Table 2. Body Weight , Daily Food Intakes and Feed Efficiency of Rats Fed the AIN-76 Diet with or without 0.01% β-carotene*,,# (N = 6)**

group	week	without β-carotene Mean ± SD	with β-carotene Mean ± SD	ANOVA
Food intake (g/d)	1	14.48 ± 1.59 ^{C}	13.79 ± 1.12 ^{C}	NS
	3	17.92 ± 1.18 ^{B}	17.59 ± 1.25 ^{B}	NS
	6	19.80 ± 0.36 ^{A}	20.00 ± 0.12 ^{A}	NS
Body weight (g/wk)	1	66.74 ± 9.60 ^{c}	64.39 ± 5.25 ^{c}	NS
	3	154.38 ± 25.29 ^{b}	166.02 ± 4.60 ^{b}	NS
	6	287.26 ± 17.55 ^{a}	284.87 ± 12.26 ^{a}	NS
Feed efficiency # (%)		29.42 ± 3.11	29.58 ± 3.69	NS

*Values with different superscripts in the same column are significantly different from one another at $p<0.05$ as determined by Duncan`s multiple range test. A, B, C: food intake, a, b, c: body weight.

**Results were analyzed by one-way ANOVA to determine the effects of food intake, body weight gain, and feed efficiency. Differences of $p<0.05$ were considered significant for main food intake, body weight, and feed efficiency. NS = not significant ($p>0.05$).

#Feed efficiency = (daily weight gain/daily food intake) × 100%

Liver tissues (0.150-0.200 g) were homogenized in 2 mL of absolute ethanol (3.0 g/L pyrogallol) and saponified by the addition of 1 mL of saturated KOH followed by heat treatment at 80 °C for 20 min. After the addition of 3 mL of deionized water containing 50 g/L NaCl, the samples were extracted 3 times with 5 mL of hexane. The combined extracts were dried under nitrogen and redissolved in 4.5 mL of methanol. Trans-β-apo-8' carotenal (0.5 mL of 0.6 mg/L) were used as internal standards. A 20-μL aliquot was injected (HPLC L-6000 pump, L-4200 UV-VIS detector, D-2500 Chromato-Integrator, Hitachi) into a reverse-phase column (C-18-AR, 5 μm for β-carotene) with a mixed solvent (methanol: toluene = 3:1, v/v) with a flow rate of 1.7 mL/min. The absolute amount of β-carotene was determined by integration of the area under the curve of absorbance at 450 nm versus time and by comparison with the area obtained for a known amount of purified β-carotene. The percent of recovery of β-carotene added to the cells and liver were 99.3% and 98.6%, respectively.

Table 3. β-Carotene Content of Liver Tissue and Primary Rat Hepatocytes Isolated from Rat Fed AIN-76 Diet with 0.01% β-Carotene.

	β-carotene	
	(μg/mg protein)	(nmol/g tissue)
Liver tissue (n = 6)	0.0478 ± 0.004	0.532 ± 0.088
Hepatocytes (n = 6)	0.0178 ± 0.003	

Statistical Analysis

Statistical analysis was performed by two-way ANOVA using SAS software (SAS Institute, Cary, NC, USA), and differences among groups were compared by Duncan's multiple range test ($p < 0.05$).

RESULTS

Table 1 shows that rat diets contained as little as 50 g/kg soybean oil (polyunsaturated fat) with or without 0.1 g β-carotene. The feeding efficiency did not significantly differ between the diets with and without β-carotene (Table 2). β-Carotene contents of liver tissues and primary rat hepatocytes isolated from rats fed diet with 0.1 g/kg β-carotene were 0.0478 ± 0.004 μg/mg protein (0.532 ± 0.088 nmol/g tissue) and 0.0178 ± 0.003 μg/mg protein, respectively (Table 3).

LDH leakage of cells from rats fed the β-carotene diet increased significantly ($p = 0.0001$) relative to that in rats fed the β-carotene-free diet when primary rat hepatocytes were incubated with 0.05~0.2 mM $FeCl_3$ for 30 and 60 min as shown in Fig. 1. The LDH leakage