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Original Research Article

Effects of *Angelica sinensis* extracts on lipid oxidation in fish feeds and growth performance of juvenile Jian carp (*Cyprinus carpio* var. Jian)

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ABSTRACT

The study was to explore the effect of the extracts of *Angelica sinensis* (EAs) on lipid oxidation in fish feeds compared with ethoxyquin (EQ) and the effect of dietary EAs on growth performance of carp (*Cyprinus carpio* var. Jian). Firstly, fish feeds were respectively added with EQ, and ethyl ether extract, ethyl acetate extract (EAE), acetone extract, ethanol extract (EE) and aqueous extract (AQE) of *Angelica sinensis*, except for the control. The results showed that EAs and EQ inhibited lipid oxidation in fish feeds ($P < 0.05$). Of all of the examined EAs, EAE showed the strongest protective effects against lipid oxidation ($P < 0.05$). Moreover, EAE at high concentrations showed a stronger effect on lipid oxidation compared with EQ ($P < 0.05$). Then, 7 experimental diets respectively supplemented with 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 g/kg of EAE were fed to the respective treatment groups for 30 d. Four replicates were performed for each treatment group; 20 carp (mean weight: 12.10 ± 0.13 g) were in each replicate. The results indicated that dietary EAE improved the growth performance in carp ($P < 0.05$). The appropriate concentration of EAE for carp growth was estimated to be 3.643 g/kg diet. Thus, EAE could be used as a natural antioxidant in feeds for Jian carp.

1. Introduction

Fish feeds contain fish oil that makes them prone to lipid oxidation due to the presence of polyunsaturated fatty acids (PUFA) (Hernández et al., 2014). PUFA can undergo peroxidation in a chain reaction with reactive oxygen species (ROS) from cells (Porter, 1986). Lipid peroxidation is propagated under the catalytic actions of iron and other redox metal (Li et al., 2016a) and atmospheric oxygen (Pratt et al., 2011). It has been demonstrated that lipid oxidation leads to the breakdown of nutritional ingredients, changes in taste, scent and colour, development of toxic metabolites and decrease in shelf life in feeds (Błaszczyk et al., 2013). Dietary oxidised lipids can decrease health and growth performance in fish (Chen et al., 2013; Han et al., 2012). Thus, it is important to expand our knowledge of how to inhibit lipid oxidation in fish feeds.

Ethoxyquin (EQ), a synthetic antioxidant, has been used for many years to hinder lipid oxidation in feeds (Błaszczyk et al., 2013). However, studies have suggested that EQ is carcinogenic and toxic to animals and may bio-accumulate in farmed animal tissues and then enter into humans via consumption (Błaszczyk et al., 2013; Li et al., 2016a). Therefore, use of EQ in feeds is under strict regulation due to the potential toxicity to fish and health hazards to humans (EFSA, 2010; EFSA, 2013; EFSA, 2015). There is growing interest in replacing the synthetic antioxidant with natural ingredients (Lobo et al., 2010). Chinese angelica, *Angelica sinensis*, is

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a well-known medicinal herb and food material with multifunctional pharmacological activities (Yeh et al., 2014). Studies have indicated that the extracts of *Angelica sinensis* (EAs) scavenges ROS *in vitro* (Zhang, 2015) and in Neuro 2A cells (Huang et al., 2008). Furthermore, studies have indicated that dietary Chinese herbal compounds containing *Angelica sinensis* increase the growth performance in fish (Tang et al., 2009; Wang et al., 2008). Therefore, it is reasonable to hypothesise that EAs could mitigate lipid oxidation in fish feeds and improve the growth of fish. However, information regarding the effects of EAs on fish feeds and fish is scarce.

In this study, we explored the effects of EAs on lipid oxidation in fish feeds and the growth of fish. The purpose of the study was to evaluate the antioxidant effects of EAs on fish feeds compared with EQ and the growth-promoting effects in fish. The results may provide a basis for using EAs as a natural antioxidant in fish feeds.

2. Materials and methods

All procedures of this study were approved by the Institutional Animal Care and Use Committee of Neijiang Normal University in accordance with the Institutional Ethics Committee of the Chinese Institute of Chemical Biology guidelines.

2.1. Chemicals

Ethyl ether, ethyl acetate, acetone, and ethanol were analytical grade and purchased from the Chengdu Kelong Chemical Reagent Factory (Chengdu, Sichuan, China). Folin-ciocalteu reagent was obtained from Hefei Bomei Biotech. Co., LTD (Hefei, Anhui, China). Ethoxyquin (≥90%) was obtained from Shanghai Puzhen Biotech. Co., LTD (Shanghai, China). Ethyl carbamate (≥99%) was purchased from Shanghai Ekear Biotech. Co., LTD (Shanghai, China). All of other chemicals were analytical grade.

2.2. Preparation of extracts of Angelica sinensis

Root of *Angelica sinensis* was obtained from the Chengdu Pharmaceuticals market of China (Chengdu, Sichuan, China). Botanical identification was performed in Neijiang Normal University, where each sample was assigned a reference number and deposited. The dried plant material was treated and extracted sequentially using a range of organic solvents with a different degree of polarity, including ethyl ether, ethyl acetate, acetone, ethanol and water, recommended by Dalar et al. (2014) and Li et al. (2016b). After extraction, the dry ethyl ether extract (EEE), ethyl acetate extract (EAE), acetone extract (AE), ethanol extract (EE), and aqueous extract (AQE) were kept in sealed bottles in the darkness and stored at −70 °C until use.

2.3. Determination of total phenolic content

The phenols content of EAs was quantified according to the method of Yuan et al. (2005). The phenols content of EAs was expressed as gallic acid equivalents. Four replicates were prepared for each sample. Yield rate and phenolic content of EAs are provided in Table 1.

Table 1
Yield rate and phenolic content of extracts of *Angelica sinensis* .[1]

Item	Yield, g/kg dry herb	Phenols, mg/g dry extract
EEE	82.20 ± 4.09[b]	29.97 ± 0.97[c]
EAE	56.03 ± 2.54[c]	45.75 ± 1.77[a]
AE	21.26 ± 1.54[e]	37.39 ± 1.22[b]
EE	40.50 ± 2.28[d]	18.82 ± 1.39[d]
AQE	159.36 ± 9.64[a]	17.12 ± 1.28[d]

EEE = ethyl ether extract; EAE = ethyl acetate extract; AE = acetone extract; EE = ethanol extract; AQE = aqueous extract (AQE).
[a – e] Within a column, values with different superscripts are significantly different ($P < 0.05$).
[1] Values are means ± SD of 4 replicates.

2.4. Determination of lipid oxidation in fish feeds

Firstly, 7 experimental feeds were formulated as described in our previous study (Li et al., 2017) with slight modifications. There were one control feed (with no supplementation) and 6 feeds supplemented with one of EAs or EQ. All of these test feeds were used to screen the antioxidative capacity of different EAs compared with EQ. In brief, all solid materials were crushed in a grinder (Ronghao RHP-2000A, Zhejiang, China) and sieved through a 60 mesh screen. For 1,000 g of feeds, 1.0 g of dried EQ, EEE, EAE, AE, EE and AQE were respectively added to 5.0 g of $Ca(H_2PO_4)_2$, 10.0 g of vitamin mixture and 10.0 g of mineral mixture, except for the control, and blended in a mixer (Edei HH-2169, Shanghai, China) for 3 min. Then, the 7 mixtures above were individually blended with 270 g of fish meal, 360 g of soybean meal and 324 g of wheat flour in another mixer (Jinjie YG-3KG, Guangdong, China) and homogenized by sieving through a 40-mesh screen after 20.0 g of soybean oil were added. Distilled water was included to achieve a proper pelleting consistency, and the final mixture was further homogenized and extruded through a 2-mm die using a meat grinder (Deming DM-JRJ10, Hangzhou, China) with a cutter (Lin and Zhou, 2006). Twenty grams of particle-like feeds for each treatment were placed in a separate 100 mL open beaker. Four replicates were prepared for each treatment. The beakers were then transferred to an oven (Jinghong DHG-9030A, Shanghai, China) at 45 °C for 8 d without stirring (Li et al., 2016a). Immediately after the storage period, lipid in each replicate kept in ice was extracted and the peroxide value (POV) and malonaldehyde (MDA) levels were determined using the methods of Maqsood and Benjakul (2010). In this study, EAE showed the strongest antioxidant effects among all examined EAs. So, EAE was selected as an appropriate EAs to be compared with EQ at the different concentrations. Next, 13 experimental feeds were formulated by giving 0.0 (control), 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 g/kg EQ or EAE. The feeds were produced and treated, and POV and MDA levels were measured using the same methods described above. Formulation of the basal feed is shown in Table 2.

Inhibition (I) of lipid oxidation in fish feeds were calculated using the following equation: $I(\%) = 100 \times (1 - A_1/A_0)$, where A_0 was the content of POV or MDA of the control and A_1 was the content of POV or MDA of the sample solution (Ruberto et al., 2000).

2.5. Animal experiment

2.5.1. Experimental fish and diets

A total of 1,350 juvenile Jian carp (average weight 9.23 ± 2.10 g) were purchased from a fish farm in Neijiang (Sichuan, China) and acclimated to the following laboratory conditions: (22.0 ± 1) °C, constant aeration, daily dechlorinated water change and natural photoperiod (Jiang et al., 2011). The water was from tap water, which had an index sign matching the Standards for Drinking Water Quality (GB5749-2006), and was flowing by XL-180 water pumps (Zhongshan, Guangdong, China). According to the regression equations (Fig. 1), the maximum inhibitory effect of EQ on lipid oxidation in fish feeds was estimated to be 75.95%. Under the same conditions, for achieving the

Table 2
Composition and nutrient contents of the basal feed used in the lipid oxidation trial.

Ingredients, %	Content	Nutrients content, %	Content
Fish meal [1]	27.00	Crude protein	34.32
Soybean meal [2]	36.00	Crude lipid	6.84
Wheat flour [3]	32.50	Phosphorus	1.73
Soybean oil [2]	2.00		
$Ca(H_2PO_4)_2$	0.50		
Vitamin mixture [4]	1.00		
Mineral mixture [5]	1.00		

[1] Fish meal is freshly produced from local freshwater fish (common carp and crucian carp) by drying at 105 °C. It contained 58.98% crude protein, 8.64% crude lipid, 16.84% ash, 5.37% calcium and 4.31% phosphorus.

[2] Soybean meal and oil are produced from local soybean by mechanical compression. Soybean meal contained 40.51% crude protein, 5.68% crude lipid, 5.55% ash, 0.35% calcium and 0.64% phosphorus.

[3] Wheat flour is produced from local wheat grain by mechanical milling. It contained 11.90% crude protein, 1.51% crude lipid, 2.02% ash, 0.16% calcium and 0.43% phosphorus.

[4] Vitamin mixture provides the following per kg of vitamin mixture: $FeSO_4 \cdot 7H_2O$ (20% Fe), 69.70 g; $CuSO_4 \cdot 5H_2O$ (25% Cu), 1.20 g; $ZnSO_4 \cdot 7H_2O$ (23% Zn), 21.64 g; $MnSO_4 \cdot H_2O$ (32% Mn), 4.09 g; $Na_2SeO_3 \cdot 5H_2O$ (1% Se), 2.50 g; KI (4% I), 2.90 g; $CaCO_3$, 897.98 g.

[5] Mineral mixture provides the following per kg of mineral mixture: retinyl acetate (500,000 IU/g), 0.80 g; cholecalciferol (500,000 IU/g), 0.48 g; DL-α-tocopherol acetate (50%), 20.00 g; menadione (23%), 0.43 g; thiamin nitrate (90%), 0.11 g; riboflavine (80%), 0.63 g; pyridoxine HCl (81%), 0.92 g; cyanocobalamin (1%), 0.10 g; ascorhyl acetate (93%), 7.16 g; D-calcium pantothenate (90%), 2.73 g; niacin (99%), 2.82 g; D-biotin (2%), 5.00 g; meso-inositol (99%), 52.33 g; folic acid (96%), 0.52 g.

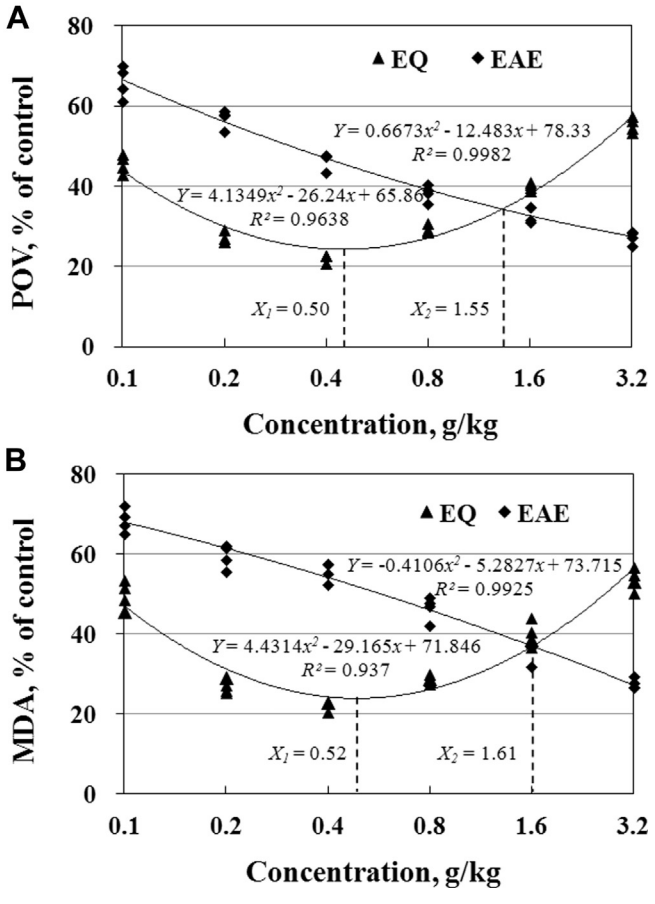

Fig. 1. Polynomial regression of peroxide value (POV; A) and malonaldehyde (MDA; B) levels in fish feeds treated with graded levels of ethoxyquin (EQ) and ethyl acetate extract (EAE) of *Angelica sinensis*. The data represent the value of each replicate, with 4 replicates in each treatment. Based on POV and MDA levels (Y) in fish feeds, the corresponding concentrations (X) of EQ and EAE were calculated using the equations in the figure, where $X = \log(x)$.

same effect of EQ, the required concentration of EAE was estimated to be 6.13 g/kg diet. So, 7 experimental diets were formulated to give 0.0 (basal diet), 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 g/kg EAE and produced as described above. The basal diet contained 34.17% crude protein and 5.63% crude lipid. Formulation of the basal diet is shown in Table 3.

2.5.2. Feeding trial

The trial procedures were based on those described by Lin and Zhou (2006) with slight modifications. A total of 640 fish (average initial weight [12.10 ± 0.13] g) from the acclimatisation aquariums were randomly distributed into 7 groups including 0 (control group), 1, 2, 3, 4, 5 and 6 g EAE/kg diet groups. Twenty fish were stocked in each aquarium (55 cm × 32 cm × 40 cm) with 61.6 L water. The control group was with 8 replicate aquariums and the EAE diet groups were with 4 replicate aquariums in each group. The fish in each group were fed 8 times daily and carefully observed to ensure satiation without overfeeding for 30 d. The experimental conditions were similar to the acclimation conditions. The water in the experimental aquariums was from tap water, which had an index sign matching the Standards for Drinking Water Quality (GB5749-2006). The water was renewed with dechlorinated tap water after constant aeration daily. At the end of the feeding trial, the fish in each aquarium were counted and weighed. Data on initial number, final number, initial weight, final weight and feed intake (FI) of fish were used to calculate survival rate, weight gain (WG), specific growth rate (SGR), feed efficiency (FE) and protein efficiency ratio (PER). The fish were anaesthetized in water containing 50 mg/L ethyl carbamate. Intestines and hepatopancreas of 10 fish in each aquarium were quickly removed and weighed for analysis of hepatosomatic index (HSI) and intestosomatic index (ISI).

2.6. Statistical analysis

The data were expressed as means ± standard deviation (SD). The data were subjected to two-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences. The significance level adopted was 5%. The statistical analysis was carried out using SPSS 13.0 for Windows software (SPSS, USA). The correlations of POV and MDA levels in fish feeds with EQ and EAE levels were established by polynomial regression analysis. The correlations of I on POV and MDA levels in fish feeds with phenols content in EAs were established by linear regression analysis. The dietary EAE requirement of juvenile Jian carp based on SGR was estimated by broken-line regression analysis.

Table 3
Composition and nutrients content of the basal diet used in the feeding trial.

Ingredients [1], %	Content	Nutrient content, %	Content
Fish meal	25.00	Crude protein	34.17
Soybean meal	30.55	Lys	2.07
Wheat flour	37.17	Met + Cys	1.51
DL-methionine	0.42	Crude lipid	5.63
Threonine	0.40	n-3 + n-6	2.20
Fish oil	1.16	Total phosphorus	0.69
Soybean oil	1.80		
$Ca(H_2PO_4)_2$	1.50		
Vitamin mixture	1.00		
Mineral mixture	1.00		

[1] All are commercial raw materials.

3. Results

3.1. Yield rate and phenolic content of EAs

As shown in Table 1, AQE showed the highest yield rate among the extracts examined. The rank order of yield rate in these extracts was AQE > EEE > EAE > EE > AE ($P < 0.05$). The EAE presented the maximum content of phenolic compounds among the extracts examined ($P < 0.05$). The descending order of phenolic content in these extracts was EAE > AE > EEE > EE = AQE ($P < 0.05$).

3.2. Effects of extracts of Angelica sinensis on lipid oxidation in fish feeds

The effects of EAs on lipid oxidation in fish feeds are presented in Fig. 2A and 2B. The POV and MDA levels in fish feeds treated with EQ, EEE, EAE, AE, EE and AQE were effectively decreased in comparison to the control ($P < 0.05$). When the fish feeds contained EQ, the POV and MDA levels were estimated to be the lowest values for the examined compounds (Figs. 2 A and 2B) ($P < 0.05$). The POV and MDA levels in fish feeds treated with EAE were respectively estimated to be 38.56% and 44.55% of the control, which were the lowest values for the examined EAs (Figs. 2A and 2B) ($P < 0.05$). Moreover, the present data suggested that the POV and MDA levels gradually decreased with increasing levels of EQ below concentrations of 0.50 and 0.52 mg/mL, respectively (Figs. 1A and 1B) ($P < 0.05$). However, the POV and MDA levels gradually increased with increasing levels of EQ above concentrations of 0.50 and 0.52 mg/mL, respectively (Figs. 1A and 1B) ($P < 0.05$). It should be noted that the POV and MDA levels continuously decreased with increasing levels of EAE in fish feeds (Figs. 1A and 1B) ($P < 0.05$). Furthermore, respectively increasing the EAE concentrations to 1.55 and 1.61 mg/mL resulted in much lower POV and MDA levels in fish feeds (Figs. 1A and 1B) ($P < 0.05$) compared with EQ.

Linear regression of I on POV and MDA levels in fish feeds treated with EAs containing different levels of phenols was presented in Fig. 3. The results suggested that I of EEE, EAE, AE, EE and AQE on POV and MDA levels in fish feeds were positively correlated with their phenols contents.

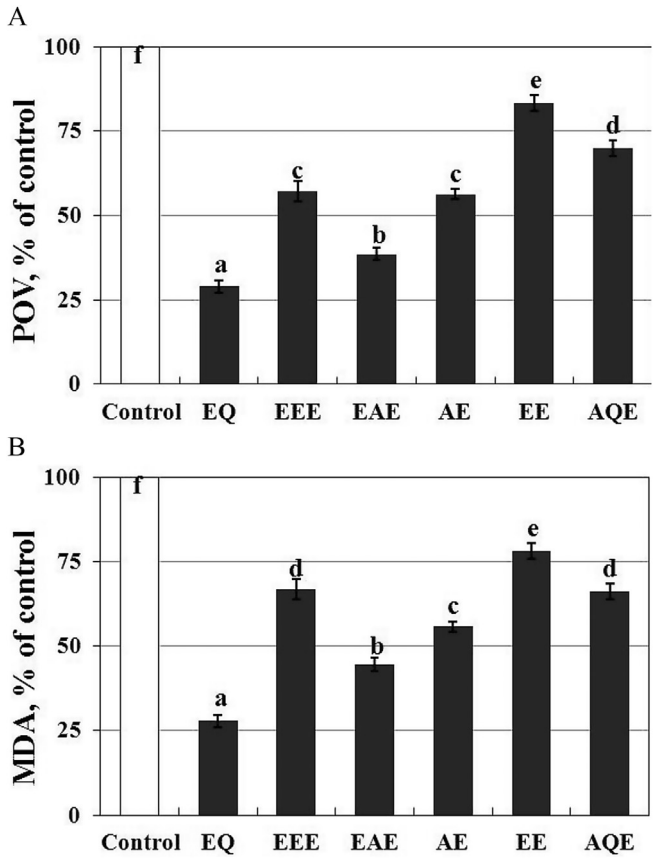

Fig. 2. Influence of ethoxyquin (EQ) and extracts of *Angelica sinensis* on peroxide value (POV; A) and malonaldehyde (MDA; B) levels in fish feeds. The data represent the means ± SD of 4 replicates. $^{a-f}$ Bars with different superscripts are significantly different ($P < 0.05$). EEE = ethyl ether extract, EAE = ethyl acetate extract, AE = acetone extract, EE = ethanol extract and AQE = aqueous extract (AQE).

3.3. Effects of dietary ethyl acetate extract on growth performance in fish

Effects of dietary feeds containing graded levels of EAE on growth parameters are provided in Table 4. Compared with the control group, dietary EAE significantly increased final body weight (FBW), WG, SGR, FI, FE and PER in juvenile Jian carp ($P < 0.05$). Moreover, FBW, WG, SGR, FI, FE and PER gradually increased with increasing dietary EAE levels (Table 4) ($P < 0.05$). After these parameters reached the highest level, no differences were found with a further increase of EAE level (Table 4) ($P < 0.05$). The FBW and WG reached the highest level for fish fed diets containing 4.0 g EAE/kg diet. The SGR, FI, FE and PER were the highest for fish fed diets containing 3.0 g EAE/kg diet. However, according to the broken-line regression method based on SGR (Fig. 4), the appropriate concentration for growth is estimated to be 3.643 g EAE/kg diet. The regression equation was $Y = 0.129\,X + 2.560$, $R^2 = 0.981$ (Fig. 4).

3.4. Effects of dietary ethyl acetate extract on intestine and hepatopancreas growth in fish

Intestine and hepatopancreas growth of juvenile Jian carp fed the diets containing graded levels of EAE are shown in Table 4. The HSI of fish fed EAE-supplemented diets at concentrations of 2 g/kg was significantly higher than that of fish fed the basal diet (Table 4)

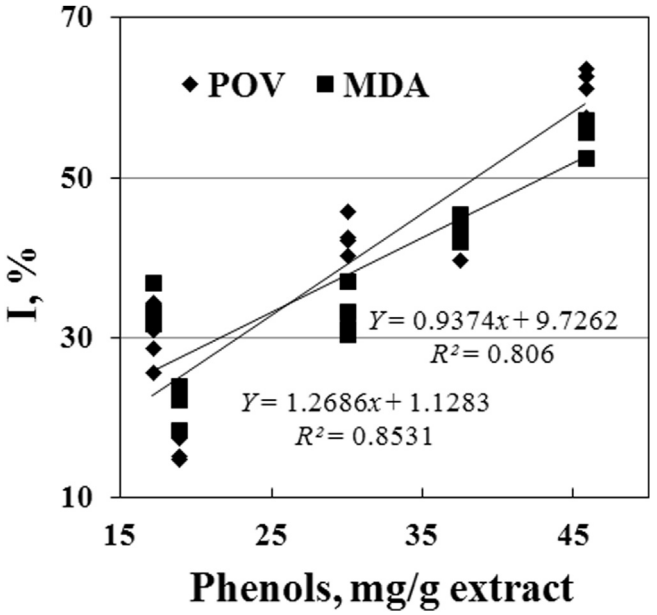

Fig. 3. Linear regression of the inhibition (I) on peroxide value (POV) and malonaldehyde (MDA) levels in fish feeds treated with the extracts of *Angelica sinensis* containing different levels of phenols. The data represent the value of each replicate, with 4 replicates in each treatment.

Table 4
Growth performance, feed utilization and development of digestive organs of juvenile Jian carp fed diets containing graded levels of ethyl acetate extract (EAE) of *Angelica sinensis* for 30 d.[1]

EAE, g/kg diet	IBW, g/fish	FBW, g/fish	WG, g/fish	SGR, %	FI, g/fish	FE, %	PER	HSI, %	ISI, %
0.0	12.15 ± 0.37^{a}	26.29 ± 1.13^{a}	14.14 ± 0.57^{a}	2.57 ± 0.11^{a}	20.64 ± 1.04^{a}	68.50 ± 2.98^{a}	2.01 ± 0.09^{a}	3.27 ± 0.14^{a}	2.37 ± 0.14^{a}
1.0	12.08 ± 0.33^{a}	27.10 ± 1.08^{ab}	15.03 ± 0.79^{ab}	2.70 ± 0.14^{ab}	21.31 ± 0.87^{ab}	70.48 ± 2.71^{ab}	2.07 ± 0.08^{ab}	3.36 ± 0.20^{ab}	2.46 ± 0.16^{ab}
2.0	12.13 ± 0.31^{a}	28.10 ± 1.22^{bc}	15.98 ± 0.41^{bc}	2.80 ± 0.15^{bc}	22.00 ± 1.16^{bc}	72.63 ± 2.54^{bc}	2.13 ± 0.09^{bc}	3.52 ± 0.20^{bc}	2.56 ± 0.15^{abc}
3.0	12.13 ± 0.32^{a}	28.99 ± 1.37^{c}	16.86 ± 1.04^{cd}	2.91 ± 0.16^{cd}	22.69 ± 1.11^{cd}	74.32 ± 3.83^{bc}	2.18 ± 0.11^{bc}	3.59 ± 0.27^{bc}	2.65 ± 0.12^{bc}
4.0	12.10 ± 0.29^{a}	30.76 ± 1.61^{d}	18.66 ± 1.03^{e}	3.11 ± 0.13^{d}	23.70 ± 0.78^{d}	78.76 ± 4.87^{c}	2.31 ± 0.14^{c}	3.75 ± 0.19^{c}	2.78 ± 0.11^{c}
5.0	12.18 ± 0.32^{a}	30.16 ± 1.43^{d}	17.99 ± 1.12^{de}	3.02 ± 0.17^{d}	23.44 ± 1.05^{d}	76.81 ± 5.55^{bc}	2.25 ± 0.16^{bc}	3.76 ± 0.23^{c}	2.76 ± 0.18^{c}

IBW = Initial body weight; FBW = final body weight (FBW).
WG (g/fish) = FBW (g/fish) − IBW (g/fish).
SGR (%) = 100 × {ln[mean FBW (g/fish)] − ln[mean IBW (g/fish)]}/Days.
FE (%) = 100 × WG (g/fish)/FI (g/fish).
PER = WG (g/fish)/Protein intake (g/fish).
HSI (%) = 100 × Wet hepatopancreas weight (g/fish)/Wet body weight (g/fish).
ISI (%) = 100 × Wet intestine weight (g/fish)/Wet body weight (g/fish).
[a – e] Data in the same column with different superscripts are significantly different ($P < 0.05$).
[1] Data are means ± SD of 4 replicates, and 20 fish are in each replicate.

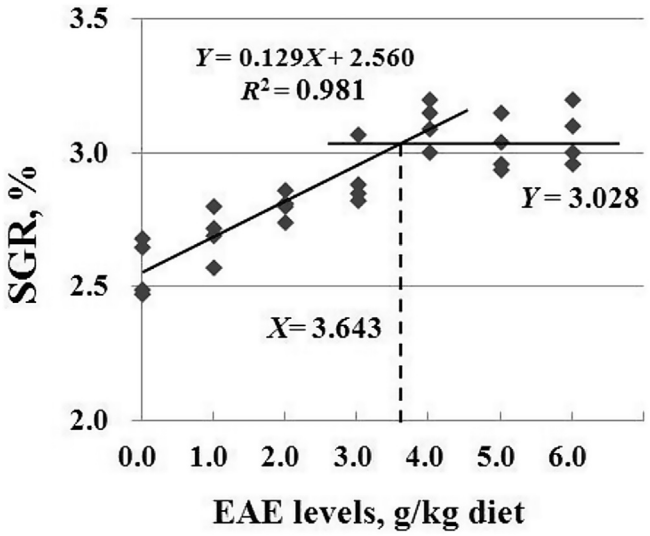

Fig. 4. Broken-line analysis of specific growth rate (SGR) for juvenile Jian carp fed diets containing graded levels of ethyl acetate extract (EAE) of *Angelica sinensis* for 30 d. Based on SGR, the regression equations were $Y = 0.129X + 2.560$ ($R^2 = 0.981$), $Y_{max} = 3.028$. The optimal EAE requirement of juvenile Jian carp was 3.643 g/kg diet. The data represent the value of each replicate, with 4 replicates in each treatment.

($P < 0.05$). However, the ISI of fish fed the basal diet was significantly lower than that of fish fed diets containing EAE at concentrations of 3.0 g/kg (Table 4) ($P < 0.05$). No differences were found with a further increase of EAE level.

4. Discussion

4.1. Extracts of Angelica sinensis inhibited lipid oxidation in fish feeds

The autoxidation of PUFA is an important factor leading to the lipid oxidation in food and feeds (Pratt et al., 2011). Fish feeds contain high amount of PUFA that makes them prone to lipid oxidation (Hernández et al., 2014). The peroxide is the primary oxidation product at the initial stage of lipid oxidation (Sharma and Vig, 2014). Malondialdehyde is the degradation product of peroxides at the later stage of lipid oxidation (Sharma and Vig, 2014). In the present study, EQ and EAs significantly protected against the formation of peroxide and MDA in fish feeds. These results indicated that EQ and EAs inhibited lipid oxidation in fish feeds. This finding was in agreement with the report that EQ suppresses lipid oxidation in fish feeds (Błaszczyk et al., 2013). Furthermore, the EAE showed the strongest effects among all examined EAs in this study.

The antioxidant effects of EAs in fish feeds may be closely associated with their active ingredients. In this study, a positive correlation was found between the antioxidant effects and the phenols content in EAs, which suggested that the antioxidant activity of EAs in fish feeds may be caused by the presence of phenolic compounds. This finding was consistent with the reports that the plant-derived phenolic compounds possess potent antioxidant activity (Sharma and Vig, 2014; Yuan et al., 2005). However, EQ that belong to phenolic compounds showed pro-oxidant actions in fish feeds at high concentrations in this study. This result was in accordance with the reports of Błaszczyk et al. (2013) and Błaszczyk and Skolimowski (2015). The pro-oxidant action of EQ may be ascribed to its free radical form. Ethoxyquin was found to exist partly in a free radical form in its solutions and in the compound itself (Skaare and Henriksen, 1975). These free radicals are involved in pro-oxidant activity (Sakihama et al., 2002). Therefore, using EQ concentrations that are too high could cause pro-oxidative properties in fish feeds. However, there are few papers describing the pro-oxidant action of EAs in fish feeds. Moreover, EAE at high concentrations showed stronger inhibitory effects on lipid oxidation in fish feeds than EQ. Thus, it is possible that the EAEs are used as natural antioxidants for inhibiting lipid oxidation in fish feeds.

4.2. Dietary ethyl acetate extract of Angelica sinensis improved the growth of fish

Tang et al. (2009) and Wang et al. (2008) reported that dietary Chinese herbal compound containing *Angelica sinensis* improves the growth in fish. However, few papers described the effects of EAs on the growth of fish. In the present study, dietary EAE significantly increased FBW, WG, SGR, FI, FE and PER of juvenile Jian carp. These results suggested that dietary EAE improved the growth performance of fish. Based on the broken-line regression analysis for SGR, the appropriate concentration of EAE for growth of carp was estimated to be 3.643 g/kg diet. The improvement of growth may be partly attributed to the growth and development of digestive organs in fish (Hong et al., 2015). In this study, diets containing EAE significantly increased HSI and ISI in carp, which suggested that dietary EAE improved the growth and development of the digestive organs of fish.

5. Conclusion

In summary, the present study showed that EAs blocked lipid oxidation in fish feeds. Ethyl acetate extract of *Angelica sinensis* showed the strongest effects on protecting against the lipid oxidation among all of the EAs examined. Moreover, EAE at high concentrations showed stronger inhibitory effects on lipid oxidation in fish feeds compared with EQ. Furthermore, our results confirmed that dietary EAE improved the growth performance in fish. Therefore, EAE could be used as a potential natural antioxidant in fish feeds.

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