JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 7877–7882 7877
DOI:10.1021/jf901367u

LDL Oxidation, Antioxidant Capacity and Growth of Cultured Grey Mullet (*Mugil cephalus*) Fed Dietary Sorghum Distillery Residue Pretreated with Polyethylene Glycol

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Dietary sorghum distillery residue (SDR) showed antioxidant and blood thinning effects on grey mullet during winter, but inhibited their growth. The objective of this study was to establish a preliminary treatment of the dietary SDR with polyethylene glycol (PEG), a tannin-binding agent, to enhance growth and blood antioxidant capacity of grey mullet (*Mugil cephalus*) feed. The feeding trial was carried out from June to November. The water temperature was between 25 and 30 °C; the specific growth rate of mullet was reduced significantly by feeding diet containing 20% SDR in comparison to fish fed the control diet or diet containing 20% SDR and PEG. In the period of October−November, the water temperature decreased to 19−25 °C; the specific growth rates of the 20% SDR-PEG group and the 20% SDR group were 0.13 and 0.19% day^{-1}, respectively, significantly higher than those fed the control diet (0.07% day^{-1}). Feeding with 20% SDR or 20% SDR-PEG diets resulted in prolonged lag phase of low-density lipoprotein (LDL) oxidation compared to fish fed the control diet. The total antioxidant capacity of the plasma of the grey mullet fed 20% SDR-PEG was 1.24 mmol/L, significantly higher than those in the fish fed 20% SDR diet (0.84 mmol/L) or the control (0.72 mmol/L). In vivo observations found that preliminary treatment of SDR with PEG eliminated the endogenous undesirable growth inhibitory factors but maintained its protective effects against LDL oxidation in blood and improved the total antioxidant capacity and cold adaptation of grey mullet. The ethanol extract of SDR contained 31.9 ± 7.8 mg/g gallic acids equivalent. The concentration needed to scavenge 50% of the DPPH radicals (IC_{50}) was 0.86 mg/mL. Increased gallic acid equivalent and decreased IC_{50} of DPPH scavenging activity of SDR fed to fish increased the total antioxidant capacity in blood plasma of grey mullet significantly.

KEYWORDS: Sorghum; distillery residue; polyethylene glycol; mullet; low-density lipoprotein (LDL); total antioxidant capacity; gallic acid; tannin; DPPH

INTRODUCTION

Antioxidants can defend against free radical mediated reactions. Mobilizing antioxidants, for example, vitamin E, from storage in situ to the target sites plays an important role for tolerance, repair, and recovery of the body (*1*). High antioxidant capacity in the body must be maintained to cope with environmental oxidative stress. Human studies and animal models have reported that oxidative stress is related to common degenerative diseases, such as cancer and cardiovascular pathologies (*2*). Antioxidants, for example, β-carotene, α-tocopherol, and some natural compounds such as isoflavonoids and flavonoids from plants of the soybean family, can prevent low-density lipoprotein (LDL) oxidation (*3*−*5*) and delay the development of atherosclerotic plaques in animals. Human and laboratory animals are homoiothermal, but fishes are poikilothermal, and their blood viscosity is sensitive to temperature changes. grey mullet (*Mugil cephalus*) fed sorghum distillery residue (SDR) showed improved cold resistance and increased specific growth rate during winter and Atlantic salmon (*Salmo salar* L.) macrophage cell line treated with ethanolic extract of I-Tiao-Gung (*Glycine tomentella*) from the soybean family showed anti-inflammation (*6*, *7*). Hardly any studies related cold adaptation to antioxidant capacity of fish. Our previous studies confirmed that feeding on SDR improved blood fluidity and cold adaptation of grey mullet cultured through winter (*5*). The blood thinning effect was in agreement with epidemiological data that polyphenols from tea reduced the risk of stroke (*3*, *8*). SDR contains abundant polyphenols including tannins (*6*), which have strong antioxidant activity (*9*) in fish (*10*).

However, SDR had a negative effect on the growth of grey mullet cultured at their optimal temperatures (*11*). Studies have suggested that the high concentrations of condensed tannins present in forages consumed by animals were associated with decreases in feed intake, in vivo digestibility, and in situ and in vitro degradability of nutrients (*12*, *13*). Our previous results also showed that the tannin in SDR had an adverse effect on protein

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Table 1. Ingredient Formulation and Proximate Composition of Grey Mullet Feed Containing Sorghum Distillery Residue (SDR)

ingredient	control	20% SDR	20% SDR-PEG
fish meal (kg)	20	20	20
soybean meal (kg)	30	26	26
rice bran (kg)	40	24	24
SDR (wet weight) (kg)		20	20
PEG (kg)			0.1
wheat flour (kg)	5	5	5
nineral premix[a] (kg)	0.5	0.5	0.5
vitamin premix[b] (kg)	0.1	0.1	0.1
dicalcium phosphate (kg)	0.5	0.5	0.5
lecithin (kg)	0.5	0.5	0.5
choline (kg)	1	1	1
fish oil (kg)	1	1	1
yeast (kg)	1	1	1
vitamin E (kg)	0.04	0.04	0.04
moisture (%)	9	8	8
crude protein (%)	33	32	33
crude lipid (%)	10	8	8
ash (%)	10	9	9
crude fiber (%)	3	4	4
soluble salt (%)	0.4	0.6	0.6
gross energy [c]	3790	3700	3770

[a] Mineral premix (g/kg of premix): $CaCO_3$, 336; KH_2PO_4, 502; $MgSO_4 \cdot .7H_2O$, 162; NaCl, 49.8; Fe(II) gluconate, 10.9; $MnSO_4 \cdot H_2O$, 3.12; $ZnSO_4 \cdot 7H_2O$, 4.67; $CuSO_4 \cdot 5H_2O$, 0.62; KI, 0.16; $CoCl_2 \cdot 6H_2O$, 0.08; NH_4-molybdate, 0.06; $NaSeO_3$, 0.02. [b] Vitamin premix (mg/g of premix): retinol palmitate, 500,000 IU; thiamin, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10; cholecalciferol, 50,000 IU; α-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25. [c] Gross energy calculated using values of 4.5, 8.6, and 3.5 kcal/g for protein, lipid, and carbohydrate, respectively (*20*).

digestion (*11*), due to the fact that tannins bind protein. Other studies have shown inhibitory effects of tannins on ruminal enzymes, attributed to the formation of tannin−enzyme complexes with subsequent inactivation of the enzymes (*14*, *15*). Therefore, it is necessary to reduce the protein binding effect to appreciate the beneficial effects of tannins.

Polyethylene glycol (PEG) is nontoxic and is used in a variety of products. PEG with added electrolytes is used for bowel preparation before surgery or colonoscopy and drug overdoses as well as in various protein medications. PEG allows a slow clearance of the carried protein from the blood. It makes for a longer acting medicinal effect, reduces toxicity, and allows longer dosing intervals (*16*, *17*).

In vitro tests indicated that SDR treated with PEG improved protein digestibility, which was inhibited by tannin of SDR (*11*). PEG has been known to increase growth in some species of warm-blooded animals (*18*), but little has been known about its effects on fish. It is the objective of this study to determine if treating SDR with PEG eliminate the undesirable growth inhibitory factors of SDR but maintain its protective effects on blood properties and cold adaptation of grey mullet cultured through winter.

MATERIALS AND METHODS

Diet Formulation and Preparation. SDR was obtained from Kinmen Kaoliang distiller. The SDR was dried by double drum dryer and then ground with a blender to pass a 1 mm sieve to 60 mesh. Prior to diet formulation, the proximate compositions of feed ingredients were analyzed using AOAC methods (*19*). Gross energy was calculated (*20*) from the proximate composition as shown in **Table 1**. Fish meal, mineral premix, vitamin premix (Yi Cheng, Taoyuan, Taiwan), and other feed ingredients were mixed to make pellets 3 mm in diameter. The control feed was formulated (**Table 1**) by us and manufactured by a commercial source for mullet farmers. The test diets were evenly mixed according to **Table 1**, added with water in a 1:1 (w/v) ratio into dough form, and then pelleted (Nippon Career Chopper, 2.5 mm diameter) for growth trials.

SDR (100 g) was extracted with 2 L of 95% ethanol, which was dried in a rotary evaporator to obtain SDRE. Another portion of SDR (100 g) was first extracted with petroleum ether (bp 60−80 °C) to remove lipid. The residue after filtration was dried to remove ether followed by extraction with 95% ethanol like SDRE to obtain SDRE-2 for total phenolics analysis.

Growth Trial. The growth trial of grey mullet (*M. cephalus* Linnaeus) was started in July 2006 and lasted for 5 months. A total of 90 grey mullet individuals (378 ± 68 g) were divided into three groups. Each group of 30 fish was cultured in a cage net in an open pond (30 m × 30 m × 2 m). In the test diets, soybean meal and rice bran were replaced by 20% SDR or 20% of SDR pretreated with PEG (SDR-PEG) to give the same levels of protein and gross energy (**Table 1**). Water salinity was 30 ppt. Water temperature was measured daily at 8:30−9:00 a.m., whereas water quality was monitored weekly at the same time. Dissolved oxygen was maintained at 6.5−8.0 mg/L, NH_3-N at 0.1−0.3 mg/L, and pH at 7.5−8.8. The diets were manually fed twice daily, each time at 2% of mullet body weight. The fish were allowed to acclimate to the experimental system for a period of 2 months before the growth trial was begun. During the adaptation period, control diet was fed daily to all fish. Fish were individually weighed and recorded for feed adjustment every month. Body weight and length of mullet were measured, and blood was collected for analyses of glucose, triacylglycerol, and cholesterol. The specific growth rate (SGR) was calculated following the equation of Hossain et al. (*21*)

$$\mathrm{SGR}\ (\%\mathrm{day}^{-1}) = (\ln \mathrm{FBW} - \ln \mathrm{IBW})/\mathrm{days} \times 100$$

where FBW is final body weight and IBW is initial body weight.

Fish Blood Collection. Fish were collected from aquaria by net. Each fish was put on a tray. A wet cloth was used to cover the fish eye. Blood samples were immediately withdrawn from the caudal vein using a syringe with a needle (TOP Surgical, Taiwan Corp., Kaohsiung, Taiwan, ROC) containing lithium heparin (357 units/mL) as anticoagulant. After thorough mixing, each blood sample was transferred to a 2.0 mL microtube containing 0.1 mL of heparin. The blood samples were kept on ice, brought back to the laboratory, and then centrifuged at 600 *g* for 15 min to obtain plasma.

Biochemical Analyses of Blood Plasma. Concentrations of plasma glucose, triacylglycerol, total cholesterol, and LDL-cholesterol were determined enzymatically using commercial kits (Randox Laboratories, San Francisco, CA).

The *total antioxidant capacity* (TAC) of plasma was measured using an enzymatic kit (Randox Diagnostics, NX 2332, County Antrim, U.K.) according to the method of Miller et al. (*22*), using 2,2′-azinobis(3-ethylbenzthiazoline sulfonate) (ABTS) and metmyoglobin plus H_2O_2. The mixture produced a blue-green color of the $ABTS^{\bullet+}$ ion that absorbs at 600 nm. Antioxidant inhibited the production of $ABTS^{\bullet+}$. TAC of plasma was calculated as millimolar in reference to the antioxidative activity of Trolox.

DPPH Radical Scavenging Activity and Total Phenolic Estimation. A method according to Shimada et al. (*23*) was used to test for DPPH radical scavenging activity. Freshly prepared α,α-diphenyl-β-picrylhydrazyl (DPPH) methanolic solution was used to dissolve the ethanolic extract of SDR, SDRE, or Trolox, a positive control. The resulting solutions were left to stand for 30 min in the dark prior to being detected at 517 nm. The absorbance (A_{517nm}) was measured using an ELISA reader (μ Quant, Bio-Tek Instruments, Inc., Winooski, VT). A lower A_{517nm} indicates a higher DPPH scavenging activity.

Folin−Ciocalteu phenol reagent was used to react with SDRE or SDRE-2 in methanolic solution at 25 °C for 5 min. Na_2CO_3 was added to the mixture to pH 9.70−9.85 and allowed to stand for 2 h at 25 °C. Absorbance at 675 nm was measured. Gallic acid was used to make a standard curve for quantitation of gallic acid equivalent (mg/g of SDRE).

Isolation and Cu^{2+}-Induced Oxidation of LDL. LDL (200 μL) was obtained by ultracentrifugation of grey mullet blood plasma (6 mL) from a density range of $1.019 < d < 1.063$ adjusted with NaBr (*24*).

The concentration of dialyzed LDL was adjusted to 0.15 mg of cholesterol/mL. The kinetics of LDL oxidation were determined by

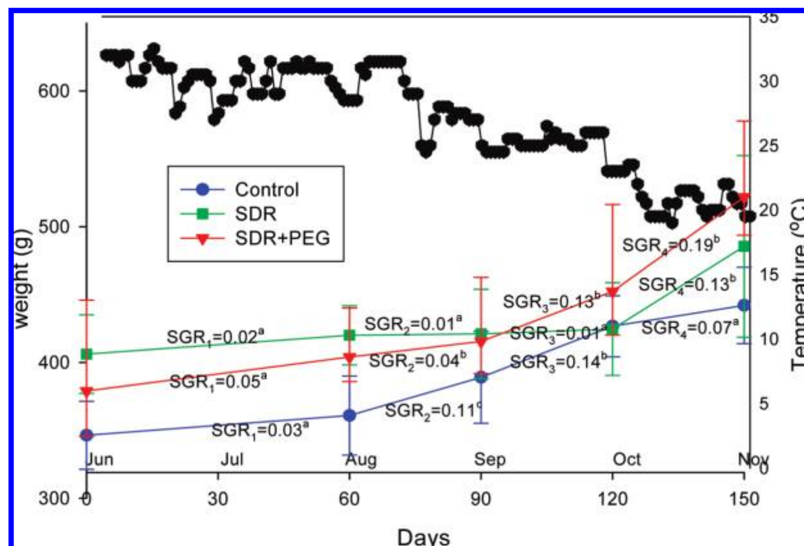

Figure 1. Grey mullet growth and water temperature profile during June–November.

monitoring the changes in absorbance at 234 nm (ΔA_{234nm}) at 25 °C in a 96-well microliter plate (Spectramax 190, Maxline Microplate Reader System Devices, Sunnyvale, CA). One hundred microliters of LDL was added in each well in which the final volume was adjusted to 240 μL with PBS. LDL oxidation was initiated by adding 10 μLof 0.125 mM $CuSO_4$ to a final volume of 250 μL. The plot of ΔA_{234nm} versus time generally showed three consecutive phases: lag phase, propagation phase, and decomposition phase. The lag phase is defined as the intercept of the tangent drawn to the steepest segment of the propagation phase to the horizontal axis according to the method of Kleinveld et al. (*24*).

Satistical Analysis. The results were expressed as mean ± SD. Data were analyzed by a one-way analysis of variance followed by checking all differences between pairs of means by Duncan's multiple-range test and SPSS (Statistics Package for Social Science) software. Pearson correlation coefficients were used to measure the relationship between individual parameters of LDL and its oxidation indices. $P < 0.05$ was regarded to be statistically significant.

RESULTS AND DISCUSSION

Growth Rate. This study was performed in an open culture pond. Water temperature, body weight, and specific growth rate of grey mullet fed the control diet or test diets are shown in **Figure 1**. Due to the environmental effect, the standard deviations of the results were quite large, as would have been seen in aquaria. Although aquaria in laboratories could provide a stabilized environment for culture trials, fish growth would be limited by the size of the aquaria.

Throughout the 5 months of culture time, the growth of grey mullet seemed to have four different stages (SGR_1–SGR_4). From June to mid-August, the average water temperature was 30 ± 2 °C. SGR_1 of fish fed the SDR diet and the SDR-PEG group were not significantly different from the control group ($P > 0.05$). However, during August, SGR_2 values of the three groups were significantly different ($P < 0.05$). The SDR group was 0.01% day^{-1}; the SDR-PEG group, 0.04% day^{-1} and the control group, 0.11% day^{-1}. During September–October, the average water temperature was 26 ± 2 °C; SGR_3 of the control and the SDR-PEG group increased to 0.14 and 0.13% day^{-1}, respectively, of which the growth rates were faster than that of the SDR group (0.01% day^{-1}). During October–November, while the water temperature decreased to 20 °C, SGR_4 of the control group decreased to 0.07% day^{-1}. On the contrary, that of the SDR group showed a noticeable increase in SGR_4 to 0.13% day^{-1}, whereas that of the SDR-PEG group was even higher, being 0.19% day^{-1}. Both the SDR and SDR-PEG diets yielded faster growth rate than that of the control group during the cold season.

The previous results indicated that SDR without PEG treatment had a negative effect on the growth of grey mullet cultured at their optimal water temperatures (*6*). This growth trial showed that dietary SDR subjected to preliminary treatment with PEG improved SGR, which was higher than those of the control group and the SDR group during winter time.

The preliminary treatment of SDR with PEG was proved to be feasible to eliminate the in vivo undesirable effects of SDR. The SDR-PEG group showed higher specific growth rate than the SDR group or the control group. SDR contains polyphenols including tannin. The amount of nonextractable polyphenols is almost double that of the extractable polyphenols in a whole diet (*25*). Nonextractable polyphenols are high molecular weight proanthocyanidins and phenolics that associate with dietary fiber and indigestible compounds. Nonextractable polyphenols or polyphenols associated with indigestible fractions are the major fractions of dietary polyphenols (*26*, *27*).

Polyphenols with a high degree of polymerization and the polyphenols associated with high molecular weight compounds become bioactive once they are released from food matrix by the action of digestive enzymes in the small intestine and bacterial degradation in the large intestine (*28*). An estimated 48% of dietary polyphenols is bioaccessible in the small intestine, whereas 42% becomes bioaccessible in large intestine (*25*). Certain proteins can bind and precipitate polyphenols (*9*, *18*), resulting in reduction of growth rate. However, after PEG treatment, the tannins are bound to PEG (*11*) and form strong hydrogen bonding with the phenolic and hydroxyl groups in tannins. The surface conjugation of PEG increases the hydrophilicity of villi and lowers their surface free energy (*29*), providing controlled release of the polyphenols. The improved specific growth rate of grey mullet by feeding SDR or SDR-PEG during October and November is indicative of the combined bioactivities of the polyphenols and the polyethylene glycol bound SDR.

Our previous results showed that the dietary intake of SDR was able to reduce hydroperoxide formation in grey mullet gill, inhibited hemolysis, induced a blood-thinning effect, and improved cold adaptation and, thus, increased SGR during the winter (*6*). Treating SDR with PEG thus eliminated the undesirable growth inhibitory effects of SDR in this in vivo study (**Figure 1**). On the other hand, PEG increased the bioaccessibility

Table 2. Growth, Plasma Glucose, and Plasma Lipid of Grey Mullet Fed Diet Containing 20% SDR or 20% SDR-PEG for 5 Months[a]

parameter	time	control	20% SDR	20% SDR-PEG
body length (cm)	initial	33 ± 02 a	33 ± 2 a	33 ± 2 a
	final	34 ± 03a b	35 ± 2 ab	35 ± 2 a
body mass (g)	initial	346 ± 83 ab	410 ± 83 ab	378 ± 106 a
	final	452 ± 29 ab	465 ± 106 ab	516 ± 95 a
blood glucose (mg/dL)	initial	147 ± 29 b	178 ± 45 b	103 ± 43 a
	final	77 ± 19 a	77 ± 20 a	77 ± 9 a
cholesterol (mg/dL)	initial	289 ± 063 ab	315 ± 095 aa	287 ± 067 a
	final	361 ± 072 bb	288 ± 083 ab	241 ± 055 a
triacylglycerol (mg/dL)	initial	402 ± 104 ab	305 ± 105 ab	299 ± 136 a
	final	388 ± 294 a	650 ± 326 b	609 ± 224 ab

[a] All values are expressed as mean ± SD ($n = 10$). Values with different letters in the same row are significantly different ($p < 0.05$) analyzed with Duncan's multiple-range test.

Table 3. Yield, Total Phenolic Contents in Gallic Acid Equivalent, and DPPH Scavenging Activity Indicated by IC_{50}[a] of the Ethanol Extract of Sorghum Distillery Residue (SDRE)

treatment	yield (% SDR)	gallic acid equivalent (mg/g of SDR)	IC_{50}[a] (mg/mL)
SDRE[b]	18.6	31.9 ± 7.8	0.86 ± 0.41
SDRE-2[c]	14.2	38.8 ± 5.2	0.55 ± 0.15

[a] IC_{50}, amount of antioxidant required to reduce the initial DPPH concentration by 50%. Values are mean ± SD ($n = 6$). Values are not significantly different ($p > 0.05$) analyzed with Student's t test. [b] SDRE, 95% ethanol extract of sorghum distillery residue (SDR). [c] SDRE-2, SDR extracted with petroleum ether was followed by extraction of the residue with 95% ethanol.

Table 4. Total Antioxidant Capacity (TAC) in Plasma of Grey Mullet Fed for 5 Months[a] with Diet Containing SDR or SDR Pretreated with PEG (SDR-PEG)

diet	TAC (mmol/L)
control	0.72 ± 0.24 a
20% SDR	0.84 ± 0.33 a
20% SDR-PEG	1.24 ± 0.26 b

[a] All values are expressed as mean ± SD ($n = 10$). Values with different letters in the column are significantly different ($p < 0.05$) analyzed with Duncan's multiple-range test.

of dietary SDR consisting of tannins/polyphenols extracted from SDR with ethanol (SDRE) acting as antioxidant (**Table 3**) in fish fed. SDRE is rich in isoflavones, gallic acid, 3,4-dihydroxybenzoic acid, and caffeic acid (data not shown); absorption into fish enhanced the total antioxidant capacity of blood plasma (**Table 4**) and exerted protective effects on LDL oxidation (**Figure 2**). The polyphenols in SDR are antioxidative against free radical mediated oxidation (**Table 5**). The dietary SDR also kept the red blood cell membrane intact and suppressed hemolysis and lowered blood viscosity, thus enhancing blood fluidity or blood circulation in fish (*6*). These effects explain why the fish fed this diet showed higher growth rate in the winter than the control. SDR-PEG eliminated the undesirable growth inhibitory effects of SDR but maintained blood fluidity and cold adaptation of grey mullet cultured through the winter. All of these effects seemed to result in grey mullet more adaptive to cold temperature. The mechanism and the active compounds responsible for the efficacy of SDR are currently being investigated.

Final Body Mass, Blood Glucose, and Blood Lipid. From June to November, feeding grey mullet with diets containing 20% SDR or 20% SDR-PEG for 5 months, the final body length, body

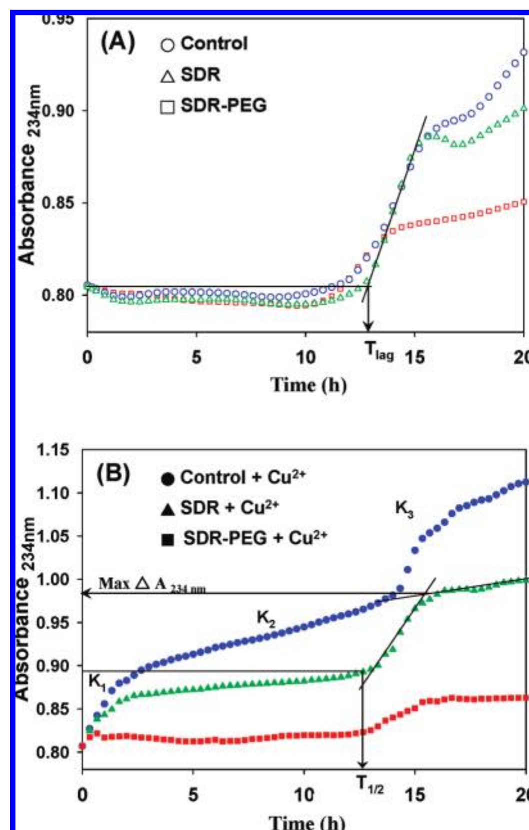

Figure 2. Kinetics of ex vivo LDL oxidation in plasma of grey mullet fed for 3 months with diet containing 20% SDR or 20% SDR-PEG.

mass, and final blood glucose were similar between the three groups (**Table 2**). Total cholesterol of fish from the SDR-PEG group was 241 mg/dL, significantly lower ($P < 0.05$) than those of the control group (361 mg/dL), and there was no significant difference between the SDR-PEG and SDR. Final triacylglycerol (TG) showed that SDR and SDR-PEG were higher in TG but not significantly different. SDR had a negative effect on the growth of grey mullet cultured at their optimal water temperatures. Feeding 20% SDR or 20% SDR-PEG improved SGR, which was higher than those of the control group during winter time. Our results indicated that SDR could be developed into functional feed additive for formulation of anti-cold-stress feed. In addition, the soybean meal and rice bran could be replaced by 20% SDR or 20% SDR-PEG in grey mullet diets.

DPPH Scavenging Activity and Total Phenolic Content. Two SDRE samples were used to test for total phenolic content and DPPH scavenging activity (**Table 3**). The SDRE contained lower gallic acid equivalent (31.9 ± 7.8 mg/g) and had weaker inhibition against DPPH radicals as shown by an IC_{50} of 0.86 ± 0.41 mg/mL, whereas the SDRE-2 defatted by extraction with petroleum ether, having 38.8 ± 5.2 mg/g gallic acid equivalent, showed stronger inhibitory effect indicated by a smaller IC_{50} (0.55 ± 0.18 mg/mL) than the SDRE. Therefore, increased gallic acid in SDR could possibly result in a stronger scavenging effect on DPPH radicals, but the statistical analysis did not show significant difference.

Plasma Total Antioxidant Capacity (TAC). The TAC of plasma of the cultured grey mullet fed 20% SDR-PEG for 5 months was 1.24 mmol/L, which was significantly ($P < 0.05$) higher than that of the group fed 20% SDR diet (0.84 mmol/L) and that of the control (0.72 mmol/L) (**Table 4**). The TAC values of the SDR group and the control were not significantly different ($P > 0.05$) from each other. The higher TAC in blood plasma of fish fed 20%

Table 5. Ex Vivo Effects[a] on Plasma LDL Oxidation of Grey Mullet Fed for 3 Months with Diet Containing 20% SDR or 20% SDR-PEG

parameter	control	SDR	SDR-PEG
t_{lag}[b] (min)	733.8 ± 3.2	774.9 ± 9.50*	713.6 ± 4.8
$t_{1/2}$[c] (min)	552.6 ± 5.9	792.9 ± 9.50**	829.10 ± 9.80**
rate ($\times 10^{-3}$) (ΔA_{234nm}/min)[d]	0.71 ± 0.04	0.46 ± 0.07**	0.07 ± 0.03**
max ΔA_{234nm}[e]	0.25 ± 0.01	0.16 ± 0.01**	0.05 ± 0.01**

[a] The data are shown as mean ± SD (n = 3); *, P < 0.05, and **, P < 0.01, significantly different from control. [b] t_{lag} is the lag time to reach propagation phase from the initial time (**Figure 2A**). [c] $t_{1/2}$ is the time needed to reach halfway between the maximum A_{234nm} and the initial A_{234nm} (**Figure 2B**). [d] Oxidation rate of LDL is calculated from the slope of the absorbance curve during propagation phase (**Figure 2A**). [e] The maximum oxidation extent is the increment of absorbance at 234 nm (A_{234nm}) from the initial A_{234nm} to the tangent line of the first decomposition phase (**Figure 2B**).

SDR-PEG indicates that the presence of PEG with SDR causes the antioxidative compounds to be more available to function as antioxidants. The PEG-bound components in SDR were potentially effective antioxidants. Release of these compounds could enhance their absorption and noticeably improved mobilizing the antioxidant to plasma LDL. Therefore, higher TAC provides more protection on lipid from oxidant attacks (*30*). Similar evidence showed that feeding tilapia with an isoflavone-rich extract of I-Tiao Gung, the root of a plant of the soybean family, resulted in higher TAC in plasma and higher α-tocopherol in LDL (13.0 ± 3.3 molecules/molecules LDL) than control (8.8 ± 2.5 molecules/molecules LDL) (*5*). This showed that natural antioxidants absorbed into blood plasma can elevate the antioxidant status of plasma and protect α-tocopherol in situ from being oxidized.

LDL Oxidation. The ex vivo autoxidation of grey mullet LDL without Cu^{2+} induction showed limited increase in A_{234nm} for up to 13 h of exposure to air (**Figure 2A**). Then the A_{234nm} increased significantly, indicative of the initiation of LDL oxidation. The LDL from the three groups of fish showed typical oxidation kinetics with a lag phase and a propagation phase in the absence of Cu^{2+} catalysis. In the SDR-fed fish the lag phase of the LDL oxidation was 774.9 ± 9.5 min, significantly longer than that of the control group and the SDR-PEG group (P < 0.05) (**Table 5**). The LDL oxidation rate ($\times 10^{-3}$ ΔA_{234nm}/min) of the SDR group was 0.46 ± 0.07 (P < 0.05) and that of the SDR-PEG group was 0.07 ± 0.03 (P < 0.01), significantly slower than the control (0.71 ± 0.04 $\times 10^{-3}$ ΔA_{234nm}/min). The maximum extent (max ΔA_{234nm}) of LDL oxidation of either the SDR or SDR-PEG group was also significantly (P < 0.01) lower than the control group. The SDR-PEG group that showed the highest TAC of plasma had the slowest oxidation rate and lowest oxidation extent of LDL, being significantly (P < 0.01) different from those of the control.

In the presence of Cu^{2+} induction, the oxidation of the control group progressed rapidly without lag phase (**Figure 2B**). A term $t_{1/2}$ was designated to indicate the apparent lag phase by measuring half of the time required to increase the absorbance at 234 nm from time zero (the start) to the beginning of the termination stage (k_3). The $t_{1/2}$ of Cu^{2+}-induced oxidation of LDL of the SDR or SDR-PEG group was significantly (P < 0.01) longer than that of the control group, whereas the SDR-PEG had the longest $t_{1/2}$. The propagation phase exhibited a fast rate (k_1), lasted for up to 2 h, and reached a second propagation phase with a slower oxidation rate (k_2) followed by a third propagation phase with a faster propagation rate (k_3). Among the three groups of fish, the control group showed the most pronounced ex vivo LDL oxidation, followed by the SDR group. The SDR-PEG group appeared to be most stable to the ex vivo LDL oxidation. The three different oxidation rates (k_1-k_3) may indicate different antioxidative and pro-oxidative agents present in LDL.

The inhibitory effects against the onset and the rate of oxidation resulted in prolongation of the lag phase of LDL oxidation, strongly indicating the presence and the nature of the antioxidants present in SDR and SDR-PEG. The key efficacy of SDR-PEG was to elevate the plasma antioxidant capacity and thus reduce the LDL oxidation rate and the maximal oxidation extent of LDL.

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Received April 27, 2009. Revised manuscript received July 15, 2009. Accepted July 16, 2009. This study was funded by the National Science Council of Taiwan, ROC, with Grant NSC 95-2317-B-019-001.