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EFFECT OF RED BEET AND BETAINE MODULATING OXIDATION AND BIOACTIVITY OF RAINBOW TROUT.

Julia Pinedo-Gil[1,2*], Ana Tomás-Vidal[2], Daniel Rico-Barges[1], Brijesh K. Tiwari [3], Carlos Álvarez García[3], Miguel Jover-Cerdá[2], Miguel Ángel Sanz-Calvo[1], Ana Belén Martín-Diana[1]

[1] Subdirection of Research and Technology. Agro-Technological Institute of Castilla y León. Consejería de Agricultura y Ganadería. Finca de Zamadueñas, Ctra. Burgos km. 119, 47171, Valladolid, Spain.

[2] Research Group of Aquaculture and Biodiversity, Institute of Animal Science and Technology, Universitat Politècnica de València, Camino de Vera, 14. 46071-Valencia, Spain.

[3] Department of Food Quality and Sensory Science. Teagasc Food Research Centre. Ashtown, Dublin 15.

[*]Correspondig author: julia.pinedo.gil@gmail.com, atomasv@dca.upv.es

Phone number: +34609720461, +34963879434

ABSTRACT

The present study compares a control diet to 4 experimental diets, in which 2 red beet (14 and 28%) and 2 betaine levels (0.9 and 1.63%) were incorporated on rainbow trout diets according to a factorial design. The effects of the inclusion of different red beet and betaine concentrations on fatty acid profile (FA), lipid peroxidation and antioxidant activity on rainbow trout fillets were investigated. Although no significant differences were observed with the control group, results indicated that red beet and betaine improved fish fillet FA profile, producing an increase on PUFA, mainly DHA. Higher red beet and betaine concentrations increased flavonoid and phenolic content on the diets; however, no effects were observed on the antioxidant properties of rainbow trout fillets.

Keywords: Red beet, betaine, rainbow trout, fillet lipid oxidation, antioxidant activity.

1. INTRODUCTION

Fish lipids contain high levels of polyunsaturated fatty acids (PUFAs), which are susceptible to oxidation, resulting in a loss of fish quality (Chaiyapechara *et al*. 2003a, Baron *et al*. 2009, Pereira de Abreu *et al*. 2012, Gao and Koshio 2015). For this reason, fresh fish is a highly perishable product (Medina *et al*. 2009). Fish spoilage results from three basic mechanisms: enzymatic autolysis, oxidation and microbial growth (Aubourg 2008). However, these processes can occur alone or in combination and occurrence largely depends on fish species (size, lipid content, etc.), environmental conditions (feeding availability, temperature, etc.), post-mortem handling, storage and processing conditions (Medina *et al*. 2009, Fuentes *et al*. 2010). Rainbow trout (*Oncorhynchus mykiss*) is a fatty fish species with high commercial value and very appreciated by

European consumers (Rezaei and Hosseini 2008, Özogul *et al*. 2013). Trout, as fatty fish variety, is very prone to deterioration (Pereira de Abreu *et al*. 2012, Özogul *et al*. 2013), precisely to its high oil/fat content (Fraser and Sumar 1998).

Different strategies have been proposed to prevent lipid and protein oxidation; some of these strategies are focused in processing process such as packaging and/or the use of antioxidants incorporated on the food products. However, recently, special attention has been paid in using antioxidant ingredients in fish diets. These ingredients have been reported as a strategy to maintain fish quality (Baron *et al*. 2009, García-Romero *et al*. 2014, Secci and Parisi 2016). Diet supplementation with antioxidants enables these substances to be incorporated into the phospholipid membrane, where they can effectively inhibit oxidation reactions (Lauridsen *et al*. 1997). Previous studies have reported the use of different antioxidants such as α-tocopherol, astaxanthin or canthaxanthin enhancing the quality of different fish species, by protecting fish muscle against oxidative degradation. Jensen *et al*. (1998) showed that the introduction of astaxanthin on rainbow trout diets protects against lipid oxidation during the early stages of oxidative deterioration. Choubert *et al*. (2011) reported the same effect of astaxanthin on rainbow trout diets during long-term frozen storage. Other authors have confirmed the role of α-tocopherol or ascorbic acid as antioxidants when included as ingredient in different fish species: turbot (*Scophthahus maximus*) (Stéphan *et al*. 1995), rainbow trout (*Oncorhynchus mykiss*) (Chaiyapechara *et al*. 2003b), hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) (Huang *et al*. 2003), or red sea bream (*Pagrus major*) (Gao and Koshio 2015). New natural antioxidants have been utilised as feed additives such as thymol (Giannenas *et al*. 2012) or rosemary extracts (Álvarez *et al*. 2012, Hernández *et al*. 2014). However, the use of alternative natural ingredients, with bioactive compounds that can enhance fish quality and oxidative stability, have not been investigated. Previous work confirmed that the inclusion of barley on rainbow trout diets had an enhancing effect on quality parameters, probably associated to the presence of antioxidant compounds (Pinedo-Gil *et al*. 2017A).

Red beet (*Beta vulgaris* L.) has gain relevance in recent years, especially by its health-promoting and bioactive properties (Clifford *et al*. 2015, Nistor *et al*. 2017). Red beet is rich in valuable active compounds such as carotenoids, polyphenols, flavonoids, betalains (which represents the principal pigment in red beet) and betaines. All these compounds make red beet an interesting source of antioxidant ingredients (Nistor *et al*. 2017). Betaines and betalains have been widely studied for their nutritional and health benefits; since present a high radical scavenging and antioxidant activity (Paciulli *et al*. 2016). More specifically, betaine has been reported widely to have antioxidant, antimicrobial and antiviral activities (Pedreno and Escribano 2001, Attia *et al*. 2013). Due to the beneficial effects found on red beet and betaine, in the present study was considered of interest to investigate the effect of these two ingredients on the ~~quality of fresh rainbow trout fillets,~~ lipid stability and antioxidant activity of fresh rainbow trout fillets when included as ingredient in the diet.

2. MATERIAL AND METHODS

2.1. Experimental design

Rainbow trout were provided by a local trout farm (Cien Fuentes Fishfarm, 19420, Cienfuentes, Gadalajara, Spain). The average fish weight for each fish was 69 ± 2.21 g (least-square mean ± SEM). Fish were fed with 5 isoproteic (40% crude protein) and isolipidic (18% crude fat) diets, which contained different red beet and betaine concentrations (0-28% red beet and 0-1.69% betaine). Diets formulation and composition are published on Pinedo-Gil *et al*. 2017B. Groups of 60 fish were housed in 15 tanks of 500 L (three tanks per treatment, n=3). Three fish per tank were randomly sampled after 105 days (when fish reached their commercial weight) for the determination of lipid oxidation and bioactivity parameters. For each fish, the skin was removed and the skinless fillets were frozen until analysis.

2.2. Fatty acid profile (FAME)

Fatty acid profile (FA) was determined in red beet, diets and fish fillets. Blight and Dyer (B&D) method (Blight and Dyer 1959) was used for lipid extraction. Lipid-containing chloroform phase was separated and after evaporated. The remaining phase was dissolved in 1 mL of hexane and a methylated procedure was carried out by adding 100 μL of 0.5 M methanolic KOH and leaving the reaction for 10 min at room temperature (RT). The upper layer was transferred to a 2 mL vial. Analysis of FA methyl esters (FAME) was carried out on a gas chromatograph Agilent 7890A (Agilent Technologies, PA, California, USA) equipped with a flame ionization detector. For the analysis the method was run on helium, oven ramp temperature was set from 50 ºC to 200 ºC during the first 7 min at a rate of 3 ºC min^{-1} and held for 26 min. Injector and detector temperature were 250 ºC and 280 ºC, respectively. A sample volume of 1 μL was injected in split mode (ratio 25:1), and FAMEs were identified by comparison of retention times with those of 37 FAMEs standard mix (Supelco, Sigma-Aldrich, CO). Results have been expressed as percentage of the area.

2.3. Alpha-tocopherol content

Alpha-tocopherol content in fish fillets was determined according to the AOCS (American Oil Chemistry Society) official method (1992), using Agilent 1200 series HPLC equipped with a diode array detector. Two grams of the B&D extract (Blight and Dyer 1959) was evaporated and resuspended in 2 mL of hexane with 20 μL of tocopherol acetate as internal standard. An aliquot (10 μL) was injected and a column (250 mm x 4.6mm 5 μ,) (Teknokroma Anlítica S.A., Barcelona, Spain) was used.

Elution was performed with an isocratic mixture of hexane:2-propanol (99.6:0.4; v:v) at a flow rate of 1.3 mL min^{-1}. Detection was set at 295 nm and 284 nm for tocopherol acetate. Results were expressed in μg tocopherol per gram of fillet.

2.4. Oxidative parameters

2.4.1. Peroxide value (PV)

Fish fillet PV was measured using the B&D extract according to the International IDF Standards method (1991). Results were expressed in meq of active oxygen per Kg of lipids.

2.4.2. Conjugated hydroperoxides (dienes and trienes)

Fish fillets conjugated hydroperoxides (B&D extract) were measured as described by Undeland *et al*. (1998). Results were calculated as mmol of hydroperoxides per Kg of lipids.

2.5. Antioxidant markers

2.5.1. Extract preparation

To measure the antioxidant activity, 1 g of blended sample was dissolved in 10 mL of 90% methanol. The extraction was accelerated using a ceramic homogenizer on the test tubes by stirring for 30 s. Following samples were centrifuged at 1.635 x g for 10 min at 4 ºC and the supernatants were collected, filtered and stored at -80 ºC. All the extracts were used for antioxidant markers.

2.5.2. Phenolic characterization using HPLC

Phenolic characterization was determined on red beet and diets. Five grams of sample were mixed with 45 mL of 80% ethanol (v/v) and after it was sonicated in a water bath for 1 h. After centrifugation (5000 x g, 20 min., 10ºC), the supernatant was removed and the extraction was repeated twice. Supernatants were mixed and then evaporated at 40 ºC under nitrogen until complete dryness; finally were reconstituted in 2 mL of 40%

acetonitrile and then were filtered through 0.45 μm membrane for HPLC analysis (Bonoli *et al*. 2004, Zhao *et al*. 2006).

The phenolic compounds were separated and quantified using the method described by Schieber *et al*. (2001) with modifications, briefly as follows. Water Alliance 2795 Chromatography Separations Module (Waters Corp., Milford, USA) coupled to a Waters 2996 PDA detector fixed at 280 nm of wavelength was employed. Column equipped was a Zorbax sb-c18 Agilent (4.6 x 150 nm; 5 microns). The mobile phases consisted in 0.5% acetic acid (buffer A) and 20% (0.5% acetic acid): 80% acetonitrile (buffer B). Initial gradient started with 5% of buffer B for 1 min, and then was increased up to a 55% for 50 minutes; the column was rinsed for 5 min by pumping 95% of buffer B and finally it was re-equilibrated for another 10 min. Calibration curves were constructed using the following standards: gallic acid, chlorogenic acid, ferulic acid, p-cumaric acids, synaptic acid, 3-coumaric acid, 4-coumaric acid, syringic acid, maleic acid, transcinamic acid, vanilic acid, caffeic acid and 4-hydroxibenzoic acid at concentration of 5, 10, 20, 40 and 80 μg mL^{-1}.

2.5.3. Total Flavonoid determination (TFC)

TFC was determined using the method described by Lin and Tang (2007) for red beet and diets. Aliquots of 0.1 g of sample were dissolved in 1 mL of 10% aluminium chloride hexahydrate ($AlCl_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of deionized water. After incubation at room temperature (RT) for 40 minutes the reaction was measured at 415 nm (Shimadzu PharmaSpec UV-1700. Milton Keynes, UK). The data were expressed as quercetin equivalent (QE) per gram of sample based on the moisture content of lyophilized powder and “fresh sample”.

2.5.4. Total phenols (TP)

TP were measured using the Folin-Ciocalteu method (Slinkard and Singleton 1977) on red beet, diets and fish fillets. Results were expressed as mg of gallic acid per gram of dried weight (dw) sample.

2.5.5. Determination of the oxygen radical absorbance capacity (ORAC)

Oxygen radical absorbance capacity (ORAC) of fish fillets was measured following the procedure reported by Ou *et al*. (2001). Results were expressed as μmol of Trolox Equivalent (TE) per gram of sample (dw).

2.5.6. Trolox Equivalent Antioxidant Capacity (TEAC) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The measurement of total antioxidant capacity was determined following the procedure proposed by Serpen *et al*. (2007). One mg of fillet was mixed into 100 mg of cellulose powder prior to measurement. TEAC results were expressed as mmol Trolox Equivalent per gram of sample (dw) and DPPH as percentage of inhibition of the DPPH radical compared to a control with no red beet on diet.

2.5.7. Relative Antioxidant Capacity Index (RACI)

Relative antioxidant capacity index (RACI), a hypothetical concept, is created from the perspective of statistics by integrating the antioxidant capacity values generated from different in vitro methods, in this case TP, ORAC, DPPH and TEAC were evaluated (Sun and Tanumihardjo 2007).

2.6. Statistical analysis

Statistical analysis was performed using SAS version 9 (SAS Institute Inc., Cary, North Caroline, USA) by a GLM (Generalized Lineal Model) procedure for the variance analysis (ANOVA) followed by a t-Student test and considering significant differences between values when P-value < 0.05.

3. RESULTS AND DISCUSSION

Proximate composition and betaine content were analysed in red beet, experimental diets and fillets are reported elsewhere (Pinedo-Gil *et al.* 2017B). The whole wheat portion substituted the highest red beet concentration. It was observed that all diets were isoproteic (40% protein) and isolipidic (18% lipids), red beet contained 0.65% betaine and natural betaine was added to reach diets with betaine concentrations range from 0.9 to 1.63%. In fillets, the inclusion of red beet and betaine significantly decreased fat content and increased betaine content.

3.1. Fatty acid profile

Table 1 shows the fatty acid profile of red beet and experimental diets. Red beet contained very small proportion of fat, so the proportion of fatty acids incorporated by ingredient was very small. The most abundant fatty acids are palmitic acid (C16:0), linoleic (C18:2 n-6) and oleic acid (C18:1 n-9) with concentrations of 0.18, 0.37 and 0.27 g per 100 g^{-1} respectively. These results are in accordance with those reported by Neelwarne and Halagur (2012). And in agreement, USDA (United State Department of Agriculture) National Nutrient database, which showed that the most abundant fatty acids in red beet are palmitic acid, oleic and linoleic acids, the same as the results obtained in the present study.

The replacement of wheat with red beet and betaine resulted in a decrease in stearic acid (C18:0) and docosahexanoic acid (DHA, C22:6 n-3) in the experimental diets with high replacement level, compared with the control and the 14% replacement diets; however, the concentration of linoleic acid (C18:2 n-6) increased on the four replacement diets with the inclusion of red beet and betaine (Table 1).

On the other hand, although there was no fatty acid replacement in the diet formulation, some modifications were observed in the fillet fatty acid profile associated with the inclusion of red beet and betaine on the diet (Table 2). The inclusion of these ingredients showed a dose-dependent effect on myristic acid (C14:0) and polyunsaturated fatty acids (PUFA), specifically docosahexanoic acid (DHA; C22:6 n-3). Myristic acid (C14:0) reached the lowest values in those fish fed at higher red beet concentrations (diets C and D) and PUFA and DHA reached the highest values in those fish fed at higher red beet concentrations (diets C and D). None of the other fatty acids were affected by diet. Results were in agreement with Welker *et al*. (2016) when fed rainbow trout with different varieties and concentrations of green tea. Also Ji *et al*. (2007) reported for Japanese flounder that fish fed with increasing levels of a mixture of herbs showed lower SFA and MUFA and higher PUFA in carcass. It seems that high content in phenolic compounds can contribute in decreasing SFAs and MUFAs, while increasing PUFAs contents.

3.2. α-tocopherol content

Fish fillets α-tocopherol content was not significantly affected by different experimental diets (data not shown).

3.3. Oxidative parameters: peroxide value (PV) and conjugated hydroperoxides (dienes and trienes)

Peroxide value (PV) was evaluated on fish fillets since is one of the most common method for analysing primary lipid oxidation (Özogul *et al*. 2013). Similar to data observed on α-tocopherol content, PV and conjugated dienes and trienes hydroperoxides for fish fillets were not significantly affected by the concentration of red beet and betaine. Thus, these ingredients did not have any effect on fillets lipid oxidation (results not shown).

3.4. Antioxidant activity

3.4.1. Total flavonoid content (TFC) and total phenolic content (TP)

Ninfali *et al*. (2013) reported values of TFC on red beet root between 0.88 and 1.44 mg g^{-1}, which were in agreement with values of this study (1.82 mg QE g^{-1}) (Figure 1). When the total flavonoid content was determined in the different experimental diets it was observed that the substitution of wheat for red beet and betaine increased the TFC (Figure 1). Red beet improved the concentration of TFC on rainbow trout diets what could increase the bioactive properties of fish fillets.

TP of red beet was 5.61 mg of GAE (Gallic Acid Equivalent) g^{-1} (Figure 2). Kujala *et al*. (2000) reported that the TP of red beet was 4.2 mg g^{-1} and Bavec *et al*. (2010) 4.94 mg g^{-1}, so results are in the same order of magnitude to previous findings. It is necessary consider that the value obtained in the present study was over dry samples and the values given by other authors were in fresh. Similar to TFC, TP also increased with increasing red beet concentrations on rainbow trout diets (Figure 2). The substitution of wheat for red beet on the experimental diets increase TFC and TP, which can provide bioactive properties to fish fed with those diets. However, TP of rainbow trout fillets was not affected by the concentration of red beet and betaine, contrary to what was expected (Figure 3).

3.4.2. Individual phenolic compounds

Individual phenolic compounds were determined in red beet and experimental diets. HPLC red beet profile appears in Figure 4. Eight phenolic compounds were identified and quantified in red beet. The results showed that maleic acid (198.57 µg mL^{-1}) was the main compound followed by syringic acid (26.47 µg mL^{-1}), chlorogenic acid (25.58 µg mL^{-1}), vanillic acid (17.18 µg mL^{-1}), gallic acid (16.45 µg mL^{-1}), 4-hydroxibenzoic acid (10.42 µg mL^{-1}), ferulic acid (3.30 µg mL^{-1}) and caffeic acid (2.59 µg mL^{-1}). All these phenolic compounds were also identified in red beet by Georgiev *et al*. (2010) and Ravichandran *et al*. (2012).

When the different phenolic compounds were quantified in the experimental diets results showed some differences compared with the control diet; for instance, 4-hydroxibenzoic acid was not detected on the control diet and increasing concentrations of this compound was observed in diets at higher red beet and betaine levels. Ferulic acid was only observed on those diets at higher red beet concentration (diets C and D). Vanillic acid content was higher at higher betaine concentration diets (B and D). And although transcinamic acid was not detected on red beet, it was on the experimental diets; probably these compounds are present due to the presence of other ingredients of the diet.

3.4.3. Antioxidant activity of fish fillets

The antioxidant capacity of rainbow trout fillets was analysed by DPPH, ORAC, TEAC and RACI.

The antioxidant capacity measured by the different parameters was not significantly modified by the concentration of red beet and betaine on diet (Table 3). These results were different to what was expected, since fish fed with diets containing higher betaine concentrations (B and D) presented significantly higher values of betaine on flesh than those with lower concentration (A and C) or control, and betaine is a compound with high antioxidant activity (Pedreno and Escribano 2001, Attia *et al*. 2013, Paciulli *et al*. 2016). Also, it was found that the inclusion of red beet and betaine increased TFC and TP of the experimental diets, which could be involved on the antioxidant activity of the rainbow trout fillets. However, probably these compounds although acted as antioxidant they are no incorporated in the fish that is why is not possible to observe any effect on the fish extracts.

CONCLUSION

Results indicated that the inclusion of red beet and betaine on rainbow trout diets decreased total fatty acids concentration on fish muscle, but increase their PUFAs content, mainly DHA. On the other hand, although increasing concentration of red beet and betaine on diet increased its flavonoid and phenolic content, no effect was observed on the antioxidant and oxidative properties of rainbow trout fillets.

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Table 1. Red beet and experimental diets fatty acid profiles.

	Red beet	DIETS[1]				
		CONTROL	**A**	**B**	**C**	**D**
SFA (%)						
C14:0	0.02	0.23	0.22	0.21	0.26	0.27
C16:0	0.18	2.40	2.40	2.35	2.41	2.44
C18:0	0.04	0.50	0.47	0.51	0.45	0.42
MUFA (%)						
C16:1	0.02	0.26	0.25	0.21	0.25	0.26
C18:1 (n-9)	0.27	3.73	3.66	3.73	3.60	3.51
PUFA (%)						
C18:2n6	0.37	5.29	5.66	6.00	6.02	5.62
C18:3n3	0.03	0.41	0.49	0.40	0.47	0.47
C20:5n3	0.02	0.21	0.21	0.18	0.18	n.d.
C22:6n3	0.02	0.34	0.35	0.30	0.31	0.31

[1] Experimental diets: CONTROL (0% red beet, 0% betaine); A (314% red beet, 0.9% betaine); B (14% red beet, 1.69% betaine); C (28% red beet, 0.9% betaine); D (28% red beet, 1.69% betaine).

SFA (saturated fatty acid); MUFA (monounsaturated fatty acid); PUFA (polyunsaturated fatty acid); DHA (docosahexaenoic acid, 22:6 n-3); EPA (Eicosapentaenoic acid, 20:5 n-3).

n.d. means not detected value.

Table 2. Effect of red beet and betaine on the fatty acid profile of rainbow trout fillets. Data are shown as least-squares means ± standard error of the mean (SEM) of triplicate groups (n=3)

	DIETS					SEM	Sign.
	CONTROL	**A**	**B**	**C**	**D**		
Σ SFA (%)	**21.50**	**21.77**	**21.33**	**20.72**	**21.10**	**0.34**	**N.S.**
C14:0	1.75^{b}	1.69^{ab}	1.72^{ab}	1.64^{a}	1.64^{a}	2.14	**
C16:0	14.58	14.76	14.37	13.96	14.23	0.26	N.S.
C18:0	4.04	4.26	4.20	4.05	4.17	0.09	N.S.
C20:0	0.81	0.72	0.70	0.72	0.72	0.03	N.S.
C22:0	0.31^{a}	0.33^{ab}	0.36^{b}	0.34^{ab}	0.33^{ab}	0.02	*
Σ MUFA (%)	**34.44^{a}**	**34.30^{a}**	**35.72^{b}**	**34.43^{a}**	**33.66^{a}**	**0.43**	**
C16:1	2.66^{b}	2.64^{b}	2.59^{ab}	2.40^{a}	2.39^{a}	0.08	*
C18:1 n-9 trans	27.41	27.30	28.68	27.54	26.83	0.35	N.S.

C18:1 n-9 cis	1.91	1.90	1.96	1.97	1.97	0.07	N.S.
C20:1 n-9	1.14^{a}	1.21^{ab}	1.26^{b}	1.23^{b}	1.20^{ab}	0.03	*
C22:1	0.40	0.38	0.41	0.41	0.41	0.01	N.S.
C24:1	0.91	0.86	0.82	0.88	0.85	0.04	N.S.
Σ n-9	**30.46^{a}**	**30.41^{a}**	**31.90^{b}**	**30.73^{a}**	**29.99^{a}**	**0.38**	**
Σ PUFA (%)	**44.07^{ab}**	**43.92^{ab}**	**42.95^{a}**	**44.85^{b}**	**45.25^{b}**	**0.59**	*
C18:2 n-6 cis	26.47	27.02	26.59	27.06	27.20	0.52	N.S.
C18:3 n-3	2.77	2.82	2.92	2.89	2.79	0.05	N.S.
C20:2	0.94^{a}	1.14^{b}	1.13^{b}	1.14^{b}	1.17^{b}	0.06	*
C20:3 n-6	0.75	0.85	0.80	0.78	0.88	0.05	N.S.
C20:3 n-3	0.18	0.15	0.15	0.16	0.17	0.02	N.S.
C20:4 n-6 (ARA)	1.11^{c}	0.89^{ab}	0.81^{a}	0.89^{ab}	1.05^{bc}	0.07	**
C20:5 (EPA)	2.44	2.23	2.23	2.37	2.39	0.09	N.S.
C22:6 (DHA)	9.40^{bc}	8.84^{ab}	8.32^{a}	9.55^{bc}	9.60^{c}	0.32	*
Σ n-6	**28.34**	**28.75**	**28.20**	**28.73**	**29.13**	**0.55**	**N.S.**
Σ n-3	**2.94**	**2.97**	**3.07**	**3.06**	**2.96**	**0.05**	**N.S.**
n-6/n-3	**9.64**	**9.71**	**9.21**	**9.40**	**9.87**	**0.25**	**N.S.**
EPA/DHA	**0.26**	**0.25**	**0.27**	**0.24**	**0.25**	**0.02**	**N.S.**
ARA/EPA	**0.33**	**0.33**	**0.31**	**0.31**	**0.30**	**0.02**	**N.S.**

[1] Fish fed with experimental diets: Fish fed with CONTROL diet (0% red beet, 0% betaine); fish fed with A diet (14% red beet, 0.9% betaine); fish fed with B diet (14% red beet, 1.69% betaine); fish fed with C diet (28% red beet, 0.9% betaine); fish fed with D diet (28% red beet, 1.69% betaine).

SFA (saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); ARA (arachidonic acid, 20:4 n-6); DHA (docosahexaenoic acid, 22:6 n-3); EPA (Eicosapentaenoic acid, 20:5 n-3).

Different superscript letters indicate significant differences ($P < 0.05$) between the experimental diets.

Table 3. Effect of red beet and betaine on the antioxidant activity of rainbow trout fillets. Data are shown as least-squares means ± standard error of the mean (SEM) of triplicate groups (n=3)

	DIETS					SEM	Sign.
	CONTROL	**A**	**B**	**C**	**D**		
DPPH	34.89	36.46	34.61	35.74	38.83	1.60	N.S.
TP	23.50	21.24	19.89	20.45	20.55	2.03	N.S.
ORAC	997.09	844.48	857.81	957.36	827.72	74.01	N.S.
TEAC	1266.68	1132.53	1032.19	1110.81	1054.79	99.27	N.S.
RACI	0.20	-0.01	-0.17	0.01	-0.01	0.22	N.S.

[1] Fish fed with experimental diets: Fish fed with CONTROL diet (0% red beet, 0% betaine); fies fed with A diet (314% red beet, 0.9% betaine); fish fed with B diet (14% red beet, 1.69% betaine); fish fed with C diet (28% red beet, 0.9% betaine); fish fed with D diet (28% red beet, 1.69% betaine).

DPPH (1,1-diphenyl-2-picrylhydrazyl); TP (Total phenols), ORAC (Oxygen radical absorbance capacity), TEAC (Trolox Equivalent Antioxidant Capacity); RACI (Relative antioxidant capacity index).

Absence of superscripts letters indicates no significant differences (P>0.05) between the different experimental diets.

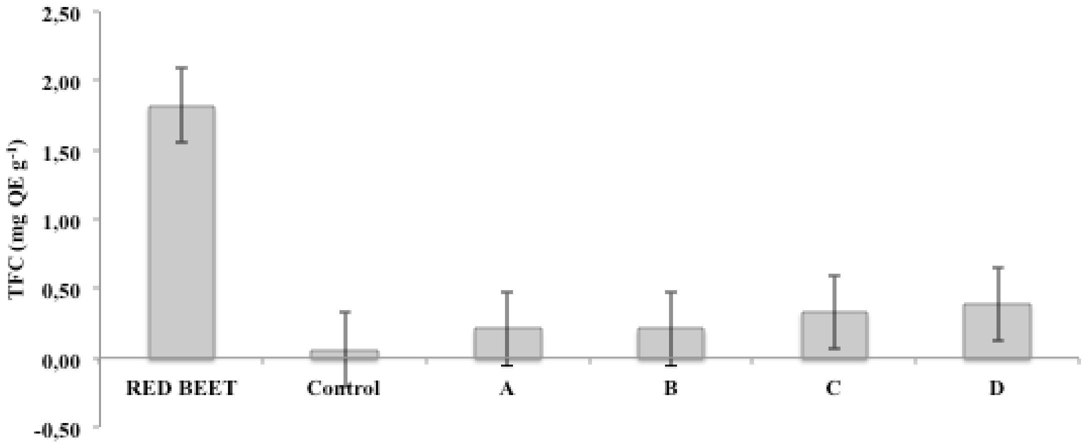


Figure 1. Red beet and experimental diets total flavonoid content (TFC). CONTROL (0% red beet, 0% betaine); A (14% red beet, 0.9% betaine); B (14% red beet, 1.69% betaine); C (28% red beet, 0.9% betaine); D (28% red beet, 1.69% betaine) are the different experimental diets.

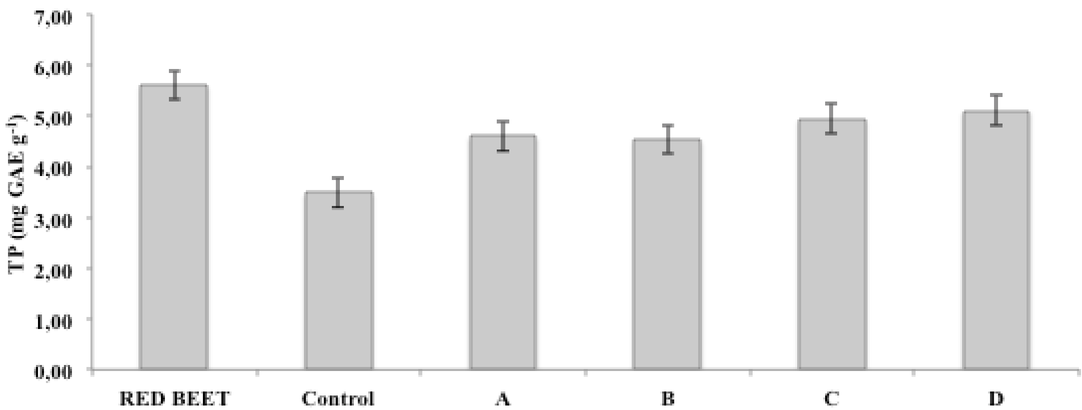


Figure 2. Red beet and experimental diets total phenolic content (TP). CONTROL (0% red beet, 0% betaine); A (14% red beet, 0.9% betaine); B (14% red beet, 1.69% betaine); C (28% red beet, 0.9% betaine); D (28% red beet, 1.69% betaine) are the different experimental diets.

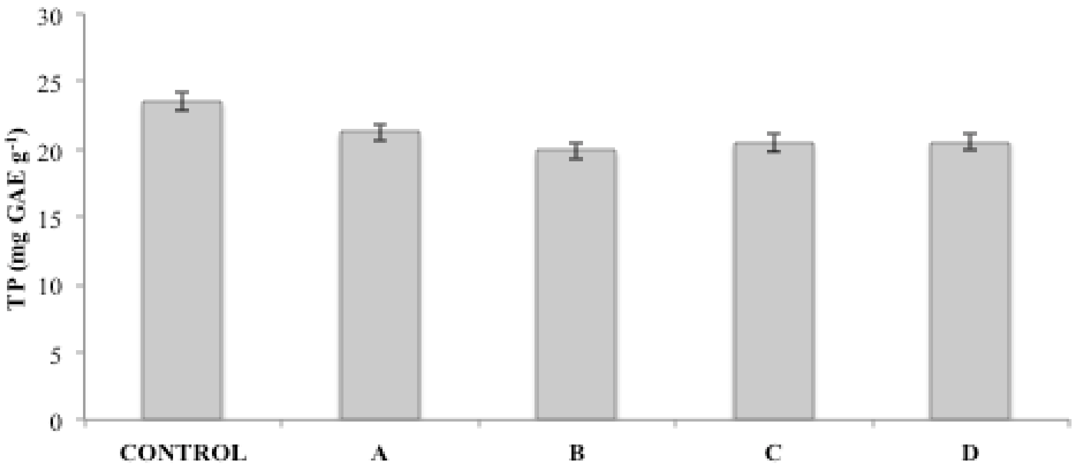
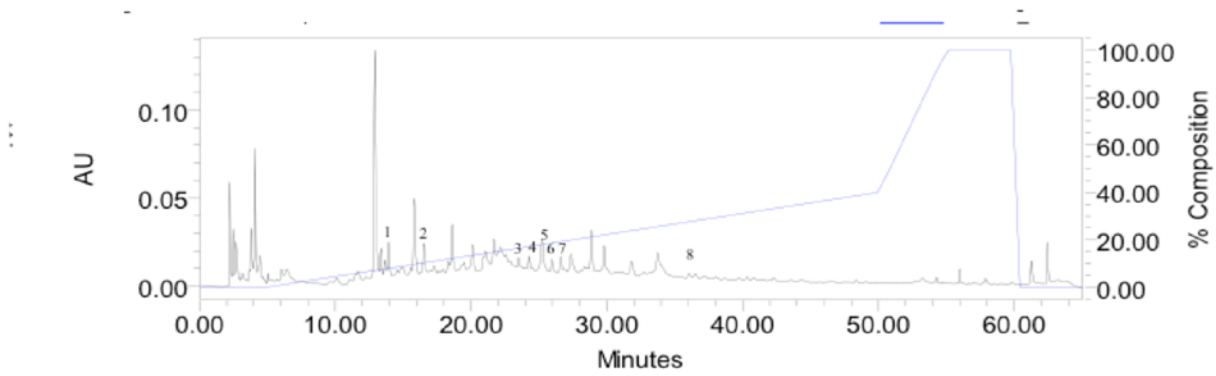


Figure 3. Fillets total phenolic content (TP) of fish fed with different experimental diets (n=3). CONTROL (0% red beet, 0% betaine); A (14% red beet, 0.9% betaine); B (14% red beet, 1.69% betaine); C (28% red beet, 0.9% betaine); D (28% red beet, 1.69% betaine) are the different experimental diets. Absence of different small letters (a, b) correspond to no significant differences ($P > 0.05$) between different samples.



	Red beet	DIETS[1]				
		CONTROL	A	B	C	D
1 Gallic acid	16.45	307.86	288.04	278.39	107.74	320.64
2 Maleic acid	198.57	19.42	22.84	33.52	17.84	40.92
3 4-hydroxybenzoic acid	10.42	n.d.	0.06	0.32	9.37	10.29
4 Chlorogenic acid	25.58	9.35	31.91	0.06	4.13	18.66
5 Vanillic acid	17.18	5.36	n.d.	10.11	n.d.	15.10
6 Caffeic acid	2.59	2.45	2.54	2.45	2.49	2.68
7 Syringic acid	26.47	7.76	10.31	3.42	2.39	0.68
8 Ferulic acid	3.30	n.d.	n.d.	n.d.	0.67	0.78
9 Transcinamic acid	n.d.	4.52	17.17	1.76	5.32	11.38

Figure 4. HPLC chromatogram of phenolic compounds in red beet extracts. 1. Gallic acid; 2. Maleic acid; 3. 4-hydroxybenzoic acid; 4. Chlorogenic acid; 5. Vanillic acid; 6. Caffeic acid; 7. Syringic acid; 8. Ferulic acid; n.d. means not detected value.