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Mineral Compositions in the Feces of Some Silkworms

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This study was carried out to investigate the mineral content of the feces of the 5th instar larvae in a few silkworm species such as a parent domestic silkworm (Jam134), a hybrid (Kumok-jam), the Japanese oak silkworm, and the Chinese oak silkworm. The major minerals of all silkworms throughout all 5th instar larval period are K, P, Mg and Ca, and the result is consistent with the previous study of the mineral content in the mulberry leaves. Although the calcium content decreased sharply at 7th day of the 5th instar, the crude protein content significantly increased at the same age in both domestic silkworms, suggesting a direct relationship between feeding behavior of the silkworms at the larval period and mineral/protein contents. However, this trend was not observed in both oak silkworms. In the comparison of the mineral content among silkworm species, two domestic silkworms were significantly higher in the calcium content compared with two oak silkworms, and the hybrid Kumok-jam was further higher significantly than the feces of parental Jam 143. Excluding the calcium content, overall no significant content variation in other minerals was observed among four silkworm species studied in this study.

Key words: Silkworm, Silkworm feces, Minerals, Calcium

Introduction

Traditionally, silkworm feces have been known to have several therapeutic effects for relieving pain, inflammation of joint, neuralgia, hemiplegia by paralysis, scabies, pains caused by stomach chill, stomach pain, fever, lumbar myalgia, facial neuralgia, breeding in the uterine, menstrual irregularity and so on (Lee and Kim, 2000; Gui *et al.*, 2003). Further, the feces have traditionally been utilized for the stuffing of a pillow in China, because the pillow enables to sleep deeply by facilitating blood cycling (Yang *et al.*, 2002). At present, the silkworm feces are utilized for the blood glucose lowering agent with other sericultural products, such as silkworm powder and mulberry root cortex (Chung *et al.*, 1997), and utilized as an ingredient for soap and cosmetics. According to the users, the soaps are thought to remove the old corneous tissue and soften hands, and smooth rashes.

Silkworm feces are known to contain about 84% of organic acids, 9 ~ 16% of ash, 2 ~ 4% of nitrogen, and high amount of several amino acids like histidine, several steroid like beta cytosterol (Ryu *et al.*, 2003). Further, the feces are known to contain urea, phosphoric acid, calcium, potassium, vitamins A and B, plant growth hormone, and chlorophyll (Gui *et al.*, 2003). Considering the staple food for silkworms is mulberry leaves, the feces may also contain many ingredients present in the mulberry leaves.

Recently, silkworm feces are also a subject of research for pheophytin, which is a derivate of chlorophyll, as a potential anticancer agent. Ahn *et al.* (2001) reported that the ethanol extracts from silkworm feces have a potential of anticancer activity, particularly from the freeze-dried feces obtained at 3rd day of the 5th instar larvae. Further, Ahn *et al.* (2002) reported the pheophytin extracts obtained from 7th day of the 5th instar larvae contained the highest percentage of pheophytin and better firinolytic activity among those from other larval periods.

With the increasing interest for utilizing the silkworm feces in many fields, more systematic study on the silkworm feces is required, but not much study is available yet. In the long run, an accumulation of the silkworm feces data on the useful ingredients will be an important start point to grasp superiority of the Korean sericultural product. In this study, we, therefore, analyzed a few mineral contents of a few silkworm species such as a parent

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domestic silkworm (Jam 134), a hybrid silkworm (Kumok-jam), the Japanese oak silkworm, and the Chinese oak silkworm. These silkworm species data are hoped to promote future study on the reserved, several hundreds domestic silkworm breeds in the future.

Materials and Methods

Sample collection

Silkworms from which feces were obtained were a parent domestic silkworm, *Bombyx mori* (Jam 134), bred by cross breeding, a F_1 hybrid silkworm, *B. mori* (Kumok-jam), obtained by crossing between Jam 125×Jam 140, the Japanese oak silkworm (*Antheraea yamamai*), and the Chinese oak silkworm (*Antheraea pernyi*). The domestic silkworm feces were obtained only during 5th instar larval stages before mounting to the cocooning frame. The silkworm rearing was performed as usual at the Department of Sericulture & Entomology, The National Institute of Agricultural Science & Technology, Suwon, Korea during May ~ July, 2003, the period of which is a spring rearing season in Korea. The Japanese and the Chinese oak silkworms were reared on the branches of oak tree. On every the other days of 5th instar larval period, silkworm feces were collected and dried at 60°C for 2 hrs.

Analysis of mineral content

Crude protein was analyzed according to the method of Bradford (1976). That is, to the 0.1 g of the dried feces, 1 ml of potassium phosphate buffer was added, centrifuged for 40 min at 13,000 rpm, and supernatant was obtained. Ten micro-liter of supernatant was mixed with 200 μl of coloring reagent and 790 μl of ddH_2O. Absorbency was measured at 596 nm using spectrophotometer, and protein content was estimated from the standard curve. Mineral content was analyzed by atomic absorption spectroscopy (AAnalyst 300, Perkin Elmer, USA). Phosphorus content was determined by colorimetric method, which utilizes ammonium molybdate, hydroquinone, and sodium sulfate (AOAC, 1980). All samples were analyzed three times using independently prepared silkworm feces.

Statistical analysis

Using SAS program, Duncans multiple range test ($p < 0.05$) was performed to test if any significant difference exists in the mineral content among samples of silkworm feces.

Results and Discussion

Mineral content and crude protein content of the 5th instar larvae of the Japanese oak silkworm (Table 1), the Chinese silkworm (Table 2), Jam 134 (Table 3) and Kumok-jam (Table 4) are presented in the respective tables. The major minerals of the feces of the 5th instar larvae of all silkworms are K, P, Mg and Ca throughout 5th instar larval period (Tables 1, 2, 3, 4). High content of those minerals

Table 1. Mineral content of the 5th instar larvae of the Japanese oak silkworm (g/100 g)

Age (day)	Protein	P	K	Ca	Fe	Mg	Na	Cd	Zn	Mn	Cu
1st	8.63a	0.547a	0.809b	0.403a	0.025b	0.234b	0.015c	0.020d	0.006a	0.064b	0.00105b
3rd	5.72b	0.784a	0.865b	0.356b	0.035a	0.232b	0.036b	0.057c	0.004b	0.098a	0.00115b
5th	2.73c	0.512a	1.15a	0.340b	0.035a	0.276a	0.036b	0.084c	0.004b	0.058b	0.00095b
7th	7.48a	0.277b	1.208a	0.412b	0.020b	0.22b	0.058a	0.212a	0.004b	0.055b	0.0008b
9th	1.97c	0.299b	1.18a	0.532a	0.023b	0.199b	0.009c	0.134b	0.004b	0.05b	0.00525a

The values with different alphabets indicate significant difference at the level of $p < 0.05$.

Table 2. Mineral content of the 5th instar larvae of the Chinese oak silkworm (g/100 g)

Age (day)	Protein	P	K	Ca	Fe	Mg	Na	Cd	Zn	Mn	Cu
1st	16.75a	0.560a	0.644c	0.556a	0.017b	0.201cd	0.008b	0.169a	0.003b	0.122a	0.00025c
3rd	4.62c	0.277b	0.165e	0.556a	0.015cd	0.185d	0.010b	0.19a	0.003ab	0.081c	0.0002c
5th	3.72dc	0.362b	0.673c	0.255c	0.021a	0.241ab	0.008b	0.115b	0.003a	0.063dc	0.00055ab
7th	2.16c	0.321b	0.957b	0.291bc	0.015c	0.244a	0.023a	0.042c	0.003a	0.046c	0.0005b
9th	2.11c	0.530a	1.112a	0.336b	0.014cd	0.252a	0.012b	0.057c	0.003a	0.046c	0.0006a
11th	7.91b	0.301b	0.458d	0.507a	0.013c	0.22bc	0.019a	0.007d	0.003ab	0.107b	0.00055ab

The values with different alphabets indicate significant difference at the level of $p < 0.05$.

Table 3. Mineral content of the 5th instar larvae of the Jam134 (g/100 g)

Age (day)	Protein	P	K	Ca	Fe	Mg	Na	Cd	Zn	Mn	Cu
1st	5.51b	0.551a	0.487d	1.468a	0.020b	0.422c	0.037ab	0.100a	0.002b	0.009ab	0.00015a
3rd	4.59b	0.488a	1.854c	1.090ab	0.020b	0.450bc	0.064b	0.016c	0.002ab	0.008b	0.00025a
5th	5.22b	0.523a	2.103b	1.275ab	0.024a	0.461b	0.058ab	0.007c	0.003a	0.009ab	0.00025a
7th	13.43a	0.269b	0.073a	1.111b	0.024b	0.559a	0.053a	0.062b	0.002c	0.011a	0.00025a

The values with different alphabets indicate significant difference at the level of $p<0.05$.

Table 4. Mineral content of the 5th instar larvae of the Kumok-jam (g/100 g)

Age (day)	Protein	P	K	Ca	Fe	Mg	Na	Cd	Zn	Mn	Cu
1st	9.84b	0.280a	0.664d	2.361a	0.023ab	0.585a	0.066a	0.189a	0.003a	0.009a	0.0002ab
3rd	6.99bc	0.374a	1.237c	1.813b	0.024a	0.614a	0.038b	0.189a	0.002a	0.006a	0.00025a
5th	8.55c	0.304a	1.945b	1.724b	0.020c	0.601a	0.035b	0.173a	0.003a	0.01a	0.00015b
7th	13.97a	0.303a	2.406a	1.728b	0.021bc	0.578a	0.027b	0.061b	0.003a	0.012a	0.0002ab

The values with different alphabets indicate significant difference at the level of $p<0.05$.

is roughly consistent with the mineral composition of both cultivated and wild mulberry leaves collected from several geographic locations in Korea and Tsushima Island in Japan. Kim *et al.* (2001) reported that the major minerals in the mulberry leaves were K (2.494 g/100 g DW), P (2.255 g/100 g) and Ca (1.835 g/100 g), and minor ones were Mg (0.627 g/100 g), Na (0.0298 g/100 g) and Fe (0.0245 g/100 g). Considering the staple food for silkworms is mulberry leaves, the data obtained from the domestic silkworms (Jam 134 and Kumok-jam) appear to be reasonable in that some undigested components of mulberry leaves can be excreted through feces. In the case of both oak silkworms, merely the same speculation can be made at this point, until the mineral composition data of oak leaves are available.

Overall there was no obvious trend in the changes of protein and mineral contents as larval age progresses, particularly for both oak silkworms (Tables 1 and 2). For example, the calcium content was 0.403 (g/100 g) at 1st day of the 5th instar of the Japanese oak silkworm and decreased to 0.340 (g/100 g) until 5th day of the 5th instar, but it again increased to the higher level than that of 1st day of the 5th instar (Table 1). Similar pattern was observed from the Chinese oak silkworm feces as larval period continues (*i.e.*, increase-decrease-increase pattern) (Table 2). On the other hand, the mineral and protein contents of the domestic silkworms showed somewhat consistent result. For example, the calcium content was 1.468 (g /100 g) at 1st day of the 5th instar of Jam 134 and it significantly decreased to 1.111 (g/100 g) at 7th day of the 5th instar. Also, the calcium content of the Kumok-jam showed a significant decrease at 7th day of the 5th instar (from 2.361 at 1st day to 1.728 at 7th day). This trend probably can be explained in terms of feeding behavior of the two last staged-larval domestic silkworms, at which the silkworms do not eat any more mulberry leaves until mounting to the mounting frame (Kim *et al.*, 1966). In the case of crude protein, two domestic silkworms showed rapid increase at the last larval period (Tables 3 and 4). The crude protein content was 5.505 (g/100 g) at 1st day of the 5th instar of Jam 134, but it significantly increased to 13.425 at 7th day of the 5th instar (Table 3). The same is true for the Kumok-jam in that the crude protein content increased from 9.835 at 1st day of the 5th instar to 13.97 at 7th day of the 5th instar (Table 4). This result is consistent with the physiological changes of the silkworm body, in which most of the silkworm body is filled with silk grand during last larval period. However, no obvious trend on the crude protein content was observed in both oak silkworms (Tables 1 and 2). For example, the protein content of the feces of the Japanese oak silkworm at 1st day of the 5th instar was 8.63 (g/100 g), but the content significantly decreased until at the end of larval period except for non-substantial decrease at 7th day of the 5th instar (Table 1). Although the changing pattern is somewhat different, the Chinese oak silkworm also shows overall substantial decrease in the crude protein content during whole larval period (Table 2). Thus, the feces at 1st day of the 5th instar of the Chinese oak silkworm were highest in the crude protein content during whole 5th instar larval period. Currently, we do not have an obvious answer for the substantial decrease during 5th instar larval period in both oak silkworms. Although limited, one possible expiation on the lack of consistent trend in both oak silkworm data appears to have been stemmed because the two species of oak silkworms are wild, but the artificially provided "semi-

Table 5. Comparison of mineral content of the 5th instar larvae among silkmoths (g/100 g)

Species	Protein	P	K	Ca	Fe	Mg	Na	Cd	Zn	Mn	Cu
Japanese oak silkworm	5.31a	0.484a	1.042a	0.409c	0.028a	0.232c	0.031a	0.101a	0.004a	0.065a	0.00184a
Chinese oak silkworm	6.21a	0.392a	0.668a	0.417c	0.016b	0.224c	0.013b	0.097a	0.003b	0.078a	0.000442b
Jam143	7.19a	0.458a	1.129a	1.236b	0.022ab	0.473b	0.053a	0.046a	0.002b	0.009b	0.000225b
Kumok-jam	9.83a	0.315a	1.563a	1.907a	0.022ab	0.595a	0.042a	0.153a	0.003b	0.009b	0.0002b

The values with different alphabets indicate significant difference at the level of $p<0.05$.

domestic rearing environment" may have not provided stable enough physiological environment for full feeding. Thus, more scrutinized rearing environment possibly can result in somewhat different chemical content than ours. Thus, an extensive research on this aspect is required.

Mineral content in the feces among silkworm species are presented in Table 5. Crude protein content was higher in the domestic silkworms (7.816 and 9.834 g/100 g) than two oak silkworms (5.305 and 6.21 g/100 g), but the difference was not statistically significant ($p>0.05$). On the other hand, the calcium content was significantly higher in two domestic silkworms (1.236 and 1.907 g/100 g) than two oak silkworms (0.409 and 0.417 g/100 g). Among two domestic silkworms, the feces of the Kumok-jam were further higher significantly (1.907 g/100 g) than the feces of Jam 143 (1.236 g/100 g). These data appear to suggest that silkworm feces also are a rich source of calcium as mulberry leaves at least in the domestic silkworms. Excluding calcium, most other minerals did not show any significant content difference among species. Nevertheless, potassium and magnesium contents were somewhat higher in the two domestic silkworms, particularly in the Kumok-jam (Table 5). On the other hand, phosphate content was somewhat higher in the Japanese oak silkworm, but the estimate was not statistically significant.

In conclusion, it is evident that the feces from two domestic silkworms contain substantially high amount of calcium compared with two oak silkworms. This result is consistent with the report of the high calcium content in the mulberry leaves (Kim *et al.*, 2001). Minerals are of significant to balance and metabolize our bodily functions. Particularly, calcium is a mineral in our body that makes up our bones and keeps them strong (Christian and Greger, 1991; Eades, 1994). Thus, silkworm feces also can be utilized as a calcium rich source. At 7th day of the 5th instar, calcium content dropped sharply in the two domestic silkworms, but reversely protein content increased significantly, suggesting that the crude protein/calcium contents are closely related to the feeding behavior of the silkworms during 5th instar larval stage. Although this study provided limited result on the chemical composition of silkworm feces, we hope this study would provide a direction for further mineral research on the silkworm feces.

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