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Postprandial Ammonia Excretion and Oxygen Consumption Rates in Olive Flounder *Paralichthys olivaceus* Fed Two Different Feed Types According to Water Temperature

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Abstract

Postprandial ammonia excretion and oxygen consumption in olive flounder *Paralichthys olivaceus* fed two different feed types, moist pellet (MP) and expanded pellet (EP) diets, to satiation were determined at 12°C, 15°C, 20°C, and 25°C for 48 h. The ammonia excretion and oxygen consumption rates increased with increasing water temperature. However, the postprandial times for the maximum rates of ammonia excretion and oxygen consumption were shortened from 12 h to 6 h after feeding with increasing water temperature. The ammonia excretion and oxygen consumption rates of fish fed EP were significantly higher ($P < 0.05$) than those fed MP at 12 h post-feeding both for 12°C and 15°C. The highest ($P < 0.05$) weight-specific ammonia excretion rates at 12°C were observed in fish fed EP and MP at 12.1 mg NH_3-N $kg^{-1}h^{-1}$ and 8.7 mg NH_3-N $kg^{-1}h^{-1}$, respectively, for 12 h and 9 h after feeding. The highest ($P < 0.05$) weight-specific oxygen consumption rates at 12°C were observed in fish fed EP and MP at 116.4 mg $kg^{-1}h^{-1}$ and 101.0 mg $kg^{-1}h^{-1}$, respectively, for 12 h after feeding. The highest ammonia excretion rates at 25°C in fish fed EP and MP increased to 16.9 mg NH_3-N $kg^{-1}h^{-1}$ and 18.3 mg NH_3-N $kg^{-1}h^{-1}$, respectively, for 6 h after feeding. The highest ($P <$ 0.05) weight-specific oxygen consumption rates at 25°C were observed in fish fed EP and MP at 184.3 mg O_2 $kg^{-1}h^{-1}$ and 197.3 mg O_2 $kg^{-1}h^{-1}$, respectively. These data are extremely valuable for the design of biofilters and development of effluent treatment technologies for land-based flounder farms.

Key words: *Paralichthys olivaceus*, Ammonia excretion, Oxygen consumption, Moist pellet, Expanded pellet, Metabolite

Introduction

Most marine teleosts are predominantly ammonotelic and 70–90% of nitrogen excretion is in the form of ammonia nitrogen (Randall and Wright, 1987; Dosdat et al., 1996). Ammonia nitrogen is harmful to fish and is considered to be a major factor limiting fish biomass and stocking densities in intensive culture systems (Cai and Summerfelt, 1992; Forsberg and Summerfelt, 1992; Lee et al., 2012). Benthic fish species excrete urea-N at higher rates compared to pelagic species to defend against ammonia toxicity (Engin and Carter, 2001; Smutna et al., 2002; Ip et al., 2004; Merino et al., 2007). Quantification of ammonia excretion and oxygen consumption is important for estimating optimum carrying capacities and designing biofilters in aquaculture systems.

The olive flounder *Paralichthys olivaceus* is the most important mariculture species in Korea. It has been cultured in land-based flow-through culture systems and fed with moist pellet (MP) and expanded pellet (EP) diets. However, there is a lack of information about ammonia excretion and oxygen consumption by olive flounder fed the two different diets with respect to seasonal culture temperatures. Based on coastal seawater temperatures, most flounder are fed in water temperatures from 12°C to 25°C. Water temperature is

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an important factor controlling fish stocking density in relation to ammonia nitrogen excretion and oxygen consumption rates (Paulson, 1980; Cai and Summerfelt, 1992; Forsberg and Summerfelt, 1992; Ruyet et al., 2004). Like olive flounder, most flatfish are cultured in land-based facilities and are stocked at high densities per unit of water volume or bottom area (Bjørnsson, 1994; Irwin et al., 1999; Bengtson et al., 2003). The high stocking densities usually require high water exchange rates to meet the oxygen needs and to carry out the nitrogenous metabolic byproducts, especially ammonia (Colt and Armstrong, 1981; Handy and Poxton, 1993; Tanaka and Kadowaki, 1995). Ammonia nitrogen excretion rates are directly related to dietary nitrogen and protein intake in fish (Rychly, 1980; Handy and Poxton, 1993; Wagner et al., 1995), and indirectly related to dietary nutrient compositions (Beamish and Thomas, 1984). Therefore, this study examined postprandial ammonia excretion and oxygen consumption rates in olive flounder as a function of water temperature and post-feeding period for the two different feed types, MP and EP, to provide useful data for the design and optimization of wastewater management technologies in land-based olive flounder culture systems.

Materials and Methods

Experimental Conditions

The experiment was carried out at the National Institution of Fisheries Research and Development (NIFS), Busan, Korea. Three hundred experimental fish (300 ± 100 g) were stocked in eight experimental tanks (fiber-reinforced plastic; FRP, 1.5 m^3). Duplicate tanks were maintained at each experimental temperature and acclimated for 3 weeks. The desired water temperatures (12°C, 15°C, 20°C, and 25°C) were maintained using heat pumps. At each temperature, fish were fed to satiation either the MP or EP diet at 09:00. Light was provided by overhead fluorescent lamps from 08:00 to 20:00. During the experimental period, salinity, pH, ammonia, and dissolved oxygen (DO) in stocking tanks were maintained at 32 ± 1 psu, 8.1 ± 0.2, <0.003 mg L^{-1}, and >7 mg L^{-1}, respectively.

Sampling and Analysis

Fish at each experimental temperature were fed to satiation either the MP or EP diet at 09:00 daily. Moist pellets (MP diet) were manufactured in the laboratory using frozen trash fish with a formulated powder compound. The MP diet contained 64.6% crude protein, 7.7% crude fat, and 12.9% crude ash. Expanded pellets (EP diet) were manufactured by SUHYUP Feed Co. Ltd., Korea. The EP diet contained 52.3% crude protein, 11.7% crude fat, and 11.4% crude ash. After feeding, three individual fish from each experimental tank were randomly selected and transported into separate 30- to 80-L water volume plastic bags filled with filtered (0.45 µm, Millipore) seawater of the same water temperature as their respective tanks. Water volumes were determined based on previous experiments that allowed fish and water quality detection with an ending effluent DO above 5 mg L^{-1} and 65% saturation, which exceeds the recommended limit for *Scophthalmus maximus* (Brown et al., 1984). Before stocking the fish, DO was measured using a DO meter (Handy Polaris, OxyGuard, Birkerød, Denmark) and water samples were collected to analyze initial ammonia nitrogen. The plastic bags were sealed and returned to the experimental tanks, which were maintained within ±0.2°C of the desired experimental temperature.

After 3, 6, 9, 12, 18, 24, 36, and 48 h, DO and ammonia nitrogen concentrations were determined immediately after the plastic bags were opened for the treatments and controls (without fish) for each water temperature. The fish in each container were then removed with a net, weighed, and returned to their experimental tanks. The water remaining in the plastic bags was homogenized and filtered through a 0.45-µm filter (Millipore) for the analysis of ammonia nitrogen. Ammonia nitrogen was determined according to Verbeeten et al. (1999) and Merino et al. (2007). At least 15 sample measurements for each temperature, diet, and post-feeding period were attempted.

Weight-specific ammonia excretion (mg NH_3-N $kg^{-1}h^{-1}$) and oxygen consumption (mg O_2 $kg^{-1}h^{-1}$) were calculated as: $\{(C_{Nf}\ (CC_{Ni})_t\ W_t^-\ CeC_{Nf}\ \ CC_{Ni})_c\}\cdot W\upsilon^{-1}$ and $\{(C_{Of}\ (CC_{Oi})_t\ W_t^-\ \ CdC_{Of}\ \ C_{Oi})_c\}\cdot W\upsilon^{-1}$, respectively, where C_{Nf} and C_{Ni} are the ammonia concentrations at the end and the beginning of the experiment, respectively, and C_{Of} and C_{Oi} are the oxygen concentrations at the end and the beginning of the experiment, respectively. W_t is the fish weight (kg), $W\upsilon$ is the water volume (L), and the subscripts t and c stand for treatment and control, which included and did not include fish, respectively.

Statistical Analysis

The data on ammonia excretion and oxygen consumption levels are expressed as means ±hSD; $n = 15$ for each temperature, diet, and post-feeding period. Effects of post-feeding period and water temperature on ammonia excretion and oxygen consumption were tested using a two-way nested ANOVA. Significant ANOVAs were followed by a Student-Newman-Keuls multiple comparison test to identify differences among treatments. The relationships between ammonia excretion rate and post-feeding period and oxygen consumption rate and post-feeding period were described by the linear regression equation y = a + bx, where y is the ammonia excretion rate or oxygen consumption rate and x is the post-feeding period for two temperatures, depending on the computed values of R^2 and the significance of the regression parameters. Signifi-

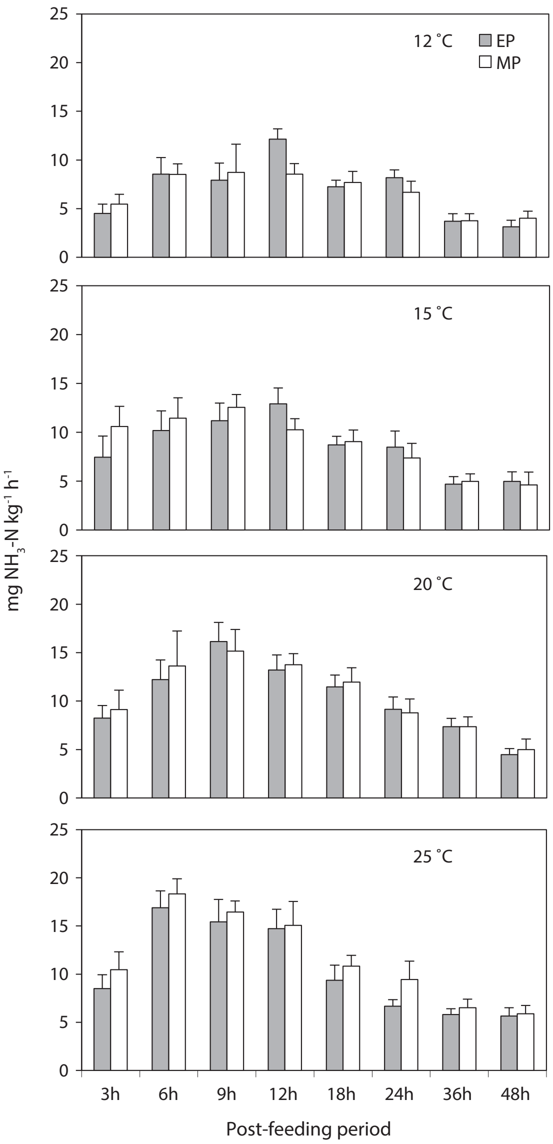

Fig. 1. Weight-specific ammonia (NH_3-N) excretion by olive flounder *Paralichthys olivaceus* fed expanded pellet (EP) and moist pellet (MP) for the variables of water temperatures and post-feeding periods. Values are means ± SD (n = 15) for each treatment.

cance was accepted at $P < 0.05$. All statistical analyses were conducted using the SAS program (SAS Institute Inc., Cary, North Carolina, USA).

Results

Postprandial Ammonia Excretion

Weight-specific ammonia excretion with respect to diet, temperature, and post-feeding period is presented in Fig. 1. At 1–48 h post-feeding, the excretion patterns were similar at 12 °C and 15°C, which exhibited lower excretion rates and a slower rate of decline. The peak hourly excretion rates of the fish fed the EP and MP diets were three to four times higher than those of the resting metabolite rates at 36–48 h for all temperatures. The peak hourly excretion rates of the fish fed EP occurred at 12 h post-feeding for 12°C and 15°C, and were significantly greater than those of fish fed MP ($P < 0.05$). However, the peak excretion rates of the fish fed MP occurred at 9 h post-feeding from 12°C to 20°C. Approximately 74%, 78%, 80%, and 84% of the 24-h post-feeding ammonia excretions of the fish fed EP were excreted within 18 h post-feeding at 12°C, 15°C, 20 C, and 25°C, respectively. However, approximately 78%, 81%, 81%, and 81% of the 24-h post-feeding ammonia excretions of the fish fed MP were excreted within 18 h post-feeding at 12°C, 15°C, 20°C, and 25°C, respectively. The highest weight-specific hourly excretion rates of 16.9 ± 1.73 mg NH_3-N $kg^{-1}h^{-1}$ and 18.3 ± 1.57 mg NH_3-N $kg^{-1}h^{-1}$ for the fish fed EP and MP, respectively, were detected at 25°C within 6 h post-feeding.

The regression between mass-specific ammonia excretion (Am) and post-feeding period (pf) was diphasic at the 9-h and 6-h post-feeding points for 20°C and 25°C, respectively. Regression lines of EP and MP for the 20°C and 25°C temperature treatments were: Am $(EP)_{20°C} = 4.29 + 3.953$ pf ($R^2 = 0.998$, $P < 0.01$), Am $(MP)_{20°C} = 6.59 + 3.02$ pf ($R^2 = 0.925$, $P < 0.01$) and Am $(EP)_{25°C} = 0.09 + 8.4$ pf ($R^2 = 0.999$, $P < 0.01$), Am $(MP)_{25\ C} = 2.57 + 7.88$ pf ($R^2 = 0.998$, $P < 0.01$) in the first phase at the 3–9-h and 3–6-h post-feeding periods, respectively, and Am $(EP)_{20°C} = 22.1 – 2.16$ pf ($R^2 = 0.9928$, $P < 0.001$), Am $(MP)_{20°C} = 22.66 – 2.21$ pf ($R^2 = 0.989$, $P < 0.001$) and Am $(EP)_{25°C} = 21.93 – 2.24$ pf ($R^2 = 0.886$, $P < 0.001$), Am $(MP)_{25°C} = 23.23 – 2.28$ pf ($R^2 = 0.965$, $P < 0.001$) in the second phase at the 12–48-h and 9–48-h post-feeding periods, respectively.

Postprandial Oxygen Consumption

Weight-specific hourly oxygen consumption with respect to diet, temperature, and post-feeding period is presented in Fig. 2. Mean hourly oxygen consumption rates at 25°C were significantly ($P < 0.05$) higher than at other temperatures. The peak hourly consumption rates occurred at 12 h and 6 h post-feeding for 12–15°C and 20–25°C, respectively. The peak hourly consumption rates of the fish fed the EP and MP diets were less than two times higher than those of the resting metabolic rates at 36 to 48 h for all temperatures.

The hourly oxygen consumption rates of fish fed the EP and MP diets did not significantly differ ($P > 0.05$) according to post-feeding period for all temperatures. Oxygen consumption patterns and rates were similar between EP and MP. Approximately 75–77% and 76–79% of the 24-h post-feeding oxygen consumption of fish fed EP and MP, respectively, occurred within 18 h post-feeding at 12–25°C. The highest weight-specific hourly consumption rates of 184.3 ± 25.14 mg O_2 $kg^{-1}h^{-1}$ and 197.3 ± 19.39 mg O_2 $kg^{-1}h^{-1}$ for fish

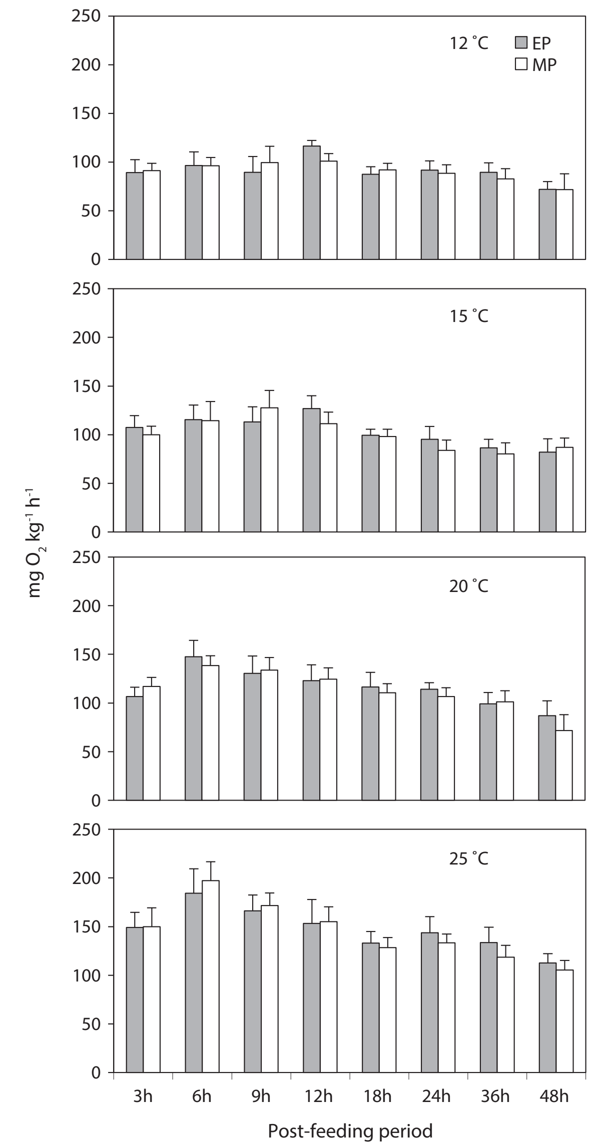

Fig. 2. Weight-specific oxygen consumption by olive flounder *Paralichthys olivaceus* fed expanded pellet (EP) and moist pellet (MP) for the variables of water temperatures and post-feeding periods. Values are means ± SD (n = 15) for each treatment.

fed EP and MP, respectively, were detected at 25°C within 6 h post-feeding.

The regression between mass-specific oxygen consumption (OC) and post-feeding period (pf) was diphasic at the 6-h post-feeding point for 20°C and 25°C. Regression lines for EP and MP for the 20°C and 25°C temperature treatments were: $OC(EP)_{20°C}$ = 66.07 + 40.65 pf (R^2 = 0.999, $P < 0.01$), $OC(MP)_{20°C}$ = 95.56 + 21.45 pf (R^2 = 0.998, $P < 0.01$), $OC(EP)_{25°C}$ = 113.96 + 35.19 pf (R^2 = 0.998, $P < 0.01$), $OC(MP)_{25°C}$ = 102.35 + 47.48 pf (R^2 = 0.999, $P < 0.01$) in the first phase at the 3–6-h post-feeding period, and $OC(EP)_{20°C}$ = 157.53 – 8.34 pf (R^2 = 0.949, $P < 0.01$), $OC(MP)_{20°C}$ = 168.28 – 10.96 pf (R^2 = 0.9142, $P < 0.01$), $OC(EP)_{25°C}$ = 190.06 – 9.03 pf (R^2 = 0.835, $P < 0.01$), $OC(MP)_{25°C}$ = 203.82 – 12.435 pf (R^2 = 0.9228, $P < 0.01$) in the second phase at the 9–48-h post-feeding period.

Discussion

Postprandial Ammonia Excretion

Postprandial ammonia excretion of *P. olivaceus* exhibited a mean hourly excretion rate between 4.5 and 18.3 mg NH_3-N kg^{-1} h^{-1}. This is consistent with the mean rates for fed flatfish of 5.1 NH_3-N kg^{-1} h^{-1} for Atlantic halibut *Hippoglossus hippoglossus* (Davenport et al., 1990), 2.7 to 30 NH_3-N kg^{-1} h^{-1} for *Pleuronectes platessa* (Jobling, 1981), 2.9 to 10.2 NH_3-N kg^{-1} h^{-1} for *S. maximus* (Burel et al., 1996; Dosdat et al., 1995, 1996), 3.4 to 4.3 NH_3-N kg^{-1} h^{-1} for *P. platessa* (Jobling, 1981), and 5.4 to 21.9 NH_3-N kg^{-1} h^{-1} for *P. olivaceus* (Kikuchi et al., 1991, 1995). Ammonia excretion rates and patterns are also affected by water temperature, feeding rate, and feeding frequency. *P. olivaceus* fed once a day exhibited an immediate increase after feeding that was four times that of 48 h post-feeding. This is consistent with the results of Kikuchi et al. (1991). *S. maximus* and *Oncorhynchus mykiss* fed twice a day had two peak excretions 6 h after being fed (Dosdat et al., 1995; Bergero et al., 2001). *Paralichthys californicus* fed continuously between 09:00 and 21:00 h exhibited a relatively constant excretion rate throughout the day (Merino et al., 2007). In the present work *P. olivaceus* were fed 1–1.5% of their body weight once a day at 09:00 h, the excretion rates and patterns correlated with water temperature. The individual feeding rates were not precisely determined in the present study. Biological estimation from digestibility, nutrient accumulation, and ammonia excretion in relation to water temperature and feeding rate will explain which temperature is energy effective for olive flounder culture. Ammonia excretion and energy budget are usually temperature dependent (Finn et al., 2002; Sun and Chen, 2009), as is generally the case in fish. In warm-water species, ammonia excretion usually increases with temperature (Claireaux and Lagardère, 1999; Ruyet et al., 2004). In the present study, the mean values of postprandial weight-specific ammonia excretions were higher at 25°C than at other water temperatures. This might be due to the feeding rate and higher metabolism. At higher water temperatures, the postprandial weight-specific ammonia excretions were higher until 48-h post-feeding. This is notable in regards to energy budget and water quality management. Olive flounder is a warm-water species and feeding rate and ammonia excretion might be affected by external temperature. The higher resting ammonia excretions at 25°C compared to other water temperatures might not be sufficient for a higher feed conversion ratio and might lead to weight loss and elevated maintenance costs.

Postprandial Oxygen Consumption

In both warm- and cold-water fish species, oxygen consumption usually increases with temperature within a limited temperature zone (Saunders, 1963; Paul, 1986; Lee et al., 2012). Postprandial oxygen consumption of *P. olivaceus* exhibited a mean hourly consumption rate between 71.8 and 197.3 mg O_2 $kg^{-1}h^{-1}$. There is lack of data to compare the postprandial oxygen consumption of flatfish species. Oxygen consumption rates and patterns are affected by water temperature, feeding rate, and feeding frequency. *P. olivaceus* fed once a day showed immediate increases in oxygen consumption after feeding that were 1.3–1.4 and 1.7–1.9 times those of 48 h post-feeding. This is consistent with the results (1.2–1.5 times) of Merino et al. (2011) for *P. californicus*. Like ammonia excretion, higher oxygen consumption rates were detected at 25°C for all post-feeding periods. This might due to the effects of higher water temperature on activity and resting metabolism. Significantly higher oxygen consumption at 25°C compared to other temperatures at 24-h post-feeding might have resulted from elevated maintenance costs.

The present study suggests that the postprandial ammonia excretion and oxygen consumption of *P. olivaceus* are comparable to those of other flatfish. The magnitudes of ammonia excretion and oxygen consumption are important for fish health and for adequate biofilter operation and sizing.

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