Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part A 142 (2005) 383 – 388
CBP
www.elsevier.com/locate/cbpa

Dietary lipid level influences fatty acid profiles, tissue composition, and lipid peroxidation of soft-shelled turtle, *Pelodiscus sinensis*

Chen-Huei Huang *, Way-Yee Lin, Jen-Hong Chu

National Chiayi University, Department of Aquatic Biosciences, 300 University Road, Chiayi 600, Taiwan

Received 28 May 2005; received in revised form 9 September 2005; accepted 9 September 2005
Available online 6 October 2005

Abstract

Dietary lipids containing equal portions of soybean oil and fish oil were fed to juvenile Chinese soft-shelled turtle, *Pelodiscus sinensis*, at supplementation level of 0 to 15% for 8 weeks. Tissue fat contents of turtles increased when dietary lipid concentration increased. Fatty acid profiles for turtles fed diets supplemented with 6% or higher levels of lipids were similar to those in dietary lipids. On absolute value basis, fatty acids of 14-, 16-, and 18-carbons in muscle of turtles fed diet without lipid supplementation were higher than those in the initial turtle muscle. Among them, C16:1 and C18:1 was approximately 4 and 2 fold higher, respectively, than that of the initial turtles. By contrast, absolute amounts of C20:5 and C22:6 in muscle of turtles fed diet without lipid supplementation were slightly less than those in the initial turtles. For turtles fed lipid supplemented diets, tissue C20:5 and C22:6, however, increased when dietary lipid level increased. These results suggest that soft-shelled turtles are capable of synthesizing fatty acids up to 18 carbons from other nutrients and that they may have limited or no ability to synthesize highly unsaturated fatty acids. Lipid peroxidation measured by thiobarbituric acid-reactive substances in tissues of turtles fed 12% and 15% lipids was greater ($p<0.05$) than that in turtles fed 3% to 9% lipids. This could be due to high lipid and unsaturated fatty acid content in these tissues. On lipid basis, lipid peroxidation in turtles fed diet without lipid supplementation was the highest among all groups suggesting the existence of antioxidant factors in the dietary lipids.

Keywords: Dietary lipid level; Fatty acid profiles; Fish oil; Highly unsaturated fatty acids; Lipid peroxidation; Soft-shelled turtle; Soybean oil; *Pelodiscus sinensis*

1. Introduction

Soft-shelled turtle is a high valued cultured animal species in Asian countries. It is considered a product with medical benefits in Chinese tradition for a long time. Compositional analysis has shown that this animal is rich in both $n-3$ fatty acids and trace elements such as zinc, iron and selenium (Wang et al., 1998). Traditional soft-shelled turtle diets would contain ingredients from marine origin that contain high levels of $n-3$ fatty acids. This would be beneficial to prevent cardiovascular diseases due to its highly unsaturated nature (Nettleton, 1995; Calder, 2004). It is not known whether $n-3$ fatty acids are essential for soft-shelled turtles. However, the high $n-3$ fatty acids level in turtle body would be expected to enhance the nutritional value of the turtle. Further, because of the highly unsaturated nature of these fatty acids, their presentation in turtle tissues would increase the susceptibility of turtle tissues to the attack from active oxygen species.

In addition to providing the essential fatty acids, another major function of dietary lipids is to supply the energy for growth. When dietary lipid is in short supply, proteins might be used as an energy source thereby reducing the utilization of protein for growth of animals. The protein sparing effect of dietary lipids has been reported for many fish species that are unable to utilize dietary carbohydrates efficiently (Kikuchi et al., 1992; NRC, 1993; Vergara et al., 1996; Company et al., 1999; Storebakken, 2002). Therefore, it is necessary to maintain suitable level of dietary lipids in animal diets for their optimal growth. The purpose of the present study is to investigate the effects of dietary lipid concentration on growth, body composition, fatty acid profiles and lipid peroxidation in soft-shelled turtles.

* Corresponding author. Tel.: +886 5 2717842; fax: +886 5 2751704.
E-mail address: chuhuang@mail.ncyu.edu.tw (C.-H. Huang).

1095-6433/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2005.09.004

2. Materials and methods

Six experimental diets (Table 1) supplemented with 0%, 3%, 6%, 9%, 12%, and 15% of lipids were fed to juvenile soft-shelled turtles for eight weeks. A 1:1 blend of soybean oil and fish oil was used as the dietary lipid source in this experiment. Estimated metabolizable energy is 3.2 kcal/g for the experimental diets using the biological fuel value of 4.5, 8.51, and 3.49 kcal/g for protein (Smith, 1971), lipids (Chiou and Ogino, 1975), and carbohydrates (Austreng, 1978), respectively. The ingredients were mixed in a KitchenAid multi-function mixer and then stored in a −20 °C freezer until the time of feed dough preparation. Water was added to the feed in a 1:2 (v/w) ratio to produce feed dough and fed to the turtles. The feed dough for individual turtles was prepared once every week, broken into equal portions, and stored at −20 °C until the fed.

Juvenile soft-shelled turtles were obtained from a turtle farm located in Pingtung, Taiwan. Turtles were acclimated and fed the control diet (0% lipid) for one month in our laboratory. Animal care conditions were similar to those described in previous reports (Huang and Lin, 2002, 2004; Huang et al., 2003). After acclimation, apparently healthy turtles were selected and randomly assigned to 90 plastic cylindrical containers (18 cm D × 20 cm H). One turtle was assigned to each container to prevent injury from fighting. There were 15 turtles for each treatment. A complete randomized design (CRD) was used in this experiment. Six experimental diets were randomly assigned to the 90 turtles. The initial turtle body weight for each treatment was 4.9±0.2 g. The height of the water in each container was about the same as the height of the turtle to enable turtles to air breath. The animals were kept indoors at 30±1 °C for the entire feeding period.

Turtles were fed 4% of their body weight daily at 17:00 for 8 weeks. This was the amount that juvenile turtles would consume within 1 h without causing digestion problems based on our previous observations. The containers were cleaned and water was replaced daily in the morning. Body weight of the turtles was measured every 2 weeks. The daily feed allowance of experimental animals was adjusted according to the measurements.

All turtles were sacrificed at the end of each trial. Four turtles from each group were randomly taken for fatty acid composition analysis. Lipids were extracted from tissues (Folch et al., 1957). Fatty acids were methyl-esterified (Lee et al., 1990). Fatty acid composition in tissue lipids were analyzed with a Hewlett-Packard HP 5890 II plus Gas Chromatograph equipped with a flame ionization detector and an HP-INNOWax capillary column (30 m × 0.25 mm, 0.5 μm film thickness) as described by Huang et al. (1998). Nitrogen was used as the carrier gas with 0.5 mL/min flow rate and 30:1 split ratio. The initial temperature was 210 °C. The temperature was increased 1 °C /min to 240 °C, where it was held constant for 17 min. Injector and detector temperatures were 250 °C and 300°C, respectively. A 1-μl sample of the methyl esters of fatty acids was injected into the gas chromatograph. Fatty acid methyl ester reference standards for gas chromatography analysis were obtained from Nu Chek (Elysian, MN).

Iron-catalyzed ascorbate-induced lipid peroxidation in fish tissues was performed following a modified method of Gatta et al. (2000). One gram of tissue was homogenized in 10 mL KCl (11.5 g/L) solution. An aliquot of 1 mL homogenized sample was transferred to a 25 mL flask and mixed with 5 mL of tris-maleate buffer (80 mM, pH 7.4), 2 mL ascorbate (20 mM), 2 mL ferrous solution (5 mM). The sample solution was incubated in a shaking water bath at 37 °C for 30 min. After the incubation, an aliquot of 2 mL sample solution was mixed in a test tube with 2 mL of reacting solution containing 15% trichloroacetic acid and 0.0375% thiobarbituric acid in 1.76% HCl and boiled for 15 min. After cooling, the absorbance of the

Table 1
Dietary formula and proximate composition of the experimental diets

	Supplemented lipid level (%)					
	0	3	6	9	12	15
Ingredient (g/100 g)						
Casein	43.5	43.5	43.5	43.5	43.5	43.5
Dextrin	39.2	31.7	23.6	15.6	7.6	0.0
α-Starch	5.3	5.3	5.3	5.3	5.3.	5.3
Lipid[a]	0.0	3.0	6.0	9.0	12.0	15.0
Betaine	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin[b]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral[c]	5.0	5.0	5.0	5.0	5.0	5.0
α-Cellulose	0.0	4.5	9.6	14.6	19.6	24.2
CMC[d]	3.0	3.0	3.0	3.0	3.0	3.0
Analyzed composition (dry matter)						
Moisture	11.3	11.1	7.3	7.9	7.5	7.3
Crude protein	44.2	44.5	43.5	43.6	42.7	42.3
Crude lipid	0.0	2.5	6.2	9.7	12.9	16.5
Crude fiber	0.4	3.8	8.4	14.3	20.2	25.0
Ash	4.3	4.3	4.2	4.2	4.2	4.1

[a]Lipid source is a 1:1 mixture of menhaden fish oil and soybean oil. [b]1 kg vitamin premix contains 2,000,000 IU vitamin A, 400,000 IU vitamin D_3, 4 g vitamin k_3, 45 g α-tocopheryl acetate, 5 g thiamine-HCl, 5 g riboflavin, 10 g calcium pantothenate, 20 g niacin, 0.6 g biotin, 4 g pyridoxine-HCl, 1.5 g folic acid, 10 mg B_{12}, 200 g inositol, 100 g ascorbyl monophoshate-Mg, 400 g choline chloride. [c]1 kg mineral premix contains 130.6 g calcium phosphate dibasic, 327 g calcium lactate, 29.7 g ferric citrate, 137 g magnesium sulfate, 239.8 g potassium phosphate dibasic, 87.2 g sodium phosphate dibasic, 43.5 g sodium chloride, 0.15 g aluminum chloride hexahydrate, 0.15 g potassium iodine, 0.1 g cupric chloride, 0.8 g manganese sulfate monohydrate, 1 g cobalt chloride hexahydrate, 3 g zinc sulfate heptahydrate. [d]CMC: carboxymethyl cellulose.

Table 2
Specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ration (PER) and survival of juvenile soft-shelled turtle *P. sinensis* fed diets containing different level of lipids

Supplemented lipid (%)	SGR (%)	FCR	PER	Survival (%)
0	1.187±0.112	1.9±0.1	1.1±0.1	86.7
3	1.196±0.125	2.2±0.2	1.0±0.1	80.0
6	1.205±0.152	2.6±0.3	0.9±0.1	86.7
9	1.281±0.162	2.0±0.2	1.0±0.1	86.7
12	1.202±0.151	2.0±0.2	1.1±0.1	93.3
15	1.199±0.151	2.1±0.2	1.0±0.2	100.0

There is no significant difference among treatment means±S.E. ($p>0.05$).