

Diurnal Ammonia and Urea Excretion Rates in the Juvenile European Sea Bass, *Dicentrarchus labrax* Fed Diets Containing Cotton Seed and/or Canola Oils

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Abstract: This study investigated the effects of total fish oil replacement by cotton seed and/or canola oils on daily total ammonia and urea-nitrogen excretion rates in the juvenile European sea bass. Iso-nitrogenous and iso-energetic diets formulated to totally replace Fish Oil (FO100) by Cotton Seed Oil (CSO100), Canola Oil (CO100) and equal combination (CSO50/CO50) of these Vegetable Oils (VOs) were fed to fish to apparent satiation and daily fluctuation of Total Ammonia Nitrogen (TAN) and urea-N were measured after 24 h. There was a delay in times of the occurrence of peak excretions in fish fed diets containing VOs. Daily TAN excretion rates decreased whereas daily urea-N excretion increased with the inclusion of VOs in diets and this was particularly the case in fish fed dietary treatment CSO50/CO50 as daily urea-N excretion expressed as percentage of Consumed Nitrogen (CN) was significantly ($p < 0.05$) higher than that of fish fed FO100 diet. There was no significant difference in the theoretically calculated Retained-N + Fecal-N values among the treatments. This study indicated that European sea bass utilized both VOs effectively as an energy source but altered dietary n-3/n-6 ratio seemed to play a part in the nitrogenous excretion mechanism in the European sea bass.

Key words: Fish oil replacement, canola oil, cotton seed oil, ammonia, urea, excretion, *Dicentrarchus labrax*

INTRODUCTION

Measurements of ammonia and urea-nitrogen excretion rates can give an insight into nitrogenous metabolism in teleost fish under various environmental conditions and nutritional regime (Jobling, 1981; Dosdat *et al.*, 1995; Remen *et al.*, 2008; Lam *et al.*, 2008). Several factors like fish species and size (Dosdat *et al.*, 1996), dietary protein level and its source (Rychly, 1980; Engin and Carter, 2001; Dias *et al.*, 2005) or nitrogen intake (Kaushik and Cowey, 1990) feeding frequency (Ramnarine *et al.*, 1987), environmental temperature (Jobling, 1981; Medale *et al.*, 1995; Person-Le Ruyeta *et al.*, 2004) and dietary limiting essential amino acids specifically arginine (Fournier *et al.*, 2003; Tulli *et al.*, 2007) and the optimal essential amino acid index (Peres and Oliva-Teles, 2006) dietary non protein nitrogen content such as nucleic acids (Oliva-Teles *et al.*, 2006) and dietary wheat gluten and high fat levels (Robaina *et al.*, 1999; Boujard *et al.*, 2004) have all been demonstrated to influence nitrogenous excretion in fish including the European sea bass *Dicentrarchus labrax*.

Following feed intake and absorption, the majority of free amino acids circling in the blood are catabolized in the liver and ammonia, a major excretory end product of nitrogen metabolism in teleost fish is produced as a result of transamination of these amino acids (Campbell, 1991; Wright, 1995; Wilkie, 1997; Tng *et al.*, 2008). However, a substantial amount of urea-nitrogen excretion was also reported in some teleost species in relation to increased feed intake (Kikuchi, 1995; Harris and Probyn, 1996; Carter *et al.*, 1998), crowding and being exposed to air (Korsgaard *et al.*, 1995; Kajimura *et al.*, 2002) and unfavorable environmental conditions such as high alkalinity (Walsh *et al.*, 1990). Extensive studies investigating the effects of dietary manipulations namely energy level and nutrient density on nitrogenous excretion demonstrated that high dietary energy had a protein-sparing effect resulting in lower ammonia productions in several farmed fish species (McGoogan and Gatlin, 2000). High energy levels in aqua feeds are achieved by adding lipids to diets as they are energy dense nutrient and readily metabolized by fish. However, the source of dietary oils (plant or animal originated) seem to be the determining factor of how well they are utilized

for energy provision and other metabolic events in fish (Montero *et al.*, 2005; Tocher *et al.*, 2008). Improved feed efficiency and protein utilization thus lowered ammonia excretion rates were also demonstrated with nutritional studies employing satiation feeding as opposed to feeding at a fixed rate in several fish species such as juvenile Atlantic cod (*Gadus morhua* L.) (Ramnarine *et al.*, 1987) and red drum (*Sciaenops ocellatus*) (McGoogan and Gatlin, 2000).

Fish meal and oil are finite resources and sustainability in aquaculture production necessitates the use of cheaper and abundant plant and animal alternatives in aqua feeds (Naylor *et al.*, 2000; Mourente and Bell, 2006; Tacon and Metian, 2008). Vegetable oil sources are increasingly being investigated to replace fish oil in diets for mariculture species including European sea bass (Richard *et al.*, 2006) and gilthead sea bream (*Sparus aurata*) (Izquierdo *et al.*, 2005). Plant seed oils are rich in C₁₈ PUFA's such as Linolenic (LNA, 18:3n3) and Linoleic Acids (LA, 18:2n6) and MUFA (mainly oleic acid, 18:1n-9) and devoid of n-3 HUFA including EPA and DHA. Marine fish species are generally recognized as having a poor ability in converting LA to arachidonic acid and LNA to EPA (20:5n3) and DHA (22:6n3) (Sargent *et al.*, 1999). Previous studies demonstrated that replacement of fish oil by VOs >60% in the European sea bass diets had no negative impact on fish growth, survival or health status (Mourente and Dick, 2002; Izquierdo *et al.*, 2003; Figueiredo-Silva *et al.*, 2005; Mourente *et al.*, 2005; Mourente and Bell, 2006; Richard *et al.*, 2006).

However, >80% substitution level was shown to have growth suppression effect in the same species (Montero *et al.*, 2005). Apart from the growth rates whole body and fillet fatty acid compositions and lipogenesis and health status there is very limited knowledge about the effects of VOs on nitrogen metabolism and excretion in fish. Since, VOs were shown to affect intestinal nutrient uptake and barrier function, plasma cortisol and prostaglandin E₂ levels, immune parameters and susceptibility to oxidative stress due to altered muscle and mitochondrial membrane n-3 HUFA levels in Atlantic salmon, *Salmo salar* (Jutfelt *et al.*, 2007; Petropoulos *et al.*, 2009; Ostbye *et al.*, 2011), it would be highly credible to speculate that nitrogen metabolism and excretion rates might be affected by inclusion of VOs in diets for fish species. The European sea bass is one of the most important farmed finfish species in the Mediterranean region (Kaushik, 2002) and any efforts towards understanding the safe use of VOs would be extremely beneficial for the cost effective feed formulation in this species. Therefore, this study aimed at demonstrating the effects of total fish oil replacement by

Cotton Seed (CSO) and/or Canola Oils (CO) in balanced feeds on the diurnal total ammonia and urea-nitrogen excretion rates of the juvenile European sea bass.

MATERIALS AND METHODS

Fish and experimental conditions: For this study, healthy juvenile European sea bass were obtained from a commercial farm (Akuvatur, Ltd., Adana, Turkey) and placed into two 1000 L fiberglass tanks until used in the experiment. The experiment was conducted in a flow through filtered seawater system that consisted of 12 circular fiberglass tanks (350 L rearing volume). Twenty juveniles (78.5±2.4 g) were randomly allocated to each tank. Fish were acclimatized to experimental diets and conditions for 2 weeks before the excretion rates were measured (Tng *et al.*, 2008). Uneaten feed and feces were siphoned out daily before beginning measurements of excretion. Water temperature and pH ranged between 26.2 and 27.4°C and between 7.5 and 8.3, respectively throughout the experimental period. Continuous aeration was utilized throughout the experiment. Water exchange was not utilized during the sampling period and the oxygen content of the outlet water remained >5 mg L⁻¹. Photoperiod was 12 h light:12 h dark.

Diets: Three iso-nitrogenous and iso-energetic diets were formulated to contain Cotton Seed Oil (CSO) and Canola Oil (CO) as fish oil replacers either totally (CSO100 and CO100) or in half (CSO50/CO50) on a dry matter basis. Control diet (FO100) contained fish oil from *Engraulis encrasicolus* as dietary oil source (Sibal Black Sea Feed Inc., Sinop-Turkey). CSO was obtained from the sale office of Cukurova Cooperative Enterprise (Cukobirlik, Adana-Turkey) whereas CO was a commercial brand sold in supermarkets. Dietary protein sources used in the diets were fish meal from *Engraulis encrasicolus* (Sibal Black Sea Feed Inc., Sinop-Turkey) and corn gluten (Sunar Inc., Adana-Turkey). Dextrin was used as a carbohydrate source (Sunar Inc., Adana-Turkey).

The other dry ingredients were CMC (Carboxy-Methyl-Cellulose), DCP (Di Calcium Phosphate) and vitamin and mineral mixtures (Sibal Black Sea Feed Inc., Sinop-Turkey) (Table 1). Diets were prepared by mixing the dry ingredients in a Hobart mixer for 45 min. The dry diet mixtures were further mixed for 30 min after the addition of dietary oil sources. Diets were then pelletized using laboratory type pellet machine. Samples of dry diets were analyzed for crude protein (Kjeldahl, selenium catalyst; Nx6,25) crude fat by chloroform and methanol extraction (AOAC, 1995). Ash contents were determined by burning the test diets at 550°C in a furnace for 16 h.

Table 1: Formulation (g kg⁻¹ diet) and chemical composition of the experimental diets

Ingredients	Diets			
	FO100	CSO100	CO100	CSO50/CO50
Fish meal	510	510	510	510
Corn gluten	225	225	225	225
Dextrin	70	70	70	70
Fish Oil (FO)	100	0	0	0
Cotton Seed Oil (CSO)	0	100	0	50
Canola Oil (CO)	0	0	100	50
CMC	47	47	47	47
DCP (Di Calcium Phosphate)	23	23	23	23
Mineral mix ¹	15	15	15	15
Vitamin mix ¹	10	10	10	10
Proximate composition (g kg⁻¹)				
Moisture	143.6	136.1	141.8	132.1
Crude protein	457.9	458.5	468.6	462.4
Crude lipid	204.4	200.3	195.4	191.8
NFE ²	62.3	81.6	71.5	88.3
Crude ash	126.8	123.3	122.8	125.4
Gross energy (MJ kg DM ⁻¹) ³	17.1	17.2	17.1	17.0
P:E ratio (g MJ ⁻¹)	26.8	26.7	27.4	27.2

¹Vitamin and mineral premix added minimum to NRC recommendations (NRC,1993), ²Nitrogen-Free Extract: Calculated as the remainder of moisture+crude protein+crude lipid+ash, ³Calculated based on the standard physiological fuel values: 19 kJ g⁻¹ for protein, 36 kJ g⁻¹ for lipid and 15 kJ g⁻¹ for carbohydrate (Smith, 1989)

Experimental procedure and measurements: Fish were fed 3 times (0830-1330-1830 h) to apparent satiation throughout the experiment. Feeding continued until fish showed no interest in feeding and utmost care was given to keep the tank water clean off uneaten pellets. Sampling of tanks for total ammonia- and urea-nitrogen was blocked over time so that all diets were sampled concurrently at 8 h sampling periods (0900-1700; 1700-0100; 0100-0900 h). During sampling water flow to tanks was turned off and each tank of each treatment was sampled over one 8 h period in each sampling day (Engin and Carter, 2001). Triplicate 10 mL samples were collected for ammonia and urea measurements into scintillation vials at 4th and 8th h in each sampling period by pipetting water samples from the middle of tanks to provide data for every 4 h over a 24 h period. The experimentation was conducted >5 days so that each tank was sampled over each of the three 8 h periods with a day in between samplings (Engin and Carter, 2001).

Excretion was calculated from the change in ammonia or urea concentration and the water volume in each tank. Sample Total Ammonia-Nitrogen (TAN) concentrations were determined by the phenol-hypochloride method (Solorzano, 1969) whereas urea was analyzed by the urease method (Elliott, 1976). Total ammonia-nitrogen concentration was calculated using a standard curve prepared from ammonium chloride solution. The difference between ammonia concentration before and after urease treatment was used to calculate urea concentrations in samples.

Statistical analysis: Data are presented as means±SD and were subjected to one-way ANOVA. A Tukey-Kramer HSD test was used to compare means when a significant treatment effect was observed. The linear relationship between Consumed Nitrogen (CN) and nitrogenous excretion rates and calculated Retained-N + Fecal N for individual tanks in each dietary treatment were also investigated (Zar, 1999). Significance was accepted at probabilities of 0.05 or less.

RESULTS AND DISCUSSION

Diurnal Total Ammonia (TAN) and urea-nitrogen excretion rates:

There was no mortality throughout the experimentation. Mean daily feed intake of fish varied between 1.9 and 2.2 % BW in the dietary treatments. The overall excretion rates were similar to previously observed rates in the European sea bass when fed balanced diets (Tulli *et al.*, 2007). There was no significant difference between daily Total Ammonia-nitrogen excretion (TAN) rates among treatments (Table 2). TAN excretion rates began to increase in all dietary treatments 4 h following the morning feed (Fig. 1). The magnitude of excretion rates following 4 h of morning and afternoon feeding followed the same trends in all the dietary treatments. However, it appeared that there was a delay in TAN excretion rates of fish fed diets in which fish oil was replaced by Cotton Seed Oil (CSO) and/or Canola oils (CO) either totally or in half (CSO/CO) (Fig. 1). The TAN excretion rates of fish were also found to be significantly lower ($p<0.05$) in dietary treatments CSO100 and CSO50/CO50 compared to FO100 and CO100 8 h following the evening meal. The excretion rates before the next morning's feeding were significantly higher ($p<0.05$) than the rates at 5 h in each dietary treatment (Fig. 1).

Urea-nitrogen excretion accounted for between 11 and 21% of TAN excretion rates at each treatment (Table 2). There was no significant difference in the daily urea-nitrogen excretions among the dietary treatments. Furthermore, the variability in the excretion rates were significantly higher ($p<0.05$) in fish fed diets containing CSO and/or CO oils over a 24 h sampling period compared to fish oil only diet and rates were found to be doubled specifically 8 h following the evening feed (Fig. 2). Contrary to TAN excretion, the urea-nitrogen excretion rates of fish returned to initial levels at 9 h sampling period in each dietary treatment (Fig. 2).

The relationship between Consumed Nitrogen (CN) and mean daily nitrogenous excretion rates and calculated retained-N+fecal-N for each dietary treatment. The highest daily mean TAN excretion rates were 576±49 and 494±114 (mg TAN/kg/day) and they were obtained from fish fed

Table 2: Mean daily rates of nitrogenous excretion by the juvenile European sea bass fed diets containing CSO (Cotton Seed Oil) and CO (Canola Oil) as fish oil replacer either totally or in half and as a percentage of Consumed Nitrogen (CN)*

Hems	Diets				F	p-values
	FO100	CSO100	CO100	CSO50/CO50		
CN (Consumed Nitrogen, mg N/kg/day)	1618±180	1730±132	1811±159	1396±40	2.893	0.102
EI (Energy Intake, kJ/kg/day)	399±52	427±32	446±39	344±10	2.885	0.103
TAN excretion (mg TAN/kg/day)	576±49	369±92	494±114	366±38	3.684	0.062
Urea-nitrogen (mg Urea-N/kg/day)	62±13	75±5	75±6	76±10	1.377	0.318
Total nitrogen (mg N/kg/day)	638±60	443±110	569±116	441±44	3.125	0.088
Retained N+Fecal N (mg N/kg/day)	979±241	1287±168	1242±275	955±43	2.194	0.166
TAN excretion (CN%)	36.0±3.4	21.3±7.7	27.8±8.9	26.2±2.4	2.827	0.107
Urea-Nitrogen (CN%)	3.9±0.5 ^a	4.4±0.1 ^{ab}	4.2±0.5 ^{ab}	5.4±0.7 ^b	5.029	0.030
Total nitrogen (CN%)	39.9±3.4	25.7±7.6	32.0±9.4	31.7±2.9	2.437	0.140

*Values are means±SD (n = 3) and means in the same row with different superscripts are significantly different (p<0.05)

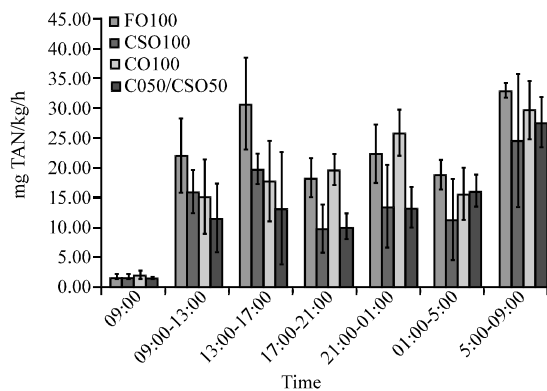


Fig. 1: Fluctuations of daily Total Ammonia Nitrogen (TAN) excretion by the juvenile European sea bass fed balanced diets in which Fish Oil (FO) was totally replaced by Cotton Seed Oil (CSO100) or Canola Oil (CO100) or the equal combination of these VO sources (CSO50/CO50). Values are means±SD (n = 3) for each treatment. *Represents initial mean TAN values for each treatment

fish oil only (FO100) and CO100 diets, respectively. Although, differences were insignificant, the mean daily TAN excretions were found to be lower in fish fed CSO100 and CSO50/CO50 diets compared to that of fish fed FO100 and CO100 diets. It also appeared that there was a strong positive and negative linear relationship between CN and daily TAN excretion rates among individual tanks in fish fed diets FO100 ($y = 0.163x + 310.9$; $n = 3$; $r^2 = 0.98$) and CO100 ($y = -0.714x + 1787$; $n = 3$; $r^2 = 0.99$), respectively. However, TAN excretion rates by CN in fish fed diets CSO100 and CSO50/CO50 showed a weaker positive linear relationship, former being the weakest.

Although, there was no significant difference among dietary treatments mean daily urea-nitrogen excretion rates tended to increase with FO replacement by CSO and/or CO in diets (Table 2). The highest and lowest excretion rates obtained from the dietary treatments CSO50/CO50 and FO100 were 76 ± 10 and 62 ± 13

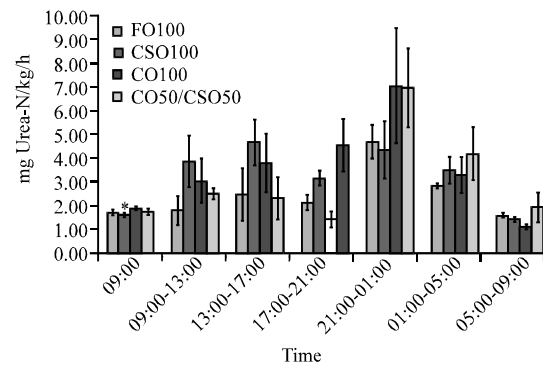


Fig. 2: Fluctuations of daily urea-N excretion by the juvenile European sea bass fed balanced diets in which Fish Oil (FO) was totally replaced by either Cotton Seed Oil (CSO100) or Canola Oil (CO100) or the equal combination of these VO sources (CSO50/CO50). Values are means±SD (n = 3) for each treatment. *Represents initial mean urea-N values for each treatment

(mg urea-N/kg/day), respectively (Table 2). Urea-nitrogen excretion rates were found to be similar in fish fed diets contained CSO and/or CO oils. Mean daily excretion rates for individual tanks in each dietary treatment demonstrated that there was a positive strong linear relationship between CN and urea-nitrogen excretion rates in fish fed diets FO100 ($y = 0.038x - 0.518$; $n = 3$; $r^2 = 0.73$) and CSO100 ($y = 0.034x + 14.7$; $n = 3$; $r^2 = 0.96$) whereas very weak converse relationships between CN and urea-nitrogen excretions were observed in fish fed diets CO100 and CSO50/CO50.

Daily mean urea-nitrogen excretion rates expressed as the percentage of Consumed Nitrogen (CN) were significantly higher ($p < 0.05$) in fish fed the CSO50/CO50 diet (5.4 ± 0.7) compared to that was observed in fish fed FO100 diet (3.9 ± 0.5) (Table 2). Although, the differences were insignificant mean daily urea-nitrogen excretion rates expressed as the percentage of Consumed Nitrogen (CN) tended to increase with the inclusion of CSO and CO and

the equal combination of both (Table 2). There were converse linear relationships between CN and daily urea nitrogen excretion rates expressed as the percentage of CN for individual tanks in dietary treatments CSO100 ($y = -0.002x + 9,402$; $n = 3$; $r^2 = 0.75$) and CO100 ($y = -0.000x + 4,873$; $n = 3$; $r^2 = 0.51$). However, weak positive linear relationships were observed between CN and daily urea-nitrogen excretions expressed as the percentage of CN for individual tanks in dietary treatments FO100 and CSO50/CO50 former being weaker than the latter. Theoretical calculations of daily Retained-N + Fecal-N for individual tanks in each dietary treatment were utilized to estimate the fate of the dietary nitrogen in the body of European sea bass. The calculations were made according to a formula indicated as:

$$\text{Retained-N} + \text{Fecal-N} = \text{CN} - (\text{TAN} + \text{urea-N})$$

Where Retained-N + Fecal-N represent the calculation of total hypothetical nitrogen retained in the body plus the nitrogen excreted in the fecal matter. CN represents daily consumed nitrogen whereas TAN and urea-N represent daily Total Ammonia Nitrogen and urea Nitrogen excretion rates in each tank of each dietary treatment, respectively.

Although, mean daily Retained-N + Fecal-N did not differ significantly among dietary treatments, fish fed diets in which the fish oil was replaced totally by CSO (1287 ± 168 mg N/kg/day) and CO (1242 ± 275 mg N/kg/day) appeared to have higher rates compared to that of the fish fed the dietary treatments FO100 and CSO50/CO50 (Table 2). The lowest rate was 955 ± 44 (mg N/kg/day) and it was calculated for the fish fed the dietary treatment CSO50/CO50 (Table 2). There were very strong positive linear relationships between the CN and daily Retained-N + Fecal-N for individual tanks in the dietary treatments FO100 ($y = 0.797x - 310.4$; $n = 3$; $r^2 = 1$) and CO100 ($y = 1.725x - 1881$; $n = 3$; $r^2 = 0.99$). The weakest positive linear relationship among the dietary treatments was observed in the fish fed the CSO50/CO50 diet ($y = 0.452x + 322.7$; $n = 3$; $r^2 = 0.18$).

This study demonstrated that the juvenile European sea bass utilized Cotton Seed Oil (CSO) and Canola Oils (CO) as energy sources effectively since no significant differences were observed in either TAN excretion rates or calculated Retained N + Fecal N values among fish fed different dietary treatments. However, it was evident that total fish oil replacement by CSO and/or CO increased daily urea-nitrogen excretion in sea bass and the daily urea-nitrogen expressed as percentage of Consumed Nitrogen (CN) were observed to be significantly higher in

fish fed CSO50/CO50. It also, appeared that TAN excretion rates expressed as percentage of Consumed Nitrogen (CN) in the VO fed fish were found to be appreciably lower compared to that of fish oil fed fish. The metabolic conversion of ammonia to urea is a strategy available to fish and it was demonstrated to be an important pathway for teleost species which are subjected to environmental stress such as air exposure, alkaline waters, high ambient ammonia levels and crowding (Korsgaard *et al.*, 1995). To the best of the knowledge, almost no information is available about the effects of fish oil replacement by VOs on total ammonia and urea nitrogen excretion rates in fish species, specifically carnivorous ones such as European sea bass. Since, daily feed intake of fish in dietary treatments was similar, lower daily TAN excretion rates in fish fed diets containing VOs may only be explained by the higher protein and energy retention efficiency considering the highest ambient ammonia levels measured during the experiment were much lower than the maximum safe level ($6.6 \text{ mg TAN l}^{-1}$) found for juvenile European sea bass eliminating the suppressing effect of ambient ammonia on excretion rates (Lemariea *et al.*, 2004). In this respect, three 8 h sampling period during which water flow to tanks was turned off seemed to be an appropriate methodology for the European sea bass, since the accumulation of ammonia to toxic levels did not occur during this period.

This methodology was also utilized in the measurement of nitrogenous waste excretion in the marine species that have similar environmental requirements to European sea bass such as California halibut, *Paralichthys californicus* Ayres (Merino *et al.*, 2007). However, increased urea-nitrogen excretion rates obtained on dietary treatments CSO100, CO100 and CSO50/CO50 may imply alteration to nitrogenous excretion mechanism in fish induced by dietary total fish oil replacement by cotton seed and/or canola oils in this study. In rats, it was demonstrated that dietary fish oil inclusion had protective effect on uranyl-nitrate induced nephrotoxicity and oxidative damage in kidney by significantly increasing the serum glucose, phosphate and phospholipids and decreasing blood urea nitrogen, urinary phosphate and protein excretions compared to dietary maize oil inclusion (Priyamvada *et al.*, 2010).

Although, the mechanism behind dietary VOs and their effects on physiology and particularly nitrogen metabolism is far from being clearly understood in fish, a recent investigation with sea bream (*Sparus aurata*) demonstrated that fish fed diets in which fish oil was totally or in partially replaced by linseed and/or soybean oils profoundly altered the head kidney fatty acid profiles

in return affecting the stress response of fish to crowding by increasing the plasma cortisol (in the case of LO) or by changing the pattern of plasma cortisol evolution after stress (in the case of SO) (Ganga *et al.*, 2011). Ganga *et al.* (2011) also postulated that the alteration of dietary n-3/n-6 ratio might be the definitive factor behind the stress response in fish. Jutfelt *et al.* (2007) also found that fish oil replacement by Sunflower Oil (SO) (21.5% replacement level on a DM basis) significantly increased plasma cortisol levels and acted as a chronic stressor in Atlantic salmon during parr-smolt transformation and subsequent sea water transfer suggesting the alteration of cell membrane phospholipid composition thereby reduction in the availability of precursors for eicosanoids as a result. Furthermore, these findings suggest that HUFA had an important role in modulating stress response in fish mediated partly by eicosanoids that are produced from C₂₀ HUFA in stressful situations (Montero *et al.*, 2003; Ganga *et al.*, 2011).

The *de novo* synthesis of urea is metabolically costly to the fish and requires an input of at least 2.5 ATP per molecule excreted (Korsgaard *et al.*, 1995). Apart from some air breathing fishes, a soda lake tilapia and toadfishes (*Opsanus* sp.), the OUC enzymes required for hepatic urea synthesis are rarely detected in most of the teleost fish and urea produced by these species is largely derived by catabolic routes from the degradation of purines via urate and arginine (Korsgaard *et al.*, 1995; Kajimura *et al.*, 2002; Tulli *et al.*, 2007). Although, urea excretion was previously thought to be a passive diffusion, it is now clear that branchial urea handling requires an energy consuming active transport system utilizing specialized Urea Transporters (UTs) (Mistry *et al.*, 2001; Wilkie, 2002). In fact a full length cDNA (~1.9 kb) coding for a special eel UT of 486 amino acid residues was isolated from seawater raised Japanese eel (*Anguilla japonica*) gill cDNA library (Mistry *et al.*, 2001). Therefore, increased urea-nitrogen excretion in the European sea bass fed dietary treatments CSO100, CO100 and CSO50/CO50 in this study suggested that fish detoxified some of the internal ammonia into urea and probably spent energy in excreting it through the branchial membranes employing UTs (Wright, 1995; Wilkie 2002). However, it appeared that higher detoxification of internal ammonia into urea was more evident in dietary treatments CSO100 and CSO50/CO50 since urea-nitrogen (CN) was higher in fish fed these diets than that of fish fed the other two dietary treatments. Previously measured long term growth rates using the same oil sources and dietary treatments in the juvenile European sea bass (~35.5 g) (Yimaz *et al.*, 2010) indicated that fish fed dietary treatments CSO100 and CSO50/CO50,

although not significantly different had lower growth rates compared to those obtained on fish fed FO100 and CO100 dietary treatments confirming the results found in this investigation.

Ammonia excretion rates are directly related to dietary protein levels and nitrogen intake in teleosts and increasing the dietary level of non-protein digestible energy increases nitrogen retention by decreasing nitrogen losses (Kaushik and Cowey, 1990; Medale *et al.*, 1995). The European sea bass remained ammonotelic throughout this investigation as the daily urea-nitrogen excretion varied between 3.9 and 5.4% of CN among fish fed different dietary treatments reiterating the previous excretion results in this species (Boujard *et al.*, 2004; Person-Le Ruyeta *et al.*, 2004; Dias *et al.*, 2005; Tulli *et al.*, 2007). Hourly TAN excretion rates began to increase in all the dietary treatments following feeding. However, the magnitude of peak excretion rates were appeared to be lower and delayed in the fish fed dietary treatments in which the fish oil was totally replaced by CSO, CO and CSO/CO compared to fish fed FO. No direct relationship has ever been established between the level of dietary plant oils and the nitrogenous excretion rates in fish nutrition previously.

But several researchers found that similar delay occurred in TAN excretion rates in several fish species when dietary fish meal was replaced not only by plant proteins but also the combinations of plant protein and oil sources providing the EAA (Essential Amino Acid) balance and requirements are met in dietary treatments (Medale *et al.*, 1998; Dias *et al.*, 2005; Engin and Carter, 2005; Benedito-Palos *et al.*, 2008; Pratoomyot *et al.*, 2010). This could be attributed to improved utilization of dietary protein through increased contribution of non-protein sources namely fat and digestible carbohydrates for energy provision (Pratoomyot *et al.*, 2010).

The graphical relationships between CN and daily TAN excretion for each dietary treatment in this study revealed a strong converse linear relationship in fish fed CO100 diet contrary to either strong or weaker positive relationships in other dietary treatments. This could indicate that some fatty acids such as the 16:0 (Palmitic Acid, PA), 18:1n9 (Oleic Acid, OA) and 18:2n6 (Linoleic Acid, LA) in the diets might have been selectively catabolized for β oxidation as these fatty acids have already been demonstrated to be readily used for both immediate energy production and storage in fish species including the European sea bass (Mourete and Bell, 2006; Jobling *et al.*, 2008).

It also appeared that predominant fatty acid classes of 18:1n9 and 18:2n6 in canola and cotton seed oils, respectively were effectively utilized since theoretically

calculated Retained N+Fecal N values for each dietary treatment, specifically in the dietary treatments CO100 and FO100 were found to be positive linearly correlated with the CN. Positive and strong converse linear relationships between CN and Retained N+Fecal N and TAN established, respectively for the dietary treatment CO100 may probably indicate the preference of 18:1n9 fatty acid class by the fish for energy production considering the lowest amount of 18:2n6 was existed among the dietary treatments except FO100. Similarly, Mourente and Bell (2006) reported that juvenile European sea bass (~5 g) fed a diet containing 14% higher Canola Oil (CO) in the VO blend replacing 60% of Fish Oil (FO) in all the dietary treatments had significantly higher live mass and liver mass compared to that of fish fed the diet containing lower amounts of CO indicating the better metabolism of 18:1n9, a dominant fatty acid class in the fatty acid composition of the canola oil for energy production and storage in the body.

Lower daily TAN and increased daily urea-N excretion rates obtained on the dietary treatments CSO100 and CSO50/CO50 compared to those obtained on diets FO100 and CO100 in this study might also indicate an impaired nitrogen metabolism and excretion mechanism in the juvenile European sea bass. This hypothesis could also be supported indirectly by the strong positive linear relationship observed between the daily CN and urea-N excretion rates in the dietary treatment CSO100. Although, it is difficult to shed a light merely relying on the nitrogenous excretion data, it appeared that fatty acid imbalance created by cotton seed and/or canola oil inclusion in dietary treatments might have interfered with the mechanism of ammonia excretion in the European sea bass fed specifically the dietary treatments CSO100 and CSO50/CO50.

The polar lipids are central components of tissue cell membranes and tended to be dominated by selectively incorporated certain fatty acids (16:0, 18:1n9, 20:5n3 and 22:6n3) (Jobling *et al.*, 2008). Therefore, low levels of EPA and DHA in the dietary treatment CSO100 and the absence of DHA in CSO50/CO50 diet combined with the lack of ability of European sea bass to desaturate and elongate LNA (Mourente and Dick, 2002) might have resulted in the decrement of these n-3 HUFA in gill cell membrane phospholipid composition jeopardizing the membrane fluidity and integrity (Hazel and Williams, 1990).

CONCLUSION

This investigation demonstrated that the daily TAN excretion rates tended to decrease in the juvenile

European sea bass fed balanced diets in which fish oil was totally replaced by either Cotton Seed Oil (CSO100) or Canola Oil (CO100) or the equal combination of these VOs (CSO50/CO50) suggesting an alteration to nitrogen excretion mechanism. Increased daily Urea-N excretion rates with the inclusion of cotton seed oil and/or canola oils in diets also indicated that European sea bass detoxified some of the internal ammonia into urea and this was particularly important in the dietary treatment CSO50/CO50, since the daily urea-N excretion expressed as percentage of Consumed Nitrogen (CN) was higher than that of other dietary treatments.

Furthermore, it appeared that fish utilized from both VOs effectively as an energy source but altered dietary n-3/n-6 ratio seemed to play a part in the nitrogenous excretion mechanism in the European sea bass. Further research into the effects of fish oil replacement by cotton seed oil and canola oil on gill cell membrane phospholipid composition and stress response and enzymes governing the nitrogen excretion and detoxification process combined with long term growth is needed in order to clarify the safe use of these VOs in diets of European sea bass.

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NOMENCLATURE

TAN	=	Total Ammonia Nitrogen
Vos	=	Vegetable oils
FO	=	Fish Oil
CSO	=	Cotton Seed Oil
CO	=	Canola Oil
LNA	=	Linolenic Acid
LA	=	Linoleic Acid
PUFA	=	Poly Unsaturated Fatty Acids
HUFA	=	Highly Unsaturated Fatty Acids
MUFA	=	Mono Unsaturated Fatty Acids
EPA	=	Eicosapentaenoic Acid
DHA	=	Docosahexaenoic Acid

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