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Production of Silkworms with Conjugated Linoleic Acid (CLA) Incorporated into Their Lipids by Dietary CLA

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Silkworms with conjugated linoleic acid (CLA) incorporated into their lipids (designated CLA silkworms) were produced to enhance the quality of silkworms having a synergistic effect with CLA functions by dietary synthetic CLA. Silkworm larvae were fed fresh mulberry leaves (control diet) until the third instar stage and were then subjected to various levels (0%, 0.1%, 1%, 5%, and 10%) of CLA-sprayed mulberry leaves (designated CLA diet) beginning on the first day of the fourth instar stage and continuing to the third day of the fifth instar stage. CLA contents in CLA silkworms increased proportionally with increasing CLA levels of CLA diets. CLA silkworms on a 1% CLA diet contained 2.2 g CLA/100 g lipid without body weight reduction, whereas CLA silkworms on a 10% CLA diet contained 14.8 g CLA/100 g lipid with a significant reduction of body weight, relative to the control silkworms. The CLA content in the lipids of CLA silkworms on a 10% CLA diet was significantly higher than that of CLA silkworms on a 5% CLA diet. A 0.1% CLA diet was not sufficient to accumulate CLA in the silkworms. Most of the CLA (approximately 99%) of silkworm lipids was present in triglyceride (TG) with a similar ratio of *c*9,*t*11 and *t*10,*c*12 CLA isomers. These results suggest that a 1% CLA diet was suitable for the production of CLA silkworms.

KEYWORDS: Conjugated linoleic acid (CLA); lipid; silkworm; instar stage; hemolymph

INTRODUCTION

Conjugated linoleic acid (CLA) provides several health benefits for humans and several types of animals. Among these are anticarcinogenic (*1*−*5*), antiatheroscrelogenic (*6, 7*), immune stimulating (*8, 9*), hypoglycemic (*10*), and body fat-reducing activities (*11, 12*). The mechanistic actions of the biological functions of CLA are unknown; however, there is strong evidence that these are mediated by the incorporation of CLA into membrane lipids.

Dietary CLA has been shown to be readily incorporated into the triglyceride (TG) and phospholipid (PL) of experimental animals, resulting in its positive biological functions. Attempts were made to accumulate CLA in poultry (*13, 14*), pork (*15*−*18*), fish (*19, 20*), and eggs (*21, 22*) by dietary CLA. The purpose of these efforts was to enhance their food qualities with CLA functions. However, it is difficult to accumulate a large amount of CLA in their lipids because some of the CLA absorbed was metabolized via β-oxidation (*23, 24*); furthermore, the excess amount of dietary CLA reduces the growth rate of animals**.** Hence, it is very important to figure out the appropriate growth stage of animals to initiate with CLA and the CLA concentrations in the diets for the maximal accumulation of CLA. For example, pigs should be subjected to dietary CLA a month before slaughtering for maximum accumulation of CLA in their tissues (*25, 26*). Recently, Park et al. (*27*) observed the accumulation of CLA in the tissues of houseflies by dietary CLA, suggesting the possibility of CLA accumulation in the tissues of other insects, such as silkworms.

It has been found that adult silkworms, which have five developmental growth instar stages and approximately 5 g of average body weight, exhibited hypoglycemic activity in humans (*28*) and mice (*29*) by the chemical compound 1-deoxynojirimycin (*30*). The mechanistic action of this compound was found to inhibit intestinal α-glycosidase activity (*31, 32*). Hence, the powder of silkworms is widely used to treat diabetes in Korea and is also available in markets in Japan as a "Bosulin" (bombyx

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+ insulin). It is of great significance to produce silkworms containing CLA incorporated into the lipids of their bodies (designated CLA silkworms), which have synergistic effects with CLA functions. No data is available for the production of CLA silkworms in the literature.

In the present study, we determine the proper instar stage of silkworms for the initiation with synthetic free CLA-coated on mulberry leaves (designated CLA diet) and then examine the appropriate CLA level for the production of CLA silkworms.

MATERIALS AND METHODS

Materials. Fresh mulberry leaves (average size: 10 cm × 20 cm) were obtained from a local mulberry farm (Sancheong, Korea). Silkworm larvae, *Bombyx mori*, were obtained from the Gyeongnam Agricultural Research and Extension Services (Jinju, Korea). Safflower oil (SO) was purchased from Cheiljedang Co. Ltd (Seoul, Korea). Acetone, hexane, 2-propanol, silicic acid, and methanol were purchased from Aldrich (Milwaukee, WI). Bio-Rad *DC* protein assay reagents and bovine serum albumin (BSA) were purchased from Bio-Rad (Hercules, CA). All other reagents used were reagent grade.

Preparation of Synthetic Free CLA. CLA was synthesized from SO by alkaline isomerization, followed by purification at −20 °C (*33*). The CLA was composed of 37% *c*9,*t*11 CLA, 39% *t*10,*c*12 CLA, 7% palmitic acid, 8% stearic acid, 3% oleic acid, and 6% others, when analyzed by GC, as described below.

Preparation of CLA Diets. CLA solutions (0.1%, 1%, 5%, and 10%, w/v) and a SO solution (10%, w/v) in acetone were prepared and stored in refrigerator until use. CLA diets were prepared by spraying CLA solutions on both sides of fresh mulberry leaves using a hand sprayer, followed by storage in an open space for approximately 40 min to allow the acetone to evaporate. Similarly, a 10% SO diet was prepared by means of CLA diets, using fresh mulberry leaves and the 10% SO solution. The CLA and SO diets were freshly prepared before use.

Rearing of Silkworms. Two experiments were performed. In the first experiment, the proper instar stage of silkworm larvae to initiate with the CLA diets was determined without adverse effects. Silkworm larvae were grown in a rearing room at the Gyeongnam Agricultural Research and Extension Services (Jinju, Korea). Larvae were subjected to fresh mulberry leaves (control diet) and were then switched to a 1% CLA diet on the first day of the third, fourth, or fifth instar stage that was continued until the third day of the fifth instar stage. Control larvae were fed the control diet for the entire experimental period. The second experiment was conducted to investigate the amount of CLA accumulated in CLA silkworm bodies. Silkworm larvae were reared as described in the first experiment, except that they were subjected to various CLA diets (0.1%, 1%, 5%, and 10% CLA diets) and 10% SO diets on the first day of the fourth instar stage. Each diet treatment was triplicated with approximately 300 individual larvae. Diets were *ad libitum*.

Sample Preparations for Lipid and Protein Analysis. From silkworms that were terminated at the end of the experiments, hemolymph was collected in test tubes containing heparin by cutting one of the abdominal legs, and foodstuffs were collected from the alimentary canal. The silkworm bodies without haemolymphs, foodstuffs, and alimentary canal were washed three times with a physiological saline solution. The hemolymph was stored frozen at −18 °C until use. The silkworm bodies and foodstuffs were freeze-dried in a freeze-drier (Ilshin Co., Seoul, Korea), followed by grinding in a mortar to produce a fine powder for further chemical analysis.

CLA and Fatty Acid Analysis. *Lipid Extraction.* The lipid of the freeze-dried powder sample (10 g) was extracted by refluxing for 20 h in a Soxhlet apparatus using ether (100 mL), followed by removing the solvent with a rotoevaporator (Eyela, Tokyo, Japan). The total lipid of the haemolymph sample (10 mL) was extracted by homogenization in 100 mL of a hexane/2-propanol (3:2, v/v) mixture with a homogenizer (Bartlesville, Switzerland) for 1 min, followed by centrifuging for 5 min at 5000 rpm (279*g*) (Ilshin Co., Seoul, Korea). The solvent layer, which was washed three times with 50 mL of 0.47 M Na_2SO_4 by hand shaking for 30 s, was dried over anhydrous Na_2SO_4 and was then dried with a rotoevaporator (Eyela, Tokyo, Japan).

Fractionation of Total Lipids. The lipid sample (5 g) was fractionated into TG and other fractions by silicic acid column chromatography (*34*).

GC Analysis. The TG and lipid samples were methylated according to the methods of the 20% tetramethylguanidine (TMG) described by Park et al. (*35*). The composition of fatty acids, including CLA isomers, was analyzed by GC (Hewlett-Packard 5890, Little Fall, TX) equipped with FID and a fused silica capillary Supelcowax-10 column (60 m × 0.32 mm, i.d., 25 μm film thickness, Bellefonte, PA), as described previously (*35*). Fatty acids, including CLA isomers, were identified by comparison of the relative retention time of standards.

Protein Analysis. The freeze-dried powder sample (0.1 g) in a test tube containing 10 mM phosphate buffer (pH 7, 15 mL) was homogenized for 1 min with a homogenizer (Bartlesville, OK, Switzerland). The homogenate was transferred to a volumetric flask (25 mL) which was then filled with the buffer solution. After centrifugation, the supernatant was used for the analysis of protein content. The protein content in the sample solution was measured by Bio-Rad *DC* protein assay using BSA as the standard.

Statistical Analysis. Data were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test, described previously (*36*).

Table 1. Body Weight and CLA Content of CLA Silkworms Initiated with a 1% CLA Diet at Different Instar Stages[a]

diet	instar stage initiated with diet[b]	fresh body weight[c] (g/silkworm)	total lipid (% dry weight)	CLA content (%)	
				dry weight	total lipid
control	first	1.73 ± 0.12[d] a	16.7 ± 0.4 b	*e*	
1% CLA	third	1.1 8 ± 0.19 b	19.1 ± 1.0 a	0.48 ± 0.05 a	2.52 ± 0.12 a
	fourth	1.58 ± 0.14 a	18.3 ± 0.5 a	0.44 ± 0.04 a	2.38 ± 0.15 a
	fifth	1.65 ± 0.16 a	17.0 ± 0.3 b	0.19 ± 0.02 b	1.10 ± 0.09 b

[a] Silkworms were sacrificed at the end of the experiment (the third day of the fifth instar stage). [b] The initiation with diets was at the first day of the given instar stage. The total number of days fed the control diet was 21 days, whereas the total number of days fed the 1% CLA diet from the third, fourth, and fifth instar stages was 12, 6, and 3 days, respectively. [c] Fresh body weight of a silkworm, without foodstuff, alimentary canal, and haemolymph, at the end of the experiment. Each treatment group contained about 300 silkworms with triplication. [d] Mean ± SD of triplication. Means with different letters in the same column represent a significant difference at $p < 0.05$. [e] Not detected.

RESULTS AND DISCUSSION

Determination of an Appropriate Larval Instar Stage To Initiate with CLA Diets. It is of significance to determine the appropriate instar stage of silkworms to initiate with CLA diets for the production of CLA silkworms because the silkworm has five growth stages and, furthermore, no report is available for CLA silkworms. Therefore, a 1% CLA diet was employed to determine an appropriate instar stage for CLA silkworm production. **Table 1** shows the changes in the body weight and the total CLA contents of CLA silkworms that were fed a 1% CLA diet from the first day of the third, fourth, or fifth instar stage to the third day of the fifth instar stage. The body weight of the control silkworms was 1.73 g/silkworm; however, it was reduced to 1.18 g ($p < 0.05$), 1.58 g, and 1.65 g, respectively, when CLA diets were initiated at the third, fourth, and fifth instar stages. Meanwhile, the body weight when the CLA diet was initiated at the fourth instar stage was different ($p < 0.05$) from that for initiation at the third instar stage but not from that for initiation at the fifth instar stage. These results imply that the body weight was inversely associated with the length of time on the 1% CLA diet, and thus, the proper instar stage to initiate with a 1% CLA diet is the fourth or fifth.

Table 2. Body Weight, Protein Content, Lipid Content, and Food Intake of CLA Silkworms[a]

diet	fresh body weight[b] (g/silkworm)	total lipid (% dry weight)	protein (% dry weight)	food intake[c] (g/silkworm)
control	1.67 ± 0.07[d] a	16.8 ± 0.9 e	62.5 ± 2.4 bc	1.48 ± 0.12 a
0.1% CLA	1.77 ± 0.11 a	18.8 ± 0.5 d	67.4 ± 2.6 ab	1.49 ± 0.13 a
1% CLA	1.62 ± 0.12 a	18.9 ± 0.5 d	70.2 ± 2.3 a	1.43 ± 0.07 a
5% CLA	1.14 ± 0.12 b	21.3 ± 0.4 c	63.3 ± 2.0 bc	1.01 ± 0.10 bc
10% CLA	0.84 ± 0.05 c	23.5 ± 0.6 b	60.5 ± 3.0 bc	0.90 ± 0.03 c
10% SO	1.34 ± 0.11 b	25.9 ± 0.6 a	59.4 ± 2.8 c	1.29 ± 0.07 b

[a] Data were obtained from silkworms produced by feeding various diets from the first day of the fourth instar stage to the third day of the fifth instar stage. Control silkworms were fed the control diet from the first day of the first instar stage. [b] Fresh body tissue weight of a silkworm, without foodstuff, alimentary canal, and haemolymph. Each treatment group contained 300 silkworms with triplications. [c] Average amount of food taken by a silkworm for 6 h at the fifth instar stage. [d] Mean ± SD of triplication. Means with different letters in the same column represent a significant difference at $p < 0.05$.

The total amount of lipids in the dry weight of CLA silkworm bodies for which CLA diets were initiated at the third and fourth instar stages was elevated to 19.1% ($p < 0.05$) and 18.3% ($p < 0.05$), respectively, but it was not affected when the CLA diets were initiated at the fifth instar stage, as compared to 16.7% for control silkworms. The CLA content in lipids of CLA silkworms for which CLA diets were initiated at the third and fourth instar stages was 2.52% ($p < 0.05$) and 2.38% ($p < 0.05$), respectively, relative to 1.10% for CLA silkworms for which CLA diets were initiated at the fifth instar stage. Similar results were seen from the CLA content in the dry weight of CLA silkworms. No CLA was found in the control samples. Consequently, the total lipid and CLA contents of CLA silkworms were positively related to the length of time on the CLA diet; however, a significant body weight reduction was seen in CLA silkworms fed a 1% CLA diet from the third instar stage, not from the fourth instar stage. Given these results, the fourth instar stage is an appropriate larval stage to initiate the CLA diet rather than the third or fifth instar stage.

Effect of Various CLA Diets on the Growth of Silkworms. **Table 2** shows the weight and lipid content of CLA silkworm bodies fed various CLA diets from the first day of the fourth instar stage to the third day of the fifth instar stage. The average body weight of a silkworm was not affected by the diets that were less than 1% CLA, but it was dramatically decreased to 1.14 ($p < 0.05$) and 0.84 g ($p < 0.05$), respectively, by the 5% and 10% CLA diets, as compared to 1.67 g for the control diet. Body weight was also decreased to 1.34 g ($p < 0.05$) by the 10% SO diet. Similarly, the total lipid content in dry body weight was not affected by diets as low as 1% CLA, but it was proportionally increased by 5% and 10% CLA diets: control silkworms contained 16.8%, and CLA silkworms fed a 10% CLA diet contained 23.5%. As expected, silkworms fed a 10% SO diet contained a higher content (25.9%), relative to silkworms fed other diets. Relative to the 62.5% of protein content in dry weight for the control diet, the protein content was increased ($p < 0.05$) by diets as low as 1% CLA**,** but it was not affected by the 5% and 10% CLA diets, nor by the 10% SO diet. Food intake was not affected by diets as low as 1% CLA, but it was decreased ($p < 0.05$) by the 5% and 10% CLA diets, relative to 1.48 g/silkworm for the control diet. Given these data, the growth of CLA silkworms was reduced by the 5% or 10% CLA diet but not by diets containing less than 1.0% CLA.

Food intake significantly influences the maintenance of the healthy body weight and the CLA content of silkworms. Two factors, residuals of acetone and the levels of CLA in the CLA diets, might affect the food intake. When preparing CLA diets by spraying CLA dissolved in acetone on fresh mulberry leaves, the acetone must be completely removed by airing in an open space for a while; otherwise, necrosis could be induced on the mulberry leaves, resulting in a reduced food intake. In the present study, no necrosis was seen in the CLA diets prepared, indicating the absence of acetone residual on the leaves. Hence, the food intake reduction by 31.8% and 39.2% of CLA silkworms fed the 5% and 10% CLA diets, respectively **(Table 2)**, might have, in part, resulted from the unpalatability of higher concentrations of CLA.

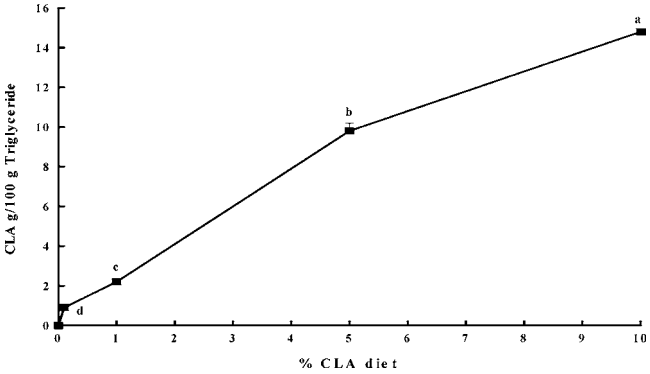

Figure 1. CLA contents in triglyceride (■) and phospholipid (▲) of silkworms fed various CLA diets from the first day of the fourth instar stage to the third day of the fifth instar stage. Means with different letters on the same line represent a significant difference at $p < 0.05$.

Table 3. Distribution of CLA Isomers to Triglyceride Fraction of CLA Silkworms[a]

diet	CLA isomer	CLA in total lipid (mg/100 g)	CLA in triglyceride[b] (mg/100 g)
1% CLA	*c*9,*t*11	1099 ± 51[c]	1085 ± 95
	*t*10,*c*12	1057 ± 62	1050 ± 88
	total	2156 ± 57	2135 ± 90
10% CLA	*c*9,*t*11	7455 ± 213	7396 ± 324
	*t*10,*c*12	7323 ± 105	7289 ± 412
	total	14778 ± 159	14685 ± 360

[a] Data were obtained from CLA silkworms produced by feeding 1% and 10% CLA diets from the first day of the fourth instar stage to the third day of the fifth instar stage. [b] Triglyceride fractionated from the total lipid. [c] Mean ± SD of triplication.

Accumulation of CLA in Lipids of Silkworms. The CLA contents in TG fraction of bodies of CLA silkworms fed various CLA diets from the first day of the fourth instar stage are shown in **Figure 1**. Approximately 99% of the CLA was distributed to the TG fraction. The CLA content in TG was proportionally increased to 0.9, 2.2, 9.8, and 14.8 g CLA/100 g, respectively, by 0.1%, 1%, 5%, and 10% CLA diets. No CLA was detected from the TG of the control and SO silkworms. As shown in **Table 3**, the TG contained approximately a 1:1 ratio of *c*9,*t*11 to *t*10,*c*12 CLA isomers. The results of the accumulation and distribution of CLA isomers to TGs of CLA silkworm bodies are in agreement with the reports on various types of experimental animals (*2, 14, 16, 37*).

The composition of major fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids) in TG is shown in **Table 4**. The alteration of fatty acid composition in TG was closely associated with the CLA level of the diets (**Table 4**). The composition of stearic and oleic acids was decreased ($p < 0.05$), whereas that of linoleic acid was increased ($p < 0.05$), compared to that of control TG. Unlike the case for silkworms, Ramsay et al. (*17*)

Table 4. Compositions of Major Fatty Acids in the Triglyceride Fraction of CLA Silkworms[a]

	fatty acid (%)					
diet	palmitic	stearic	oleic	linoleic	linolenic	total[b]
control	32.4 ± 1.5[c] a	26.0 ± 1.2 a	16.5 ± 1.0 c	4.5 ± 0.2 d	20.6 ± 1.1 b	100
1% CLA	29.4 ± 1.2 a	16.4 ± 0.8 c	20.1 ± 1.2 b	8.2 ± 0.4 c	23.8 ± 0.7 a	97.8
10% CLA	22.6 ± 0.9 b	19.5 ± 0.7 b	12.3 ± 0.7 d	10.2 ± 0.3 b	20.6 ± 0.9 b	85.2
10% SO	20.6 ± 1.0 b	11.2 ± 0.5 d	26.8 ± 1.5 a	30.5 ± 1.6 a	10.9 ± 0.6 c	100

[a] Data were obtained from silkworms produced by feeding various diets from the first day of the fourth instar stage to the third day of the fifth instar stage. Control silkworms were fed the control diet from first instar stage. [b] The remaining portion of fatty acids is *c*9,*t*11 and *c*10,*t*12CLA isomers. [c] Mean ± SD of triplication. Means with different lower case letters in the same column for each treatment represent a significant difference at $p < 0.05$.

Table 5. CLA Contents in the Total Lipid of the Haemolymph and Foodstuff from CLA Silkworms[a]

	haemolymph				foodstuff			
	total lipid[b]	CLA isomer (g/100 g lipid)			total lipid[b]	CLA isomer (g/100 g lipid)		
diet	(g/10 g dry weight)	*c*9,t11	*t*10,*c*12	total	(mg/100 mL haemolymph)	*c*9,t11	*t*10,*c*12	total
control	17.1 ± 0.5[b] c	*c*			19.8 ± 1.0 b			
1% CLA	20.3 ± 0.8 b	2.0 ± 0.1 b	2.3 ± 0.2 b	4.3	18.9 ± 0.8 b	4.3 ± 0.4 b	4.5 ± 0.2 b	8.8
10% CLA	22.4 ± 0.6 ab	6.6 ± 0.2 a	6.5 ± 0.2 a	13.1	23.5 ± 1.2 a	8.2 ± 0.3 a	8.2 ± 0.6 a	16.4
10% SO	23.4 ± 1.0 a				25.9 ± 1.5 a			

[a] Data were obtained at the end of the experiment from silkworms produced by feeding various diets from the first day of the fourth instar stage to the third day of the fifth instar stage. Control silkworms were fed the control diet from the first day of first instar stage. [b] Mean ± SD of triplicate data. Means with different letters in the same column represent a significant difference at $p < 0.05$. [c] Not detected.

reported that CLA incorporation reduces the amount of linoleic acid and increases that of oleic acid in the skeletal muscle and fat of pigs. The elevated levels of linoleic acid and reduced levels of oleic acid in the TGs of CLA silkworm lipids might be attributed to the Δ12-desaturase activity of silkworms (*38*, *39*), that synthesize linoleic acid from oleic acid, and to the oleic acid content (3%) in the diets, but further research is needed.

CLA silkworms have to maintain a healthy body condition for the therapeutic purpose of treating diabetes. The body weight of CLA silkworms on a 1% CLA diet, which was a sufficient level of CLA to produce CLA silkworms, was not affected, relative to that of control silkworms (**Tables 1** and **2**). Most importantly, the CLA silkworms on a 1% CLA diet contained 2.2 g CLA/100 g lipid, composed of 1.1 g of *t*10,*c*12 CLA, which is biologically the most active isomer for body fat reduction in humans and animals (*11*, *12*) with an elevated protein content (7.7% relative to control) (**Tables 2** and **3**). The elevation of the protein content in the CLA silkworms is in agreement with the report that mice fed synthetic CLA reduced body fats concomitantly with increasing body mass (*12*). In addition, the CLA silkworm might contain the chemical compound 1-deoxynojirimycin derived from mulberry leaves (*29*, *30*), which has the ability to lower human blood glucose levels by inhibiting intestinal α-glycosidase activity (*32*). Consequently, for the therapeutic purpose, CLA silkworms, which contain a substantial amount of CLA, a high amount of protein, and 1-deoxynojirimycin, could be produced by a 1% CLA diet.

As a CLA lipid source, CLA silkworms have a higher amount of CLA even if they have a reduced growth rate. CLA silkworms that were fed a 10% CLA diet exhibited a significant growth reduction; however, they contained significantly higher CLA content (14.8%) with 23.5% lipid, relative to 2.2% (18.9% lipid) and 9.8% (21.3% lipid) CLA contents of CLA silkworms, respectively, by 1% and 5% CLA diets (**Tables 2** and **3** and **Figure 1**), suggesting that a 10% CLA diet would be best for the production of CLA silkworms for this purpose. The incorporation of CLA isomers significantly increased the amount of linoleic acid and reduced those of palmitic, stearic, and oleic acids, a fact that must be considered when using CLA as a fat source. The lipid from CLA silkworms was composed of 99% TG, with approximately a 50% *t*10,*c*12 CLA isomer of total CLA; hence, this lipid has beneficial health effects because of a higher percentage of *t*10,*c*12 CLA isomer. The CLA content in CLA silkworms fed a 10% CLA diet far exceeded that in natural products, including milk (5.4−7.0 mg/g fat) (*40*), cheese (5.0−6.2 mg/g fat; 4.1−9.4 mg/g fat) (*41*, *42*), butter (9.4−11.9 mg/g fat) (*43*), beef (17.3 mg/g fat) (*43*), and human milk (3.6 mg/g fat) (*44*). The CLA content was a similar level to that in a fish (carp, 218.4 mg/g fat; tilapia, 180.9 mg/g fat; and rockfish, 126.2 mg/g fat) fed a diet containing 10% CLA (*19*, *20*), but it was higher than that of broilers (10.85 mg/g fat) (*45*), chickens (11.56 mg/g fat) (*45*), and pigs (6.8−7.4 mg/g fat) (*26*) fed synthetic CLA.

CLA Retention in Haemolymphs and Foodstuffs in Alimentary Canals. Table 5 shows the total lipid and CLA contents of haemolymphs and foodstuffs from alimentary canals of CLA silkworms that were fed 1% CLA and 10% CLA diets from the first day of the fourth instar stage to the third day of the fifth instar stage. The total lipid content of haemolymphs from control silkworms was 17.1 mg/10 mL, whereas it was increased ($p < 0.05$) to 20.3 and 22.4 mg/10 mL, respectively, for CLA silkworms on 1% CLA and 10% CLA diets. The lipid content of foodstuffs from CLA silkworms on a 1% CLA diet was 18.9 g/100 g, and it increased to 23.5 g ($p < 0.05$) for CLA silkworms on a 10% CLA diet, relative to that (19.8 g/100 g) of silkworms on a control diet. The lipid content of haemolymphs and foodstuffs from silkworms on a 10% SO diet was a similar amount to that with a 10% CLA diet. The CLA contents in the total lipid of haemolymphs from CLA silkworms that were fed 1% and 10% CLA diets were 5.6 and 13.1 g/100 g lipid, respectively. A substantial amount of CLA (8.8 g/100 g lipid) was retained in foodstuffs obtained from CLA silkworms with a 1% CLA diet, but it was approximately doubled in foodstuffs with a 10% CLA diet. No CLA was found in haemolymphs or foodstuffs from silkworms which ate the control and 10% SO diets. CLA isomers in lipids of haemolymphs

and foodstuffs were found to be approximately a 1:1 ratio of *c*9,*t*11 CLA to *t*10,*c*12 CLA.

It is interesting to see if CLA is synthesized by microorganisms in the intestine and/or enzymes of silkworms and is absorbed, as is the case for other fatty acids. The CLA content in the lipid of bodies and haemolymphs from CLA silkworms was proportional to the CLA level of the diet, and the composition of *t*10,*c*12 CLA and *c*9,*t*11 CLA in the lipids was close to a 1:1 ratio, but no CLA was detected in control and SO silkworms (**Figure 1** and **Tables 3** and **5**). This ratio was quite similar to that of synthetic CLA, which was used for the preparation of the CLA diet, and that of CLA accumulated in the tissues of experimental animals (*9*, *14*−*16*, *18*) and fish (*19*, *20*) by dietary synthetic CLA. No report for the presence of linoleate isomerase in silkworms is available in the literature. Thus, it is clear that the CLA in CLA silkworms was derived from dietary CLA. In addition, the midgut cells of insects absorb diglyceride, monoglyceride, free fatty acids, glycerol, and lysophospholipid without modification or further digestion (*46*). Hence, silkworms might absorb both *c*9,*t*11 and *t*10,*c*12 CLA isomers provided from dietary free CLA, followed by incorporating them into the lipids of the body tissues and haemolymphs of silkworms as is the case for other fatty acids.

In conclusion, CLA silkworms were produced by feeding a 1% or 10% CLA diet from the first day of the fourth instar stage to the third day of the fifth instar stage. CLA silkworms on a 1% CLA diet contained 2.2 g CLA/100 g lipid, composed of a similar ratio of *c*9,*t*11 to *t*10,*c*12 CLA isomers, without reduction in body weight; however, CLA silkworms on a 10% diet contained 14.8 g CLA/100 g lipid with reduction in growth rate.

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