IJIE Int. J. Indust. Entomol. 43(2) 85-93 (2021)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2021.43.2.85

ISSN 1598-3579 (Print)
ISSN 2586-4785 (Online)

Analysis of nutrient composition of silkworm pupae in Baegokjam, Goldensilk, Juhwangjam, and YeonNokjam varieties

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Abstract

Silkworm pupae have been utilized as a food source and have high-quality proteins and fatty acids. However, studies on the nutritional composition of pupae according to their variety, developmental stage, and sex have not been conducted. In this study, the nutritional composition of four Korean silk varieties (Baegokjam, BG; Goldensilk, GS; Juhwangjam, JH; and YeonNokjam, YN) were analyzed according to developmental stage and sex. The main ingredient of the pupa was protein, and the protein levels were higher in females than in males. Fat levels were high in males and increased in YN and GS during the late stage but decreased in BG. Fiber content rapidly increased in the late stages, and the lowest content was observed in BG. The pupae contained all essential amino acids, which were detected at higher ratios in females with a high protein content. Fatty acids had a different predominance depending on the variety. Oleic acid level was high in BG, linoleic acid and linolenic acid levels were high in GS, and palmitic acid and stearic acid levels were high in JH. In the mineral analysis, zinc was dominant in BG, whereas calcium and iron levels were relatively low. Zinc level was low in GS, and calcium and magnesium levels were high in JH. Potassium, sodium, magnesium, and phosphorus levels were low in YN. The silkworm pupae showed differences in components according to their variety, sex, and developmental stage; therefore, selecting a variety suitable for its purpose is necessary.

Int. J. Indust. Entomol. 43(2), 85-93 (2021)

Received : 16 Sep2021
Revised : 15 Dec 2021
Accepted : 17 Dec 2021

Keywords:
silkworm pupa,
amino acid,
fatty acid,
minerals,
variety

Introduction

Silkworms have a high value in the textile industry as silk-producing insects. They have a breeding history of more than 5,000 years, but the number of farmers has declined since the 1990s because of the replacement of silk with synthetic chemical fibers in Korea. However, the health-promoting effects of sericulture products have been reported, and the utilization methods, including functional foods and biomedical materials, are diversified (Kweon and Cho, 2001; Ryu *et al*., 1997).

Silkworm pupa, a sericulture product, is high in protein and unsaturated fatty acids and is listed in the Ministry of Food and Drug Safety (MFDS) Food Code, which is highly utilized. Pupa protein contains all eight essential amino acids that cannot be

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synthesized in the human body, including lysine and threonine, which are deficient in cereal (Longvah *et al*., 2011). Essential amino acids are building blocks of proteins and enzymes that are necessary for bodily functions. Additionally, some proteins have bioactive properties; for example, peptides from pupae hydrolysate exhibit inhibitory effects on angiotensin-converting enzyme (ACE), suggesting its potential as a therapeutic agent for hypertension (Wang *et al*., 2008). Among all pupa nutrients, the proportion of lipids is the second largest, of which 70% are unsaturated fatty acids (Kang *et al*., 2006). Fatty acids are classified as unsaturated if they have one or more double bonds. A wide range of studies on the correlation between unsaturated fatty acid intake and disease incidence has been conducted, including cross-cultural comparisons, cohort studies, case–control studies, and intervention trials. In most studies, coronal heart disease and intake of unsaturated fatty acids were found to be inversely associated (Lunn and Theobald, 2006).

Pupae are a nutritional source of essential amino acids and unsaturated fatty acids; however, studies on their compositional characteristics according to variety have not been conducted. Silkworm larvae have differences in nutritional composition and bioactivity depending on the variety or growth stage (Nguyen *et al*., 2020). Therefore, differences in nutritional components in the pupae according to the variety and pupation day are expected. Four colored silk varieties (Baegokjam of white cocoon, BG; Goldensilk of yellow cocoon, GS; Juhwangjam of orange cocoon, JH; and YeonNokjam of yellow green cocoon, YN) developed by the Rural Development Administration were used in this study and classified according to the time of pupation and sex for nutrient compositional analysis (Kang *et al*., 2011; Kim *et al*., 2020; Kweon *et al*., 2012; Lee *et al*., 1984).

Material and methods

Sample preparation

Silkworm varieties with the different cocoons colors (BG, GS, JH, and YN) were obtained from National Institute of Agricultural, Sciences (Wanju, Korea). The pupae were classified according to day of spinning (6-7 days; early stage, 8-10 days; mid stage, 11-13 days; late stage). Male and female pupae were assorted by external appearance referred to previous study (Ryu *et al*., 2002). The pupae were prepared by lyophilized and grinded for nutritional composition analysis.

Crude protein, fat, fiber and ash contents analysis

Crude protein concentration were analyzed using automatic protein analyzer (Kjeltec 2400 AUT, Poss Tecator, Mulgrave, Australia) with some modifications of semi-micro-Kjeldahl method. Crude fat was quantified using Soxhlet extraction system (Soxtec System HT1043 extraction unit, Foss Tectator, Hoganas, Sweden) after lipid extraction using diethyl ether. Crude fiber analysis was conducted using 1.25% H_2SO_4 and 1.25% NaOH digestion methods. Contents of crude ash were examined using dry ashing method at 600 °C (Lee *et al*., 2019).

Mineral composition

Mineral contents were analyzed using the protocol from the Association of Official Analytical Chemist (AOAC, 1990). Samples were transferred to crucibles and incinerated at 600 °C for 2 h. Then samples (500 mg) were cooled and mixed with the 50% HCl (10 mL). Mixtures were incubated for overnight and filtered using No. 6 filter paper (GE Healthcare Life Sciences, Chicago, IL, USA). The wavelength were measured using an inductively coupled plasma optical emission spectrometer PerkinElmer Optima 8300 (Perkin-Elmer Corporation, Norwalk, CT, USA) and the intensities of specific emitted radiant rays for each mineral were analyzed (Jo *et al*., 2020).

Amino acid composition

Samples were hydrolyzed using hydrochloric acid and phenyl isothiocyanate derivation was occurred. Then the amino acid compositions were measured by amino acid analyzer (Biochrom 20 puls amino acid analyzer, Amersham Phamacia Biotech Co., Sweden). The flow rate was 1 mL/min and detection wavelength was 254 nm (Jo *et al*., 2020).

Fatty acid composition

The samples (50 g) were mixed with 250 mL of chloroform and methanol solution (2:1, v/v). Lipids were extracted by homogenized and dehydrated by adding anhydrous Na_2SO_4. The samples were concentrated under 50-55^{o}C, then 1 mL of tricosanoic acid and 1mL of 0.5 N NaOH were added. The mixtures were boiled at 100^{o}C for 20 min, then 2 mL of methanol was added after cooling. Samples were boiled again for another 20 min and cooling. Heptane (1 mL) and NaCl (8 mL) were added, the supernatant was separated for analysis. Fatty acid composition of the sample was measured using Agilent US/HP 6890 (Agilent Technologies) equipped with flame ionization

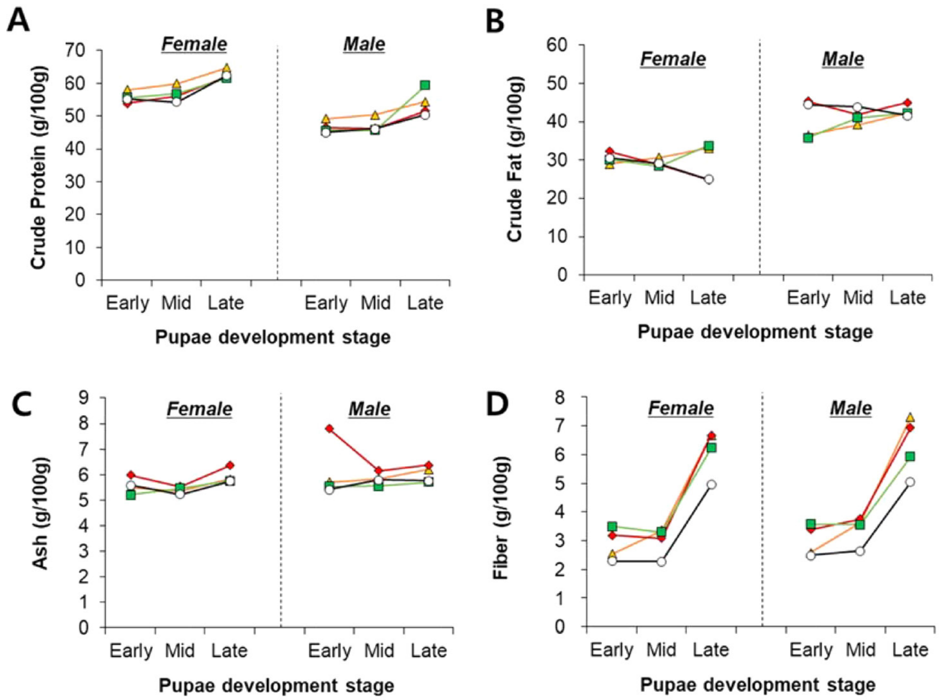

Fig. 1. Macronutrient composition in silkworm pupae. Female and male pupae of BG(○), GS(▲), JH(◆), and YN(■) varieties were compared according to early (6-7days), mid (8-10days), and late (11-13days) stages. (A) Crude protein, (B) Crude fat, (C) Ash, and (D) Fiber contents were existed in g/100 g of sample.

detector. The flow rate of the mobile phase was 1 mL/min. (Lee *et al*., 2019).

Statistical analysis

Two-way ANOVA test without replication were analyzed within varieties, sex, and developmental stages. The significance of difference among the groups were determined with the level of P<0.05.

Results and discussion

Comparison of macronutrients

Crude protein, fat, ash, and fiber contents in pupae were determined (Fig. 1). Crude protein content was higher in females (54–65 g/100 g) than in males (45–59 g/100 g). Protein levels significantly increased with the developmental stage (P<0.001, Table 1). Female GS contained the highest protein content. Crude fat content was higher in males (36–45 g/100 g) than in females (25–34 g/100 g). Fat content in BG females, JH females, and BG males decreased with the developmental stage, whereas the content in GS females, YN females, GS males, JH males, and YN males increased. Ash levels ranged from 5.2 to 7.8 g/100 g. JH males had the highest ash content at the early stage. The fiber content rapidly increased in the late stage of development (5.0–7.3 g/100 g). The fiber content was higher in JH and YN varieties than in BG variety.

There were differences in the macronutrient composition of males and females. Protein content was high in females, whereas fat content was high in males, which is consistent with the results of Ryu *et al.* (2003). In addition, the fiber content increased rapidly during the late stages. Chitin is a polysaccharide polymer structurally similar to cellulose in plants and is composed of numerous units of N-acetyl-β-D-glucosamine linked by β-1,4 glycosidic bonds. Because chitin is a major component of insect exoskeletons, its content is presumed to increase at a late stage approaching moth formation (Muthukrishnan *et al*., 2012). The varieties with the highest fiber content were JH and YN.

Comparison of essential amino acids

The levels of methionine (MET), threonine (THR), valine (VAL), isoleucine (I-LE), leucine (LEU), phenylalanine (PHE), lysine (LYS), and tryptophan (TRP) were compared (Fig. 2).

Table 1. Two-way ANOVA analysis accroding to sex, variety, and development stage.

		Female		Male		Female+male	
		F value	*P value*	*F value*	*P value*	*F value*	*P value*
protein	*Variety*	9.954	0.010	1.626	0.280	5.434	0.010
	Development stage	64.320	<0.001	10.148	0.012	45.788	<0.001
Ash	*Variety*	5.274	0.040	3.204	0.105	5.740	0.008
	Development stage	9.821	0.013	0.300	0.751	1.949	0.145
Fat	*Variety*	0.542	0.671	2.564	0.151	0.170	0.915
	Development stage	0.205	0.820	0.742	0.515	15.352	<0.001
Fiber	*Variety*	6.432	0.026	3.401	0.094	10.316	0.001
	Development stage	93.345	<0.001	43.057	<0.001	57.366	<0.001
MET	*Variety*	2.280	0.180	1.364	0.341	2.169	0.134
	Development stage	6.319	0.033	2.428	0.169	17.505	<0.001
THR	*Variety*	7.774	0.017	1.090	0.423	5.303	0.011
	Development stage	1.224	0.358	36.620	<0.001	116.458	<0.001
VAL	*Variety*	1.317	0.353	1.545	0.297	2.649	0.087
	Development stage	5.171	0.049	6.651	0.030	79.070	<0.001
ILE	*Variety*	1.618	0.282	0.821	0.528	1.878	0.177
	Development stage	7.055	0.027	3.617	0.093	55.443	<0.001
LEU	*Variety*	5.187	0.042	0.850	0.515	3.416	0.045
	Development stage	16.807	0.003	5.169	0.050	101.732	<0.001
PHE	*Variety*	11.865	0.006	0.578	0.650	3.198	0.054
	Development stage	22.736	0.002	38.574	<0.001	125.970	<0.001
LYS	*Variety*	8.257	0.015	1.448	0.319	3.095	0.059
	Development stage	10.361	0.011	38.418	<0.001	105.124	<0.001
TRP	*Variety*	0.949	0.474	1.117	0.413	1.407	0.280
	Development stage	3.388	0.104	66.452	<0.001	57.586	<0.001
Palmitic acid	*Variety*	29.445	0.001	83.736	<0.001	100.897	<0.001
	Development stage	56.058	<0.001	5.328	0.047	628.628	<0.001
Stearic acid	*Variety*	49.412	<0.001	19.703	0.002	22.231	<0.001
	Development stage	38.411	<0.001	0.395	0.690	34.266	<0.001
Oleic acid	*Variety*	433.603	<0.001	394.282	<0.001	226.142	<0.001
	Development stage	140.781	<0.001	8.604	0.017	69.909	<0.001
Linoleic acid	*Variety*	407.562	<0.001	64.462	<0.001	119.536	<0.001
	Development stage	180.803	<0.001	2.542	0.159	142.650	<0.001
Linolenic acid	*Variety*	65.512	<0.001	86.425	<0.001	130.529	<0.001
	Development stage	0.519	0.620	7.581	0.023	240.135	<0.001
Ca	*Variety*	45.328	<0.001	36.286	<0.001	79.687	<0.001
	Development stage	115.969	<0.001	58.145	<0.001	86.349	<0.001
Cu	*Variety*	7.501	0.019	0.269	0.846	3.721	0.035
	Development stage	64.057	<0.001	23.090	0.002	34.600	<0.001
Fe	*Variety*	9.483	0.011	18.871	0.002	16.504	<0.001
	Development stage	74.791	<0.001	30.810	0.001	25.514	<0.001
K	*Variety*	37.324	<0.001	13.935	0.004	40.663	<0.001
	Development stage	101.867	<0.001	21.100	0.002	42.168	<0.001

Table 1. Two-way ANOVA analysis accroding to sex, variety, and development stage.(Continued)

		Female		Male		Female+male	
		F value	*P value*	*F value*	*P value*	*F value*	*P value*
Mg	*Variety*	90.845	<0.001	21.631	0.001	62.629	<0.001
	Development stage	139.335	<0.001	23.265	0.001	90.625	<0.001
Na	*Variety*	8.074	0.016	9.952	0.010	15.438	<0.001
	Development stage	39.879	<0.001	29.242	0.001	29.154	<0.001
P	*Variety*	10.416	0.009	4.224	0.063	8.496	0.002
	Development stage	100.442	<0.001	25.166	0.001	47.217	<0.001
Zn	*Variety*	116.849	<0.001	85.660	<0.001	196.754	<0.001
	Development stage	52.612	<0.001	16.794	0.003	28.989	<0.001

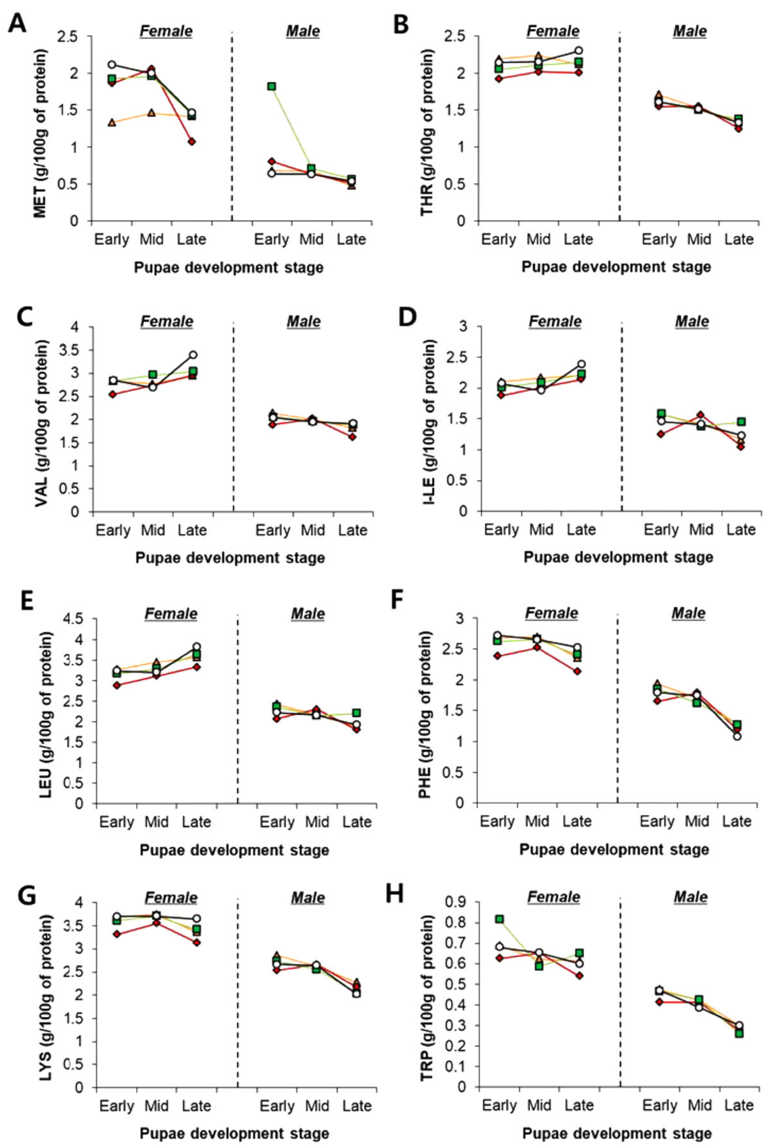

Fig. 2. Essential amino acid composition in silkworm pupae. Female and male pupae of BG(○), GS(▲), JH(◆), and YN(■) varieties were compared according to early (6-7days), mid (8-10days), and late (11-13days) stages. (A) MET, (B) THR, (C) VAL, (D) I-LE, (E) LEU, (F) PHE, (G) LYS, and (H) TRP contents were existed in g/100g of protein.

Amino acid levels were higher in females than in males. MET, PHE, and LYS levels were highest in BG females during the early stage (2.1, 2.7, and 3.7 g/100 g of protein, respectively, Table 1). THR, VAL, I-LE, and LEU levels were highest in BG females during the late stage (2.3, 3.4, 2.4, and 3.8 g/100 g of protein, respectively). YN females contained the highest TRP level (0.8 g/100 g of protein) during the early stage. In general, amino acid levels decreased with the developmental stage in male pupae.

MET, LYS, and TRP, which were at high levels during the early stages in females, were classified as amino acids with antioxidant properties (Xu *et al*., 2017). Studies evaluating the antioxidant activity of pupae have also confirmed the outstanding radical scavenging effect in the early stages of females (Lee *et al*., 2021). It is assumed that amino acids affect the antioxidant ability of pupae. TRP shares six electrons with a pyrrole ring structure, responding immediately to oxidation. MET suppresses the oxidation reaction with sulfur atoms and two lone electron pairs in the side chain. LYS also exhibits antioxidant activity because of the nitrogen atom present in the side chain (Xu *et al*., 2017). Therefore, pupae with high MET, LYS, and TRP levels are expected to have a positive effect on health because of their antioxidant activity and the inclusion of essential amino acids.

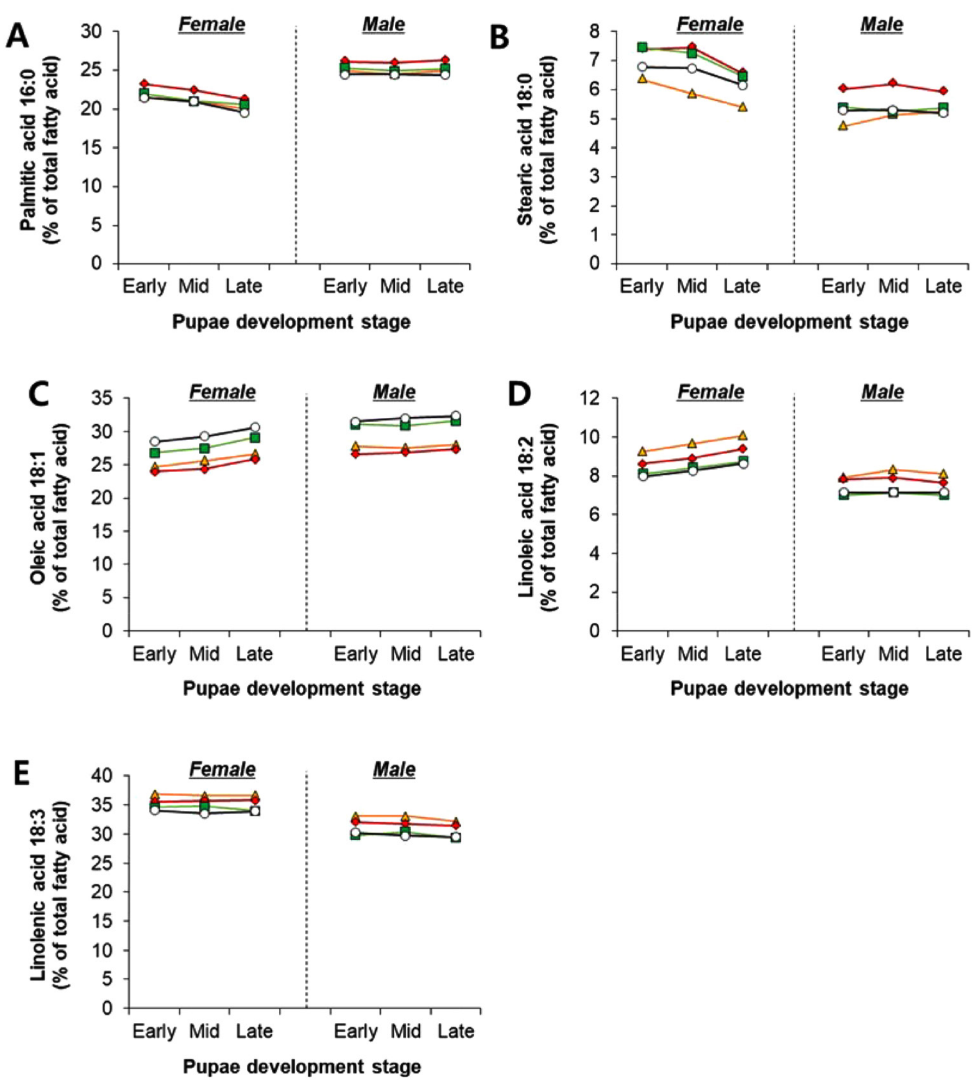

Fig. 3. Fatty acid composition in silkworm pupae. Female and male pupae of BG(○), GS(▲), JH(◆), and YN(■) varieties were compared according to early (6-7days), mid (8-10days), and late (11-13days) stages. (A) Palmitic acid, (B) Stearic acid, (C) Oleic acid, (D) Linoleic acid, and (E) Linolenic acid contents were existed in % of total fatty acid.

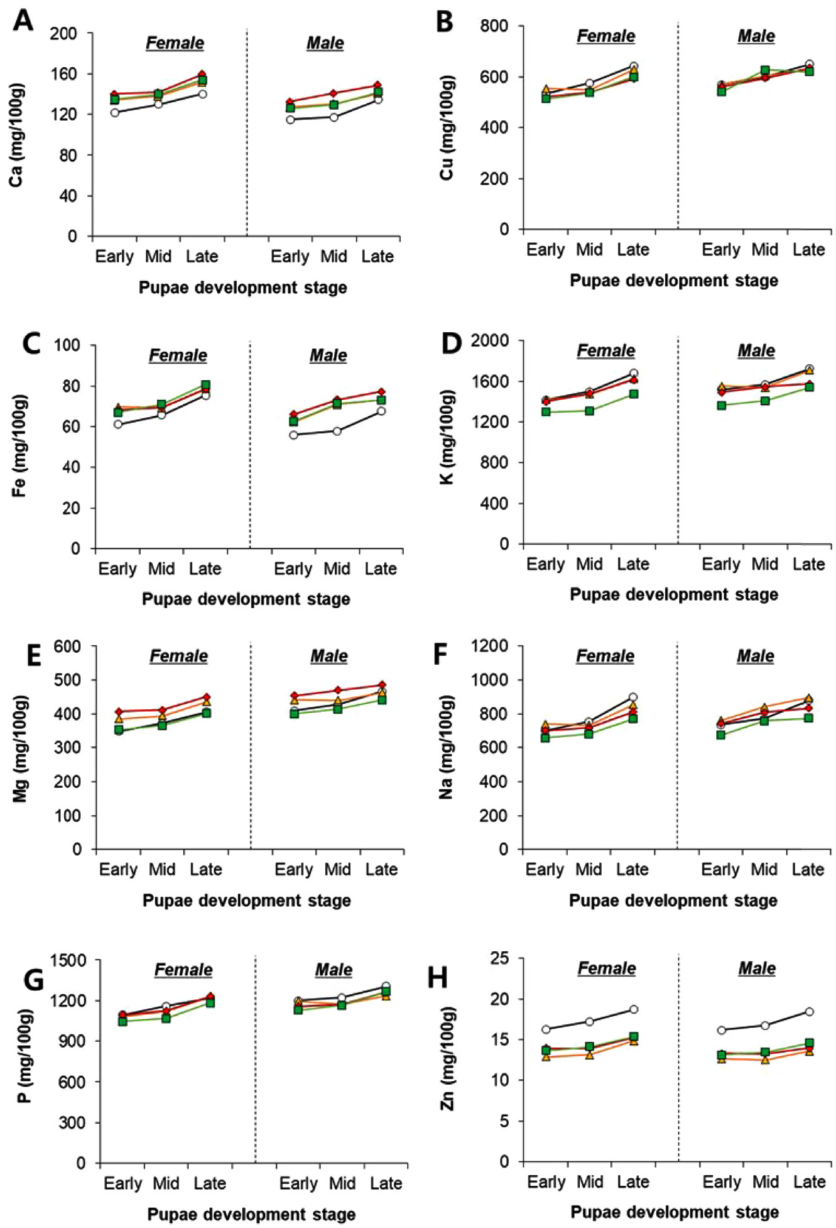

Fig. 4. Mineral composition in silkworm pupae. Female and male pupae of BG(○), GS(▲), JH(◆), and YN(■) varieties were compared according to early (6-7days), mid (8-10days), and late (11-13days) stages. (A) Calcium, (B) Cupper, (C) Iron, (D) Potassium, (E) Magnesium, (F) Sodium, (G) Phosphorus, and (H) Zinc were existed in mg/100 g of sample.

Comparison of fatty acids

The major fatty acids in pupae were palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid (Fig. 3). Palmitic acid and oleic acid were abundant in males (24–25 and 27–32%, respectively). In contrast, stearic acid, linoleic acid, and linolenic acid levels were higher in female pupae (5–7. 8–10, and 34–36%, respectively). Higher palmitic acid and stearic acid levels were observed in JH than in the other varieties with the significance ($P<0.05$, Table 1). Oleic acid was abundant in BG, and linoleic acid and linolenic acid levels were high in GS. Saturated fatty acid levels decreased with the developmental stage in females. Oleic acid and linoleic acid levels in female pupae increased with the developmental stage.

Essential fatty acids cannot be synthesized in the body and therefore must be supplied by food intake. Linolenic acid and linoleic acid, which were abundant in GS, are essential fatty acids that play a role in preventing cardiovascular disease, impaired brain function, and depression (Lunn and Theobald, 2006; Glick and Fischer, 2013). Oleic acid, which was highly abundant in BG, is a monounsaturated fatty acid. In a previous study, oleic acid reduced the risk of coronary heart disease by 20–40% when it replaced 5% of saturated fatty acid intake calories (Lopez-Huertas, 2010). The levels of stearic and palmitic acids, which are saturated fatty acids, were highest in JH. Although the difference in fatty acid content by variety was not significant, the results suggested that the predominant type of fatty acids differed by variety.

Comparison of mineral content

Mineral content increased according to pupae developmental stages (Fig. 4). BG contained less calcium, iron, and magnesium content than other varieties, whereas zinc levels were markedly higher in BG (16–19 mg/100 g). GS contained a mid-range of calcium, copper, iron, potassium, magnesium, sodium, and phosphorus content but contained the lowest zinc levels (13–15 mg/100 g). JH exhibited high levels of calcium (133–160 mg/100 g), iron (66–80 mg/100 g), and magnesium (407–486 mg/100 g). Potassium, magnesium, sodium, and phosphorus levels were lower in YN than in the other varieties.

BG had a high zinc content. Zinc is an essential factor for normal plant and animal growth and regeneration. In addition, zinc is a nutrient constituent of more than 300 enzymes and is involved in carbohydrate and fat metabolism. A deficiency of zinc causes diarrhea, dermatitis, and reduced activity of ACE and alkaline phosphatase enzymes; therefore, proper intake is required (Frassinetti *et al*., 2006). According to the Dietary Reference Intakes for Koreans 2015 report, intake of 100 g of pupa provides the daily recommended intake of zinc (7–10 mg). Additionally, intake of 100 g of pupae per day can provide the recommended daily dose of phosphorus, magnesium, and iron.

Conclusion

Protein, fat, ash, fiber, amino acid, fatty acid, and mineral contents were quantified in 24 pupa samples. Protein levels were high in females, whereas fat levels were high in males. Fiber content dramatically increased during the late stage of pupal development. The essential amino acids were more abundant in females than in males. Essential fatty acid and stearic acid levels were high in females, and oleic acid and palmitic acid levels were high in males. There were differences in fatty acid composition by variety. Mineral content increased with the developmental stage, and some varieties showed markedly different mineral compositions (BS, high zinc level; GS, low zinc level; JH, high Mg and Ca levels; and YN, low Mg and K levels). Pupae are a suitable source of essential amino acids, fatty acids, and minerals. Different varieties exhibited differences in nutritional composition, and a choice based on intended purpose is necessary.

Acknowledgments

This study was supported by the 'Research Program for Agricultural Science & Technology Development' (Grant No. PJ01502202), National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

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