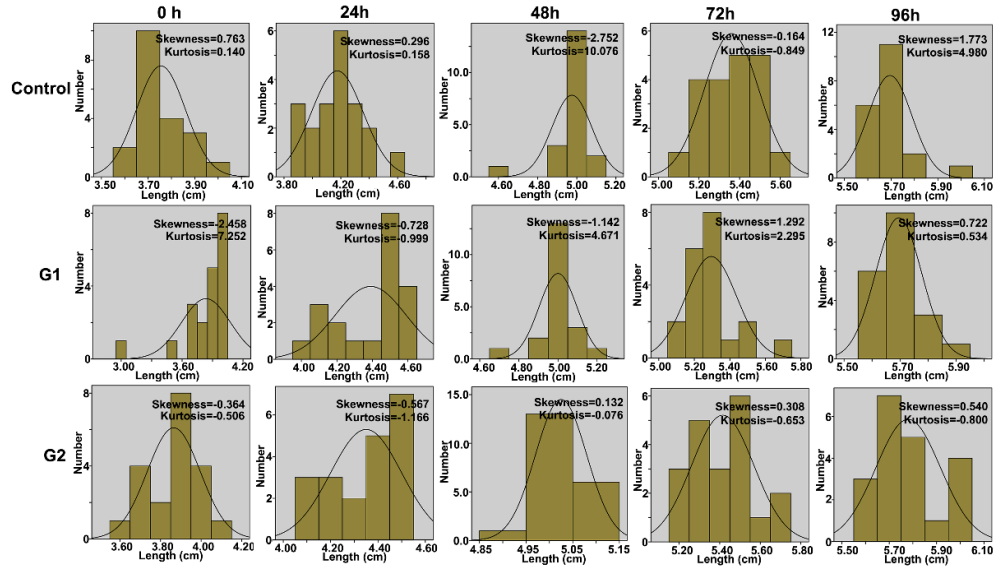
**Figure S4.** One sample Kolmogorov–Smirnov test used to assess the data of silkworm larvae (qiufeng × baiyu) length.

**Table S5.** Kruskal–Wallis Test for the data of silkworm larvae (qiufeng × baiyu)

length from different groups (cm) by changing time

		Number	Average Grade	$\chi^2$	df	Progressive Significance
0h	Control	20	35.00	4.355	2	0.113
	G1	20	24.25			
	G2	20	32.25			
	Total	60				
24h	Control	20	34.43	21.544	2	0
	G1	20	16.30			
	G2	20	40.78			
	Total	60				
48h	Control	20	31.03	2.923	2	0.232
	G1	20	25.60			
	G2	20	34.88			
	Total	60				
72h	Control	20	22.00	7.479	2	0.024
	G1	20	35.58			
	G2	20	33.93			
	Total	60				
96h	Control	20	21.63	8.102	2	0.017
	G1	20	33.93			
	G2	20	35.95			
	Total	60				

Note: When the degree of freedom (df) is 2, and the confidence level ( $\alpha$ ) is 0.05, the critical value of  $\chi^2$  is 5.991 by referring to the  $\chi^2$  distribution critical value table.  $\chi^2$  value is less than 5.991, and the progressive significance value is larger than 0.05, indicating that the conclusion is to accept the null hypothesis, and there is no significant difference among the three groups.  $\chi^2$  value is larger than 5.991, and the progressive significance value is less than 0.05, indicating that the conclusion is to reject the null hypothesis, and there is a significant difference among the three groups.



**Figure S5.** One sample Kolmogorov–Smirnov Test used to assess the data of silkworm larvae (Nistari 7019) length.

**Table S6.** Kruskal–Wallis Test used to assess the data of silkworm larvae (Nistari 7019) length from different groups (cm) by changing time.

		Number	Average Grade	$\chi^2$	df	Progressive Significance
0h	Control	20	25.50	2.682	2	0.262
	G1	20	33.70			
	G2	20	32.30			
	Total	60				
24h	Control	20	19.73	12.423	2	0.002
	G1	20	38.10			
	G2	20	33.68			



	<b>Total</b>	60				
<b>48h</b>	<b>Control</b>	20	26.23	3.631	2	0.163
	<b>G1</b>	20	30.28			
	<b>G2</b>	20	35.00			
	<b>Total</b>	60				
<b>72h</b>	<b>Control</b>	20	31.68	6.81	2	0.033
	<b>G1</b>	20	22.95			
	<b>G2</b>	20	36.88			
	<b>Total</b>	60				
<b>96h</b>	<b>Control</b>	20	26.38	6.284	2	0.043
	<b>G1</b>	20	27.15			
	<b>G2</b>	20	37.98			
	<b>Total</b>	60	-			

Note: When the degree of freedom (df) is 2, and the confidence level ( $\alpha$ ) is 0.05, the critical value of  $\chi^2$  is 5.991 by referring to the  $\chi^2$  distribution critical value table.  $\chi^2$  value is less than 5.991, and the progressive significance value is larger than 0.05, indicating that the conclusion is to accept the null hypothesis, and there is no significant difference among the three groups.  $\chi^2$  value is larger than 5.991, and the progressive significance value is less than 0.05, indicating that the conclusion is to reject the null hypothesis, and there is a significant difference among the three groups.

### The Food Intake Speed of Silkworms

To study the food intake rate of silkworms, the daily weight of mulberry leaves consumed by silkworms was measured using qiufeng  $\times$  baiyu as a model and two concentrations (2% and 8%) of BN NSs-1 and BN NSs-2. The data in **Table S7** indicate a subtle distinction between the groups of silkworms in terms of their food intake rate. Future research should focus on each silkworm's food consumption rate within each group.

**Table S7.** The raw data of weight of mulberry leaves eaten by silkworms (qiufeng × baiyu) (g).

	control	BN NSs-1		BN NSs-2	
		2%	8%	2%	8%
<b>1st</b>	47.47	47.96	46.45	51.44	44.83
<b>2nd</b>	42.02	46.1	49.61	48.48	47.24
<b>3rd</b>	43.51	43.32	43.77	44.73	43.75
<b>4th</b>	46.89	49.96	45.29	46.28	43.94

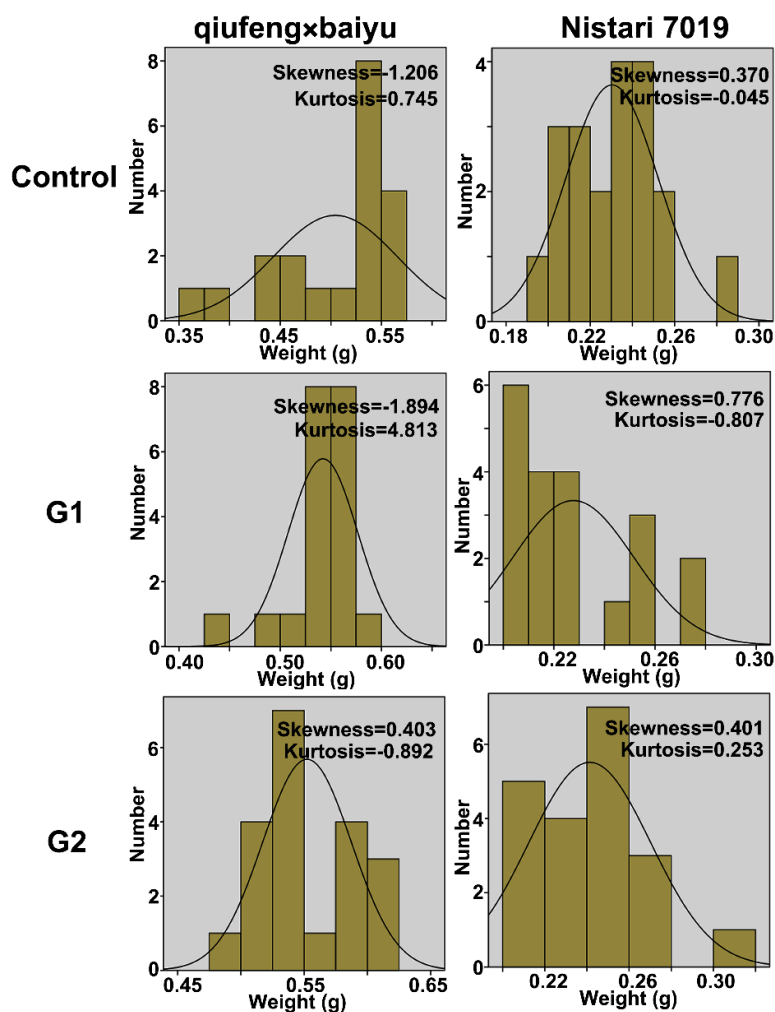
### The Concentration and Time Effect of BN NSs Toxicity

**Table S8.** The average weight and length of qiufeng × baiyu at different concentrations.

Sample	BN NS-1		BN NSs-2	
	Average Weight (g)	Average Length (cm)	Average Weight (g)	Average Length (cm)
Control (24 h)	0.825	4.3	0.825	4.3
2% (24 h)	0.954	4.3	1.028	4.5
8% (24 h)	0.944	4.4	1.011	4.6
Control (48 h)	1.263	4.8	1.263	4.8
2% (48 h)	1.347	5.0	1.408	5.1
8% (48 h)	1.478	5.2	1.390	5.1
Control (72 h)	1.629	5.3	1.629	5.3
2% (72 h)	1.679	5.2	1.716	5.4
8% (72 h)	1.913	5.5	1.949	5.6
Control (96 h)	2.020	5.7	2.020	5.7
2% (96 h)	2.272	6.1	2.114	5.7
8% (96 h)	2.322	6.0	2.418	5.8

**Table S9.** The weight of cocoon from qiufeng  $\times$  baiyu and Nistari 7019 from different groups (g).

Sample	qiufeng $\times$ baiyu			Nistari 7019		
	Control	G1	G2	Control	G1	G2
1	0.507	0.562	0.524	0.196	0.211	0.254
2	0.473	0.549	0.593	0.214	0.216	0.311
3	0.535	0.499	0.536	0.224	0.259	0.241
4	0.567	0.529	0.578	0.213	0.274	0.249
5	0.542	0.547	0.548	0.232	0.224	0.223
6	0.566	0.507	0.523	0.225	0.203	0.236
7	0.384	0.567	0.524	0.282	0.229	0.254
8	0.429	0.566	0.545	0.253	0.208	0.234
9	0.484	0.531	0.529	0.248	0.223	0.259
10	0.466	0.569	0.525	0.237	0.204	0.203
11	0.558	0.57	0.603	0.249	0.227	0.279
12	0.449	0.538	0.54	0.252	0.208	0.234
13	0.526	0.574	0.525	0.237	0.205	0.258
14	0.353	0.571	0.563	0.203	0.213	0.214
15	0.532	0.529	0.493	0.232	0.218	0.268
16	0.537	0.431	0.62	0.241	0.258	0.265
17	0.543	0.577	0.582	0.204	0.254	0.24
18	0.532	0.56	0.578	0.248	0.271	0.201
19	0.54	0.537	0.515	0.204	0.247	0.202
20	0.567	0.532	0.601	0.212	0.202	0.202
Average Standard deviation	<b>0.505</b>	<b>0.542</b>	<b>0.552</b>	<b>0.23</b>	<b>0.228</b>	<b>0.241</b>
	<b>0.061</b>	<b>0.034</b>	<b>0.035</b>	<b>0.022</b>	<b>0.024</b>	<b>0.029</b>



**Figure S6:** One sample Kolmogorov–Smirnov test used to assess the cocoon weight data from silkworm larvae (qiufeng × baiyu, Nistari 7019).

**Table S10:** Kruskal–Wallis Test used to assess the data of cocoon (qiufeng × baiyu) weight from different groups (g)

		Number	Average grade	$\chi^2$	df	Progressive significance
Weight	Control	20	22.45	6.404	2	0.041
	G1	20	34.08			
	G2	20	34.98			
	Total	60	-			

Note: When the degree of freedom (df) is 2, and the confidence level ( $\alpha$ ) is 0.05, the critical value of  $\chi^2$  is 5.991 by referring to the  $\chi^2$  distribution critical value table.  $\chi^2$  value is less than 5.991, and the progressive significance value is larger than 0.05,

indicating that the conclusion is to accept the null hypothesis, and there is no significant difference among the three groups.  $\chi^2$  value is larger than 5.991, and the progressive significance value is less than 0.05, indicating that the conclusion is to reject the null hypothesis, and there is a significant difference among the three groups.

**Table S11:** Kruskal–Wallis Test used to assess the data of cocoon (Nistari 7019)

weight from different groups (g).

		Number	Average grade	$\chi^2$	df	Progressive significance
<b>Weight</b>	<b>Control</b>	20	29.03	2.275	2	0.321
	<b>G1</b>	20	27.28			
	<b>G2</b>	20	35.20			
	<b>Total</b>	60	-			

Note: When the degree of freedom (df) is 2, and the confidence level ( $\alpha$ ) is 0.05, the critical value of  $\chi^2$  is 5.991 by referring to the  $\chi^2$  distribution critical value table.  $\chi^2$  value is less than 5.991, and the progressive significance value is larger than 0.05, indicating that the conclusion is to accept the null hypothesis, and there is no significant difference among the three groups.  $\chi^2$  value is larger than 5.991, and the progressive significance value is less than 0.05, indicating that the conclusion is to reject the null hypothesis, and there is a significant difference among the three groups.