

Review Article

THE DETERMINATION OF FAT-SOLUBLE VITAMINS, CHOLESTEROL CONTENT AND THE FATTY ACID COMPOSITIONS OF SHABUT (*Arabibarbus grypus*, HECKEL 1843) FROM KEBAN DAM LAKE, ELAZIG, TURKEY

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Abstract:

The aim of the present study is to determine the content of fatty acids (FA), fat-soluble vitamins (A, D, E and K) and cholesterol in the muscle tissue of shabut (*Arabibarbus grypus*, Heckel 1843) from Keban Dam Lake. For this purpose, 40 specimens were obtained between December and March (2013). Muscle samples (without skin) taken from each fish were homogenized. Fat-soluble vitamins (A, D, E and K) and cholesterol were analysed simultaneously using HPLC (High-performance liquid chromatography) system. The fatty acids, grouped as saturated fatty acid (SFA), mono unsaturated fatty acid (MUFA) and polyenoic fatty acids (PUFA), were analysed by gas chromatography as the methyl esters. The results of present study showed that MUFA was the highest followed by SFA and PUFA. The highest fatty acid levels found in Shabut throughout all months (December – March) were 16:0, 18:1, 22:6 n-3 (DHA) and 20:5 n-3 (EPA). Shabut had low cholesterol level. Among the fat-soluble vitamins (A, D, E and K) analysed in all months, the vitamin E content was highest followed by K, D and A. In conclusion, it can be stated that Shabut (*A. grypus*) has comparatively good fatty acid composition, fat-soluble vitamins content and low cholesterol level. Shabut (*A. grypus*) can therefore be recommended for human consumption as a comparatively good source of nutrition.

Keywords: Shabut, *Arabibarbus grypus*, Fatty acid, Cholesterol, Fat-soluble vitamins.

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Introduction

The lipids are the most important biochemical compounds of fish (Akpınar, 1986). Fish store the lipids in various organs; particularly in muscles and liver. On the contrary, the mammals store in adipose tissue. A great amount of these lipids are transferred to the different parts of the body to be used for various physiological actions (Yılmaz, 1995).

It is well known that lipids play an important role in human and animal nutrition by supplying both energy and essential fatty acids (FAs) necessary to satisfy the physiological needs of the organism. The n-3 and n-6 PUFAs are considered essential to the growth and development of children. They are precursors of composite hormones known as eicosanoids, involved in several metabolic processes of great importance for the human body, mainly related to cardiovascular activity (Eder, 1995; Inhamuns and Franco, 2008). There is a strong evidence that consumption of fish containing high levels of these fatty acids (FAs) is favourable for human health. Long-chain n-3 PUFAs cannot be synthesised by humans and must be obtained from the diet. Results of clinical and epidemiological research suggest that EPA and DHA, found only in fish and seafoods, have beneficial properties for the prevention of human coronary artery disease. Therefore fish lipids and their fatty acids have been a subject of investigation when considering human health.

The fat content and the fatty acid composition of the fish are not constant (Zlatanov and Laskaridis, 2007). The fatty acid composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Henderson and Tocher, 1987; Zlatanov and Laskaridis, 2007; Inhamuns and Franco, 2008). It is generally recognised that PUFA composition may vary among species of fish. However, little attention has been paid to the composition of different species when selecting fish for the diet.

Fish is one of the main sources of vitamins (Cahu et al., 2004). In addition, some vitamins in fish such as the fat-soluble vitamins (A, D, E and K) also have therapeutic effects toward the prevention of particular diseases (Halver, 2002). Vitamin A required in vertebrates for the regeneration of light-sensitive rhodopsin in the retina (Lovell, 1998). Vitamin D is essential in maintaining homeostasis of calcium and inorganic phosphate which humans need for the normal development and maintenance of healthy teeth and bones. It also helps maintain proper blood levels of calcium and phosphorus (Halver, 2002). Vitamin E is an antioxidant also known as tocopherol. A major function of vitamin E is its role as a metabolic antioxidant, with a specific role in preventing oxidation of unsaturated phospholipids in cellular membranes, such as erythrocytes, and subcellular membranes such as mitochondria. Vitamin K is not listed among the essential vitamins. It is necessary for normal blood clotting in all animals, including fish. Therefore, fish is valuable source of essential fatty acids, vitamins and low levels on saturated fatty acids and cholesterol (Stancheva et al., 2010).

Barbus genus of Cyprinidae is widely distributed in eastern Asia, Eastern Europe, and Africa. It is commonly called barb or

shabbout, also spelled shabbout or shabut and Shirbot. According to the records of FAO (Food and Agriculture Organization), Shabut, also known as *A. grypus*, is one of the most significant fish species listed in the fresh waters of Iraq and in the rivers along South and Southwest Iran, the Karoon river, and also in The Euphrates River and Tigris Rivers in Turkey (Zivotofskya and Amar, 2006; Dorostghoal et al., 2009).

A. grypus is mainly spread in Euphrate Basins and a vagile species that prefers rivers but is also found in estuaries. It is commercially fished and can reach nearly two meters and over 50 kg (Coad, 1996). Spawning generally occurs from May to mid-June (Geldiay and Balik, 1988). The spawned eggs are scattered above aquatic plants and cling to the vegetation (Geldiay and Balik, 1988; Epler et al., 2001).

Keban Dam was built on Euphrates River in the eastern part of Turkey. Keban Dam Lake is the second largest dammed lake in Turkey (measured by surface area). About 28 fish species belonging to eight families living in the Euphrates River and its dam lakes have been recorded (Oymak et al., 2009). *A. grypus* is one of these species in Keban Dam Lake with more and more importance in economy because of human interest.

The freshwater fish constitute a great food potential for human. It is of great importance to know fat-soluble vitamins, cholesterol content and fatty acid compositions of the fish, which is economically important and willingly consumed. Also, new information will contribute to further projects.

Several studies have investigated on *A. grypus*. Its growth, sexual maturity characteristics, reproduction biology, have been studied (Marammazi and Kahkesh, 2011; Olgunoğlu and Olgunoğlu 2011; Atar and Ates, 2010; Oymak et al., 2008; Maghami et al., 2008; Zivotofskya and Amar, 2006; Dorostghoal et al., 2009; Sahinöz et al., 2007; Kahkesh et al., 2011) but studies about fatty acid composition and fat soluble vitamins and cholesterol are limited. (Harlioğlu and Gölbaşı 2013; Olgunoğlu et al., 2011). In view of these facts, the objective of the present study was to determine fatty acid, cholesterol and fat soluble vitamin compositions of the Shabut from Keban Dam Lake, Elazığ, Turkey, because it can be more valuable and attractive fish for human consumption.

Materials and Methods

Experimental animals

The level of fatty acids, cholesterol and fat-soluble vitamins were determined in 40 Shabut (*A. grypus*) specimens obtained from Keban Dam Lake, Elazığ, Turkey between December and March (2013). The water temperatures in Keban Dam Lake were between 6.5 and 8.6°C during the time in which fish samples have been obtained. Mean total weight \pm standard error (SE) and mean total length \pm standard error (SE) of fish used in this study were 755.98 \pm 84.1 g and 409.3 \pm 4.04 mm respectively. Muscle samples (without skin) were taken from each fish for analysing of fatty acid components, fat-soluble vitamins (A, D, E and K) and cholesterol.

Extraction of lipids

Lipids of muscle samples were extracted with hexane-isopropanol (3:2 v/v) by the method of Hara and Radin (1978). Nearly 1 g tissue sample was homogenized with 10 ml hexane-isopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4 °C and parts of tissue remnants were precipitated. The supernatant part was used in the ADEK, cholesterol and fatty acid analysis.

Preparation of fatty acid methyl esters

Fatty acids in the lipid extracts were converted into methyl esters including 2% sulfuric acid (v/v) in methanol (Christie, 1992). The mixture was vortexed and then kept at 50 °C for 12 h. After it was being cooled to room temperature, 5 ml of 5% sodium chloride was added and then it was vortexed again. Fatty acid methyl esters were extracted with 2 x 5 ml hexane. Fatty acid methyl esters were treated with 5 ml 2% KHCO₃ solution and then the hexane phase was evaporated by the nitrogen flow and then by dissolving in 0.5 ml fresh hexane (Christie, 1992), they were taken to auto sampler vials.

Gas chromatographic analysis of fatty acid methyl esters: Methyl esters were analyzed with the Shimadzu GC-17 Ver. 3 gas chromatography (Kyoto, Japan). For this analysis, 25 m of long Machery-Nagel (Germany) capillary column with an inner diameter of 0.25 µm and a thickness of 25 micron film was used. During the analysis, the column temperature was kept at 120-220°C, injection temperature was kept at 240°C and the detector temperature was kept at 280°C. The nitrogen carrier gas flow was 1 ml/min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. After this process, the necessary programming was made and the Class GC 10 software version 2.01 was used to process the data.

HPLC analysis of A, D, E, K vitamins and cholesterol

Five ml supernatant was taken to 25 ml tubes with caps and 5% KOH solution was added and immediately vortexed for 20 s. The tubes were placed in a water bath at 85°C for 15 min. The tubes were then taken and cooled to room temperature and 5 ml of distilled water was added and mixed. Lipophilic molecules, that did not saponify, were extracted with 2 x 5 ml hexane. The hexane phase was evaporated with nitrogen flow. It was dissolved in 1 ml (50+50%, v v-1) acetonitrile/methanol mixture and then was taken to auto sampler vials and was analyzed. The analysis was made with the Shimadzu brand HPLC device. HPLC conditions were as follows: mobile phase 60:38:2 (v/v/v): acetonitrile/methanol/water; The mobile phase flow rate was determined to be 1 mL A UV detector was used for the analysis and as a column the Supelcosil LC 18 (15 x 4.6 cm 5 µm; Sigma USA) column was used. For vitamin E and cholesterol 202 nm, retinol, 326 nm and for vitamin D and K, 265 nm was used (L'opez-Cervantes et al., 2006; Katsanidis and Addis, 1999).

Statistical analysis

The SPSS software (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Results for the groups are expressed as mean ± standard estimation (*SEM*). Differences between the group's means were analyzed for significance using the ANOVA Duncan's Multiple Range Test. Statistical significance was defined as P<0.05.

Results and Discussion

Fatty acids composition

The fatty acid compositions (% of total fatty acids) in the muscle of Shabut are given in Table 1.

The fatty acids analysed were grouped as saturated fatty acid (SFA), mono unsaturated fatty acid (MUFA) and polyenoic fatty acids (PUFA). The results of present study showed that MUFA was the highest followed by SFA and PUFA in the muscle of all groups. The highest fatty acid levels found in Shabut throughout all months (December – March) were 16:0, 18:1, 22:6 n-3(DHA) and 20:5 n-3 (EPA).

As displayed in Table 1, Palmitic acid (C16:0) was the primary SFA in Shabut in all months followed by stearic acid (C18:0). Among the SFAs, palmitic (C16:0) and stearic acid (C 18:0) were the major SFAs in the muscle of Shabut in all months. Our results about fatty acid composition of Shabut showed similarity with the result of Olgunoglu et al. (2011).

In the present study it was also determined that oleic acid (C18:1 n-9) was the dominant MUFAs in Shabut. Through the study, oleic acid (C18:1 n-9), a monounsaturated fatty acid type (MUFA), was observed as the predominant primary fatty acid throughout four months followed by palmitoleic acid (C16:1 n-7) and *cis*-10-pentadecenoic acid (C15:1). This finding seems differences with other study (Olgunoglu et al., 2011) on Shabut. It was concluded that these differences may be attributed to the different abiotic and biotic factors such as season, the type and amount of feed available, water temperature, pH, salinity and fish age.

Omega-6 and omega-3 fatty acids are essential because humans, like all mammals, cannot synthesised them and must obtain them in their diet. Humans and other mammals, except for carnivores such as lions, can convert linoleic acid (C18:2 n-6) to arachidonic acid (C20:4 n-6) and α -linolenic acid (C18:3 n-3) to EPA and DHA, but it is slow. The results showed that Shabut contained comparatively good level of n-3 polyunsaturated fatty acids (PUFA). In the present study, docosahexaenoic acid (C22:6 n-3, DHA) was the primary fatty acids in all months followed by eicosapentaenoic acid (C20:5 n-3 EPA). This result was similarly reported by (Olgunoglu et al., 2011). The proportion of DHA and EPA were highest in March.

In the present study, the ratio of PUFA to SFA was found between 0.94 and 0.98 throughout four months and there was no statistically significance different between months (P>0.05). According to the general nutritional guidelines of the Department of Health of the United Kingdom, this ratio should be 0.4 or

Table 1. The composition of fatty acid (% of total fatty acids) in the muscle of Shabut (*A. grypus*). Therefore, the PUFA/SFA ratio results obtained in the present study was within the recommended range.

Fatty acid	December	January	February	March
C15.1	3.85 ± 0.009 ^c	3.28 ± 0.012 ^a	3.49 ± 0.032 ^b	6.08 ± 0.051 ^d
C16.0	17.38 ± 0.405 ^a	18.51 ± 0.730 ^a	22.86 ± 0.910 ^b	25.47 ± 0.595 ^c
C16.1 n-7	2.83 ± 0.024 ^a	4.10 ± 0.254 ^b	4.90 ± 0.196 ^c	6.54 ± 0.085 ^d
C18.0	7.21 ± 0.048 ^a	8.60 ± 0.070 ^b	8.79 ± 0.070 ^c	15.64 ± 0.037 ^d
C18.1 n-9	18.85 ± 0.019 ^a	19.23 ± 0.025 ^b	21.55 ± 0.116 ^c	26.64 ± 0.110 ^d
C18.2 n-6	1.89 ± 0.022 ^c	1.71 ± 0.016 ^b	1.11 ± 0.003 ^a	2.29 ± 0.047 ^d
C18.3 n-3	0.89 ± 0.010 ^a	1.46 ± 0.006 ^b	1.48 ± 0.005 ^b	2.36 ± 0.015 ^c
C18.3 n-6	1.25 ± 0.010 ^a	1.68 ± 0.008 ^b	4.31 ± 0.034 ^c	5.40 ± 0.055 ^d
C20.4 n-3	ND	ND	ND	1.98 ± 0.012 ^a
C20.4 n-6	1.28 ± 0.007 ^a	1.82 ± 0.010 ^b	2.64 ± 0.029 ^d	2.52 ± 0.065 ^c
C20.5 n-3	2.34 ± 0.036 ^a	2.74 ± 0.017 ^b	3.61 ± 0.014 ^c	4.64 ± 0.082 ^d
C22.5	1.23 ± 0.008 ^a	1.64 ± 0.029 ^b	1.75 ± 0.006 ^c	2.12 ± 0.020 ^d
C24.1	ND	2.10 ± 0.013 ^b	2.02 ± 0.006 ^a	3.01 ± 0.011 ^c
C22.6 n-3	14.45 ± 0.225 ^a	15.62 ± 0.264 ^b	16.07 ± 0.155 ^b	17.45 ± 0.163 ^c
ΣSFA	24.59 ^a	27.11 ^b	31.65 ^c	41.11 ^d
ΣMUFA	25.53 ^a	28.71 ^b	31.96 ^c	42.27 ^d
ΣPUFA	23.33 ^a	26.67 ^b	30.97 ^c	38.76 ^d
ΣPUFA/SFA	0.95 ^b	0.98 ^c	0.98 ^c	0.94 ^a
Σn-3	17.68 ^a	19.82 ^b	21.16 ^c	26.43 ^d
Σn-6	4.42 ^a	5.21 ^b	8.06 ^c	10.21 ^d
Σn-6/Σn-3	0.25 ^a	0.26 ^a	0.38 ^b	0.39 ^b

Each value is means of 40 samples with standard errors. Same letters in the same line are not statistically important ($p > 0.05$).
ND: Not determined.

more for a balanced fatty acid intake on a healthy diet (Wood et al., 2004).

Several sources of information suggest that human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids (EFA) of ~1. In a lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many of the chronic diseases of high prevalence in Western societies, as well as in the developing countries (Artemis, 2008). Some clinical studies (Lands, 1992; Okuyama, 2001) indicate that the ingested ratio of omega-6 to omega-3 (especially linoleic vs alpha-linolenic) fatty acids is important to maintaining cardiovascular health. The lower omega-6/omega-3 ratio in women with breast cancer was associated with decreased risk. A ratio of 2-3/1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5/1 had a beneficial effect on patients with asthma (Artemis, 2008). Both omega-6 and omega-3 fatty acids are essential; i.e., humans must consume them in the diets. Omega-6 and omega-3 eighteen-carbon polyunsaturated fatty acids compete for the same metabolic enzymes, thus the omega-6:omega-3 ratio of ingested fatty acids has significant influence on the ratio and rate of production of eicosanoids, a group of hormones intimately involved in the body's inflammatory and homeostatic processes which includes the prostaglandins, leukotrienes, and thromboxanes, among others. Altering this ratio can change the body's metabolic and inflammatory state. Tribble et al. (2006) healthy ratios of omega-6:omega-3, according to some authors, range from 1:1 to 1:4 (an individual needs more omega-3 than omega-6). Lands (2005) other authors believe that ratio 4:1 (when the amount of

omega-6 is only 4 times greater than that of omega-3) is already healthy. However reported that excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a lower omega-6/omega-3 ratio), exert suppressive effects (Artemis, 2008).

In the present study, n-3/n-6 proportion value was found between 0.25 and 0.39 throughout four months. According to a research (Lands, 2005) this lower omega-6/omega-3 ratio value could constitute a healthy human diet.

Fat-soluble vitamins and cholesterol content

The fat-soluble vitamins are essential nutrients controlling a diversity of biologically important processes in human body. Vitamin A, also called retinol, takes place in photoreception and regulates gene expression and cell division, bone growth, teeth development, reproduction etc. Vitamin D₃ (cholecalciferol) plays crucial role in the regulation of calcium – phosphate balance stimulating calcium absorption by the small intestine and thus regulating bone metabolism. The biologically active isomer of vitamin E - alpha-tocopherol (α-TP) acts as an antioxidant, protecting membrane structures, essential fatty acids, and vitamins A and C from oxidation. Being one of the major sources of omega-3 PUFA and fat-soluble vitamins A, D₃ and E, fish production by fish farming attains great economical importance.

On the other hand, the worldwide decline of ocean fisheries stocks has provided impetus for rapid growth in fish and shellfish farming, or aquaculture (Rosamond L, et al., 2000).

The values of A (retinol), D (D₂, D₃), E (α -tocopherol), K (K₁, K₂) vitamins are shown in Table 2. Among the fat-soluble vitamins (A, D, E and K) analysed in four months, the vitamin E content was highest followed by K, D and A.

The findings showed that there was a significant difference in vitamins throughout months.

We have not found a study related with the vitamins content of Shabut. For different species, a study on the vitamin content of fish and fish products consumed in Portugal was undertaken by Dias et al. (2003), who found that vitamins A, D and E in eel meat were 887, 16 and 2400 $\mu\text{g}/100\text{ g}$ respectively. In salmon these were found to be 33, 11 and 4000 $\mu\text{g}/100\text{ g}$ respectively. In rainbow trout these values were determined as 8.8, 19 and 130 μg per 100 g respectively (Dias et al., 2003). In addition, vitamin D (D₂ + D₃) content was 23.3 $\mu\text{g}/100\text{ g}$, and vitamin E (alpha-tocopherol) content was 4 mg/100 g in *Anguilla* sp. (USDA, 2005). In a different study (Stancheva et al., 2010) the retinol (vitamin A) content in the edible tissue of rainbow trout (*Oncorhynchus mykiss*) was $22.3 \pm 2.0\ \mu\text{g}/100\text{ g}$; cholecalciferol (Vitamin D₃) $6.0 \pm 0.29\ \mu\text{g}/100\text{ g}$ and α -tocopherol (Vitamin E) $809.1 \pm 56.0\ \mu\text{g}/100\text{ g}$. In the another study on freshwater spiny eel (*Mastacembelus simack*) vitamin A content was 0.533 $\mu\text{g}/\text{g}$, vitamin E (alpha-tocopherol) content was 1.89 $\mu\text{g}/\text{g}$, vitamin D₂ content was 0.79 $\mu\text{g}/\text{g}$ and vitamin D₃ content was 1.36 $\mu\text{g}/\text{g}$ respectively (Harlioglu et al., 2010).

Vitamin content can vary in different parts of the same tissues, and among animals collected at different times and locations. Indeed, geographic availability, seasonality, and physiological state/maturity are known to affect variability in nutrient composition, particularly for vitamins (Greenfield and Southgate, 2003).

We have not found a study related with the cholesterol content

Table 2. The contents of fat soluble vitamins in the muscle of Shabut (*A. grypus*).

Fat soluble vitamins ($\mu\text{g}/\text{g}$)	December	January	February	March
A (Retinol)	0.1 ^a	0.1 ^a	0.2 ^b	0.2 ^b
D ₂	0.1 ^a	0.1 ^a	0.3 ^b	0.4 ^c
D ₃	0.13 ^a	0.15 ^a	0.4 ^d	0.3 ^c
K ₁	0.5 ^a	1.0 ^c	0.98 ^b	1.10 ^c
K ₂	0.3 ^a	1.0 ^b	2.35 ^c	3.50 ^d
E (α -Tocopherol)	2.60 ^a	4.85 ^b	7.30 ^c	9.95 ^d
Cholesterol (mg/100 g)	10.50 ^a	11.02 ^a	13.82 ^b	15.13 ^c

Each value is an average of 40 samples with standard errors. Same letters in the same line are not statistically important ($p > 0.05$)

of Shabut. For different species, the cholesterol content of *M. simack* was found to be $52.60 \pm 4.36\ \text{mg}/100\text{ g}$ in the a study (Harlioglu et al., 2010). In a study on the cholesterol content of selected marine fishes in Malaysian waters, Osman et al. (2001) noted that the cholesterol content was 37.1 in *Gymnura* spp., 40.3 in *Pampus argenteus*, 41.1 in *Scomberomorus commersonii*, 41.8 in *Clupea fimbriata*, 45.9 in *Eleutheronema tradactylum*, 46.6 in *Magalapsis cordyla*, 46.8 in *Parastromateus niger*, 46.9 in *Plotosus* spp., 47.3 in *Selarides leptolejus* and 49.1 mg/100 g in *Rastrelliger kanagurta*.

In a different study, cholesterol content of some fish species caught from Turkish seas were investigated by İmre and Sağlık (1998). they found cholesterol content (mg/100 g) as 43.4 in the sardine (*Sardina pilchardus*) and 40.3 mg per 100 g in pandora (*Pagellus erythrinus*), 75.3 in sargo (*Diplodus sargus*), 63.4 in mackerel (*Scomber scombrus*) and 58.4 in sole (*Solea solea*). Cahu et al. (2004) were found the cholesterol content in channel catfish (58 mg/100 g) and rainbow trout (60 mg/100 g)

In the present study, the cholesterol content (mg/100 g) of the Shabut muscle was found to be between 10.50 and 15.13 in four months. The results showed that cholesterol level of Shabut is low. Osman et al. (2001) stated that the cholesterol content in fish is influenced by several factors, among them the PUFA content, and that an increase in PUFA content will be followed by a decrease in the cholesterol level.

Conclusions

It appears that no enough data on the vitamin and cholesterol compositions and fatty acid profiles of Shabut are available. Therefore the results of the present study will form the basis for further research in this field of fish chemistry for the benefits of human beings.

In conclusion, it can be stated that Shabut (*A. grypus*) has comparatively good fatty acid composition, $\Sigma n-6/\Sigma n-3$ ratio, fat-soluble vitamins content and low cholesterol level. Shabut (*A. grypus*) can therefore be recommended for human consumption as a comparatively good source of nutrition. Finally, we recommend Shabut (*A. grypus*) especially obtained in March for healthy human diet.

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