# 生餌および配合飼料で養成したクロマグロ幼魚の肝臓および背側普通筋における遊離アミノ酸組成の違い

*Aquacult. Sci.* **69**(3), 203－211 (2021)

# Differences in free amino acid compositions in liver and dorsal white muscle of juvenile Pacific bluefin tuna *Thunnus orientalis* fed raw fish and artificial feeds

Takeshi YAMAMOTO[1,*], Hiroshi HASHIMOTO[2], Koji MURASHITA[1], Hiroyuki MATSUNARI[1], Toshinori TAKASHI[3], Takeshi EBA[2] and Kazunori KUMON[2]

**Abstract:** To develop a more efficient tuna feed, free amino acid compositions in the liver and dorsal white muscle of juvenile Pacific bluefin tuna *Thunnus orientalis* fed raw fish (RF) and commercial artificial feeds (AF) were compared. Hatchery-reared juveniles (initial BW, 2 g) stocked in two net cages were fed either of the foods from late July to mid-March, and fish samples for analysis were taken monthly. In the liver, concentrations of taurine were higher in the AF group, but increased in both groups toward autumn while concentrations of other amino acids decreased. In the muscle, histidine was dominant and its concentrations were higher in the RF group whereas concentrations of anserine and carnosine were higher in the AF group. In both the liver and muscle, levels of moisture were higher and those of crude fat lower in the AF group. Daily food intake (% monthly average BW/day) in the AF group was estimated to be as high as in the RF group. These findings suggest that dietary lipid utilization was not efficient in tuna fed the artificial feeds and instead dietary protein was used as an energy source, which resulted in the differences in muscle free imidazole-compound compositions.

**Key words:** Diet; Lipid deposition; *Thunnus orientalis*; Tissue free amino acids

For sustainable aquaculture practices, it is inevitable to use feed ingredients alternative to fishmeal and fish oil so as not to threaten food for natural stocks as well as the environment (Cashion et al. 2017; Food and Agriculture Organization 2020). In the last several decades, much progress has been achieved in the use of alternative ingredients in fish feed (Daniel 2018). However, tuna aquaculture has been relying on wild-caught raw fish due to satisfactory artificial tuna feed still being under development (Ottolenghi 2008; Mourente and Tocher 2009). Current artificial tuna feeds consist largely of fishmeal and fish oil, but nevertheless the growth performance of Pacific bluefin tuna *Thunnus orientalis* is inferior to fish fed raw fish (Kondo et al. 2016). Development of satisfactory tuna feeds based on fishmeal and fish oil will be the first step to put future sustainable tuna feeds based on alternative ingredients into practice.

Utilization of nutrients from ingested food largely depends on digestion and absorption (Rust 2002). Kondo et al. (2016) found differences in the digestive enzyme activity as well as the morphology of digestive organs between Pacific bluefin tuna fed raw fish and an extruded pellet feed. Recently Murashita

Received 2 March 2021; Accepted 20 July 2021.
[1] Physiological Function Division, Aquaculture Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 224-1 Hiruda, Tamaki, Mie 519-0423, Japan.
[2] Amami Field Station, Tuna Aquaculture Division, Aquaculture Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 955-5 Sakiyamahara, Oshima, Kagoshima 894-2414, Japan.
[3] Nagasaki Field Station, Tuna Aquaculture Division, Aquaculture Research Department, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 1551-8, Taira, Nagasaki 851-2213, Japan.
[*] Corresponding author: Tel, (+81) 596-58-6411; Fax, (+81) 596-58-6413; E-mail, takejpn@affrc.go.jp (T. Yamamoto).

et al. (2021) detailed the differences in the digestive physiology of Pacific bluefin tuna fed raw fish and artificial feeds, and concluded that the changes in digestive physiology of tuna fed artificial feed could be one of the causative factors of their reduced growth performances.

Free amino acid compositions in tissues are the result of dietary supply and internal metabolism (Dabrowski and Guderley 2002), and are known to reflect the dietary amino acid composition and in certain cases the dietary amino acid insufficiency (Wilson 2002). In the liver, taurine is involved in bile acid conjugation and bile pigment excretion (Hagey et al. 2010; Salze and Davis 2015). In addition, taurine concentrations in fish tissues including the liver are suggested to be a good indicator for determining dietary taurine sufficiency (Qi et al. 2012). On the other hand, free histidine and its related di-peptides such as anserine and carnosine in fish muscle, are involved in the buffering of protons that are produced by exercise (Abe 2000). Murai et al. (1982) reported that the taurine and free histidine concentrations in the liver and muscle of wild Pacific bluefin tuna were higher than those in the tuna cultured on raw fish. In addition, muscle free amino acid pool mostly consists of free histidine, anserine and carnosine in migratory fish species including tunas (Suyama and Yoshizawa 1973).

To develop a more suitable tuna feed, this work aims at examining the effect of different foods for Pacific bluefin tuna, raw fish and artificial formulated feed, on the nutritional physiology, especially the free amino acid pools of the liver and white muscle. The samples were obtained at intervals of approximately one month from fish (initial body weight, 2 g) fed either of raw fish or artificial feeds over 7 months.

## Materials and methods

*Rearing and sampling of tuna in marine net cages*

Details of the tuna rearing and sample collection were described in Murashita et al. (2021). In brief, hatchery-reared Pacific bluefin tuna juveniles {ca. 2 g body weight (BW), 3,200 individuals/cage} which had been fed a commercial tuna feed (company name undisclosed) were transferred to two net cages (20 m diameter) at the Amami Field Station, Fisheries Technology Institute (Oshima, Kagoshima, Japan) on July 30, 2018. Then, raw fish (anchovy, *Engraulis japonicus* and sand lance, *Ammodytes personatus*) or commercial tuna feeds, the nutrient compositions of which are presented in Table 1, were supplied to fish until visual satiety two to six times a day depending on fish growth, every day until September 8 and then five days a week, for 225 days until March 13, 2019. The kind of raw fish and the size of artificial feed were changed as fish grew (Table 1). Average monthly water temperature increased from July (27.2°C) to September (28.0°C), and then gradually decreased toward February (21.9°C) (Table 2). Each month, 6 to 10 individuals from each cage were taken by fishing using an artificial bait, and the liver and from October, a portion of the dorsal white muscle (just below the dorsal fin) were sampled and stored at −20°C until analysis. The initial fish samples were pooled to one ($n$ = 1), and thereafter 3 to 6 samples from fish having intermediate condition factors (BW/fork length$^3$) within each treatment at each sampling point were selected and individually subjected to the analysis (Table 2). Before sample collection, fish were deprived of food for 15 to 16 h until the September sampling and for 3 days thereafter, to ensure that the gastro-intestinal content was completely evacuated (Murashita et al. 2021).

*Apparent nutrient digestibility trial*

To understand the digestibility of nutrients in fishmeal and fish oil-based artificial feeds, feeding trials for fecal collection were conducted using another juvenile tuna. Enzyme-treated fishmeal and fish oil-based test diets (patent pending) were prepared using a meat grinder (1.2 mm diameter). Yttrium (Y) oxide was used as an inert marker. Juveniles (ca. 2 g BW) of Pacific bluefin tuna

**Table 1.** Contents of selected nutrients (%, dry matter basis) of raw fish and artificial feeds fed to juvenile Pacific bluefin tuna in net cages

| | Raw fish | | | Artificial feeds | |
|---|---|---|---|---|---|
| | Anchovy (young) | Sand lance (small) | Sand lance (medium) | Starter[a] | Grow-up[b] |
| Day offered | 2-21[c] | 21-81 | 81-225 | 1-72 | 73-225 |
| Crude protein (%) | 73.1 | 65.7 | 66.0 | 63.9 ± 6.4 | 52.9 ± 1.8 |
| Total lipid (%) | 12.0 | 23.4 | 24.0 | 23.7 ± 1.8 | 28.2 ± 3.1 |
| Taurine (%) | 0.8 | 0.8 | 0.8 | 1.7 ± 0.5 | 0.7 ± 0.0 |
| Total histidine (%) | 1.4 | 1.6 | 1.4 | 1.6 ± 0.1 | 1.3 ± 0.1 |

[a]Values are the mean and SD of 5 starter feeds with different sizes.
[b]Values are the mean and SD of 4 grow-up feeds with different sizes.
[c]Starter feeds were also offered during day 1 to 5.

**Table 2.** Water temperature, and body weight and condition factor of sampled fish subjected to analysis

| Month | WT (°C)[a] | Raw fish | | Artificial feeds | |
|---|---|---|---|---|---|
| | | BW (kg)[b] | CF[b, c] | BW (kg)[b] | CF[b, c] |
| August | 27.8 ± 0.5 | 0.10 ± 0.01 | 17.6 ± 0.2 | 0.04 ± 0.01 | 14.6 ± 0.3 |
| September | 28.0 ± 0.7 | 0.70 ± 0.04 | 21.0 ± 0.3 | 0.24 ± 0.03 | 16.5 ± 0.9 |
| October | 26.0 ± 0.4 | 1.06 ± 0.12 | 22.6 ± 1.2 | 0.46 ± 0.12 | 18.8 ± 0.9 |
| November | 24.7 ± 0.3 | 1.64 ± 0.19 | 23.7 ± 0.6 | 0.74 ± 0.16 | 19.6 ± 0.9 |
| December | 23.8 ± 0.4 | 2.13 ± 0.34 | 24.3 ± 0.5 | 0.91 ± 0.06 | 20.0 ± 0.5 |
| January | 22.4 ± 0.5 | 2.95 ± 0.58 | 24.5 ± 0.8 | 1.41 ± 0.19 | 21.8 ± 0.4 |
| February | 21.9 ± 0.4 | 3.13 ± 0.69 | 25.5 ± 0.9 | 1.51 ± 0.17 | 20.7 ± 0.4 |
| March | 22.2 ± 0.3 | 4.74 ± 0.14 | 25.8 ± 2.1 | 1.61 ± 0.26 | 21.5 ± 0.6 |

*WT*, water temperature; *BW*, body weight; *CF*, condition factor
[a]Values are the mean and SD from the last sampling date.
[b]Values are the mean and SD ($n$ = 3 to 6).
[c]CF = BW (kg)/fork length (m)$^3$

hatchery-reared at the Nagasaki Prefectural Institute of Fisheries (Nagasaki, Japan) were transferred to tanks designed for fecal collection (1.5 k*l*, Watanabe et al. 1992) and fed the test diets to apparent satiation 4 times daily for 10 days (100 fish/tank in Trial 1 and 120 fish/tank in Trial 2). Based on the results in Trial 1 (low lipid digestibility) using only a low lipid diet, a high lipid diet was also prepared and tested in Trial 2. Sand-filtered sea water was supplied at a rate of 3 *l*/min in Trial 1 and 2 *l*/min in Trial 2. Uneaten feed pellets were drained out before the fecal collection and dead fish were removed if they were found. Feces were collected from the 5th day, pooled by treatment and freeze-dried prior to chemical analysis. The fecal collection was done without replication. The water temperature during the trials were 26.3 ± 0.4℃ in Trial 1 and 24.3 ± 0.5℃ in Trial 2. Apparent nutrient digestibility (absorption) was calculated using the following formula.

Apparent nutrient digestibility/absorption (%) = 100*(1 − Y in diet/Y in feces*Nutrient in feces/Nutrient in diet)

*Analytical methods*

Samples of raw fish and artificial feeds supplied to the caged-fish were subjected to analyses of proximate composition (moisture, crude protein, total lipid) and free and total (hydrolyzed) amino acid compositions. Samples of the liver and dorsal white muscle of tuna were subjected to free amino acid analysis, and if sufficient amounts of samples were left, to moisture and crude fat analyses. The samples of test diets and feces obtained in the digestibility trials were analyzed for proximate composition (moisture, crude protein, total lipid) and mineral composition (yttrium and phosphorus).

Chemical analyses were conducted as described in Yamamoto et al. (2020). In brief, moisture was determined by drying the samples at 110℃ for 10 h, crude protein by

the semi-micro Kjeldahl method ($N \times 6.25$), total lipid by chloroform and methanol (2 : 1) extraction, and crude fat by ether extraction. Amino acid composition was determined using an amino acid analyzer L-8800 (Hitachi High-Tech, Tokyo, Japan) after the samples were extracted in 0.6 N perchloric acid for free amino acids and vacuum-hydrolyzed in 6 N HCl at 110℃ for 22 h for total amino acids. Mineral composition was determined using inductively-coupled plasma emission spectroscopy ICPE-9000 (Shimadzu, Kyoto, Japan) after nitric acid digestion.

Nutrient content data determined for fish fed raw fish and artificial feeds at each sampling point were compared by Student's *t*-test using Microsoft Excel. A probability level of less than 0.05 was considered significant.

## Results

Contents of selected nutrients in the raw fish and artificial feeds are given in Table 1. Although the crude protein contents decreased as the size of pellet increased (results not shown), the crude protein contents of raw fish were higher than the artificial feeds. The total lipid content of young anchovy was lower than the starter feeds, but the total lipid content of small sand lance was similar to the starter feeds, both of which were offered to fish during similar periods. The total lipid content of medium sand lance was slightly lower than the grow-up feeds. The taurine contents in the starter feeds were higher than the raw fish, while those in the raw fish and grow-up feeds were similar. Total histidine contents were similar between the raw fish and artificial feeds.

Free amino acid concentrations in the liver are summarized in Fig. 1. Since taurine was dominant (16–54% of total free amino acids), followed by glutamic acid (12–17%) and alanine (8–18%), Fig. 1 shows the concentrations of taurine, the sum of glutamic acid, glutamine, glycine and alanine (AQEG), and the sum of basic amino acids (lysine, histidine, arginine). At the start and one month later, the concentrations of AQEG in both treatments were higher than taurine, while thereafter, the concentrations of AQEG decreased and those of taurine increased. The concentrations of taurine in the AF group became significantly higher ($P < 0.05$) than those in the RF group from September. The concentrations of basic amino acids followed similar changes as observed for AQEG.

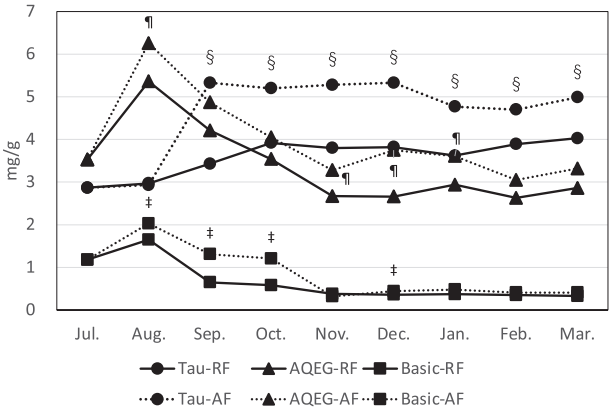


**Fig. 1.** Concentrations of major free amino acids in the liver of juvenile Pacific bluefin tuna fed raw fish (RF) and artificial feeds (AF)
*Tau, taurine; AQEG, sum of glutamic acid, glutamine, glycine and alanine; Basic, sum of lysine, histidine and arginine*
Values are means ($n = 3$ to 6; at initial, $n = 1$). Values with a symbol character (§, Tau; ¶, AQEG; ‡, Basic) indicate significant differences between the fish groups.

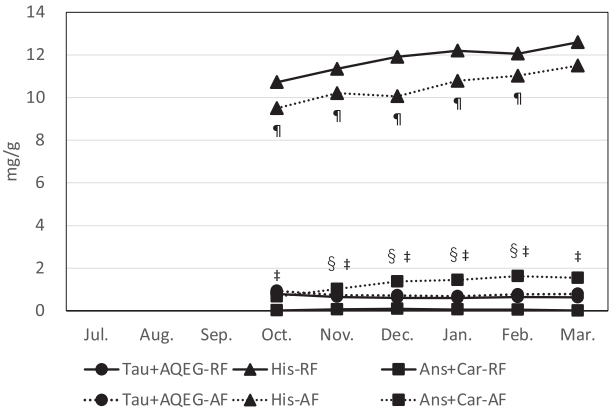


**Fig. 2.** Concentrations of major free amino acids in the white muscle of juvenile Pacific bluefin tuna fed raw fish (RF) and artificial feeds (AF)
*Tau+AQEG, sum of taurine, glutamic acid, glutamine, glycine and alanine; His, histidine; Ans+Car, sum of anserine and carnosine*
Values are means ($n = 3$ to 6). Values with a symbol character (§, Tau+AQEG; ¶, His; ‡, Ans+Car) indicate significant differences between the fish groups.

Free amino acid concentrations in the dorsal white muscle are summarized in Fig. 2. Unlike in the liver, taurine was a minor component (1–3%) and histidine was dominant (74–87%). So, Fig. 2 shows the concentration of the sum of taurine and AQEG, histidine, and the sum of anserine and carnosine. The concentration of histidine gradually increased towards the end of the feeding trial, and was always higher ($P < 0.01$) in the RF group than in the AF group except in March. The concentrations of the sums of anserine and carnosine followed similar increasing trends as observed in histidine, but always significantly higher ($P < 0.01$) in the AF group than in the RF group: approximately 90% was anserine (results not shown). The concentrations of the sum of taurine and AQEG were at low levels and similar between the groups, but the values from November to February were significantly higher ($P < 0.05$) in the AF group.

Contents of moisture, crude fat and total free amino acids including taurine, anserine and carnosine in the liver and dorsal white muscle are presented in Figs. 3 and 4, respectively. In the liver, the levels of moisture ($P < 0.01$) and total free amino acids ($P < 0.05$) of fish fed AF were always higher relative to fish fed RF, except for the total free amino acid level in March (Fig. 3). The liver crude fat levels of fish fed RF increased from September to November, while such a clear increasing trend was not observed in fish fed AF and the crude fat levels were always lower ($P < 0.01$) than fish fed RF. The moisture and crude fat levels showed mirrored changes. In the dorsal white muscle, similar trends as found in the liver were observed in the contents of moisture and crude fat (Fig. 4), while the levels of total free amino acids between the treatments were similar except in November, and gradually increased toward March.

Nutrient contents in the test diets and apparent nutrient digestibility/absorption values estimated in the fecal collection trials are given in Table 3. Apparent crude protein and total lipid digestibility values and apparent phosphorus absorption values of the low lipid diets were similar between the two trials. Compared to the low lipid diets, apparent digestibility values of a high lipid diet were lower by 3–4% for crude protein and 10–11% for total lipid, whereas apparent phosphorus absorption value was 5–6% higher.

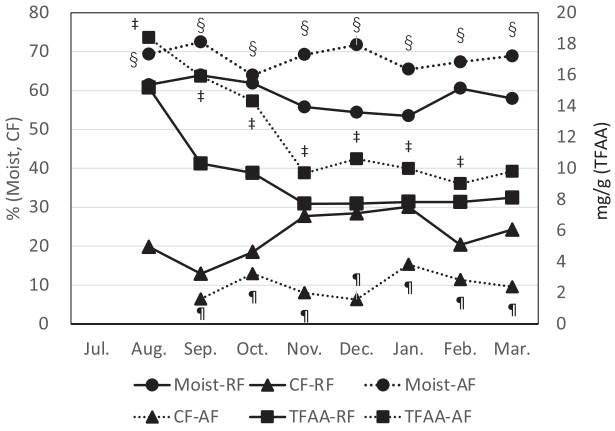


**Fig. 3.** Contents of moisture, crude fat and total free amino acids in the liver of juvenile Pacific bluefin tuna fed raw fish (RF) and artificial feeds (AF)
*Moist, moisture; CF, crude fat; TFAA, sum of free amino acids including taurine, anserine and carnosine*
Values are means ($n = 3$ to 6). Values with a symbol character (§, Moist; ¶, CF; ‡, TFAA) indicate significant differences between the fish groups. Crude fat of fish fed AF in August was not analyzed due to the limited amount of sample.

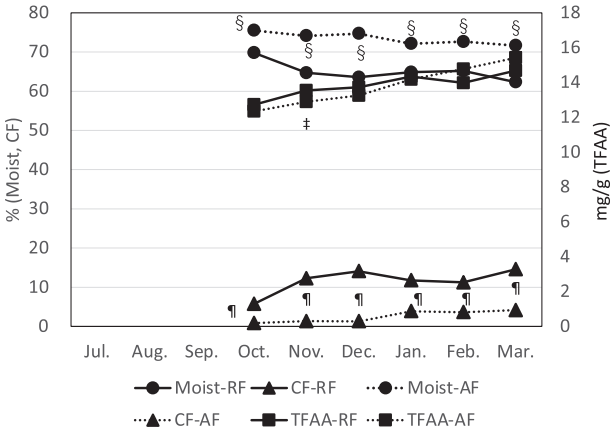


**Fig. 4.** Contents of moisture, crude fat and total free amino acids in the white muscle of juvenile Pacific bluefin tuna fed raw fish (RF) and artificial feeds (AF)
*Moist, moisture; CF, crude fat; TFAA, sum of free amino acid including taurine, anserine and carnosine*
Values are means ($n = 3$ to 6). Values with a symbol character (§, Moist; ¶, CF; ‡, TFAA) indicate significant differences between the fish groups.

**Table 3.** Contents of dietary nutrients (%, dry matter basis) and apparent nutrient digestibility/absorption (%) of juvenile Pacific bluefin tuna fed the test diets in the digestibility trials

| | Trial 1 | Trial 2 | |
|---|---|---|---|
| Diet type | Low lipid | Low lipid | High lipid |
| Dietary nutrients (%, dry matter basis) | | | |
| Crude protein | 68.8 | 67.9 | 53.5 |
| Total lipid | 16.8 | 16.6 | 25.6 |
| Phosphorus | 2.5 | 2.6 | 2.3 |
| Apparent digestibility/absorption (%)[a] | | | |
| Crude protein | 95.0 | 94.2 | 91.0 |
| Total lipid | 78.2 | 76.9 | 66.8 |
| Phosphorus | 35.4 | 35.8 | 41.2 |

[a] Values are the result of a pooled sample from a single tank ($n = 1$).

## Discussion

In this study, the taurine concentration in the liver of juvenile Pacific bluefin tuna increased from July to September (AF group) or to October (RF group), while the concentrations of other free amino acids decreased concomitantly (Fig. 1). Such a mirrored pattern between tissue taurine and free amino acids has been observed in other fish species (Matsunari et al. 2008a, b) and considered to be a physiological response to maintain the size of a tissue free amino acid pool for osmoregulation (Matsunari et al. 2008a). The higher levels of liver taurine concentrations in the AF group from September are apparently due to the higher levels of taurine in the starter feeds offered to fish until October 11. After the artificial feed was switched to the grow-up feeds, the taurine levels of which were similar to those in the raw fish, the differences in the liver taurine concentrations between the fish groups became smaller. By contrast, the concentrations of taurine in the dorsal white muscle were at very low levels (1.3–2.8% of total free amino acids) regardless of the treatments. In turn, free histidine was the dominant amino acid in the white muscle (Fig. 2) and the proportions of this amino acid in the total free amino acids in the AF group (75–79%) were lower ($P < 0.01$) than those in the RF group (84–87%), despite the fact that dietary total histidine levels in RF and AF were similar. Meanwhile, anserine and carnosine, both of which are histidine-related di-peptides, were found at higher concentrations in the AF group than the RF group.

In the white muscle of other kinds of tunas caught in the wild, appreciable amounts of anserine together with free histidine were found: the proportion of anserine to histidine was 110% in big-eye tuna *T. obesus*, 115% in southern bluefin tuna *T. maccoyii*, and 19% in yellowfin tuna *T. albacares* (Suyama and Yoshizawa 1973). On the other hand, in the dorsal white muscle of wild-caught Pacific bluefin tuna (735 g BW) had only a small amount of carnosine (2% of free histidine) without anserine being detected, which was also similar in the tuna reared on raw fish (552 g BW) (Murai et al. 1982). Later, Hirasawa et al. (1984) found in Pacific bluefin tuna cultured on raw fish that the white muscle anserine concentrations of large fish (33 kg BW) were higher than small fish (1.5 kg BW) and concluded that Pacific bluefin tuna accumulate anserine in the muscle as they grow. In yellowtail *Seriola quinqueradiata*, a similar change in the muscle anserine concentration with fish growth was suggested (Yamamoto et al. 2019).

In the dorsal white muscle of Pacific bluefin tuna reared on RF in the present study, however, the muscle anserine and carnosine concentrations remained at low levels from October (average BW, 1.0 kg) to March (4.7 kg). This suggests that the muscle anserine concentration in Pacific bluefin tuna does not solely depend on fish size. On the other hand, the muscle anserine and carnosine concentrations of Pacific bluefin tuna fed AF increased as they grew, which corresponded with the increase of free histidine concentrations (Fig. 2). In addition, the ratio of the sum of anserine and carnosine to histidine in fish fed AF increased from 7% in October to 15% in February, while the ratio in fish fed RF did not show such an increase (fluctuated between 0.1 and 0.8%). In salmonids that have a high proportion of anserine in the muscle free amino acid pool, an insufficiency of dietary histidine decreased the concentration of muscle free histidine and instead increased that of anserine

(Yamamoto et al. 2012, 2020). Pacific bluefin tuna fed AF may have increased the muscle anserine and carnosine levels to compensate for the muscle free histidine level to maintain the capacity of proton buffering (Abe 2000).

The relationship between monthly average BW of sampled fish {(initial BW + final BW)*0.5} and estimated daily food supply on a dry matter basis (% BW/day) calculated based on the monthly average BW is illustrated in Fig. 5. Compared to the RF group, the AF group was considered to feed more food on dry weight basis until 1 kg BW and then equivalent amounts of food until 1.5kg BW, meaning that the AF group was supplied with protein and lipid as much as in the RF group when the BWs were the same. However, the levels of lipid deposition in the liver and muscle of Pacific bluefin tuna fed AF were lower than fish fed RF (Figs. 4 and 5). It is well known that fish generally deposit more lipid as they grow (Shearer 1994), but the tissue lipid levels in Pacific bluefin tuna with similar BWs (e.g., October samples in the RF group and December samples in the AF group) were higher in the RF group. On the other hand, the digestibility trials using fishmeal and fish oil-based test diets and 2 g of Pacific bluefin tuna revealed that dietary protein was digested well while dietary lipid was less digested

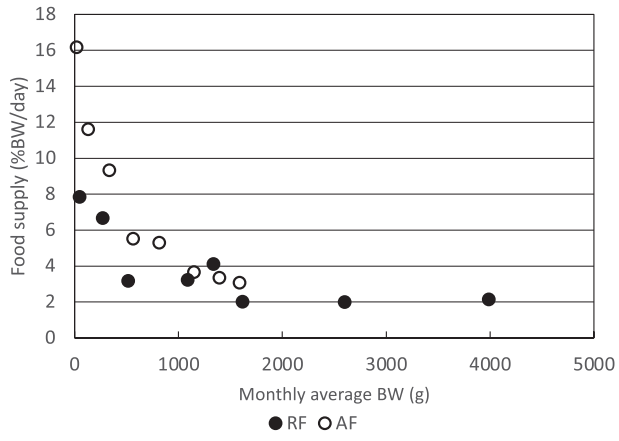


**Fig. 5.** Changes in the estimated daily food supply (dry matter basis) based on monthly average body weight of sampled juvenile Pacific bluefin tuna fed raw fish (RF) and artificial feeds (AF)
Daily food supply (% BW/day) = 100*food supplied/{(initial BW + final BW)*0.5*days}

especially from the high lipid diet (Table 3). In adult northern bluefin tuna *T. thynnus*, the apparent digestibility of nitrogen from a mixture of raw fish was estimated to be 93.7% (Aguado et al. 2004), which was almost similar to the values in the present study (91.0–95.0%). As for lipid digestibility, Takii et al. (2007) reported a value of 84.8% for a moist feed (sand lance: artificial feed = 1 : 1) in Pacific bluefin tuna, which was lower than the apparent lipid digestibility value (95.9%) determined in chub mackerel *Scomber japonicus* using a similar moist feed. The lipid digestibility values obtained in this study using artificial test diets were still lower than the values for the moist feed mentioned above, and decreased by more than 10% in the high lipid diet (66.8%) relative to the low lipid diets (76.9–78.2%). The dietary total lipid level in the high lipid test diet (25.6%) was similar to the levels of sand lance (23.4–24.0%) and AF (21.4–25.7% in the starter feeds and 25.6–31.7% in the grow-up feeds) used in the net cage trial (Table 1).

Recently Murashita et al. (2021) found that the Pacific bluefin tuna lipase has a characteristic of being activated with the presence of taurocholate, and reported that the activities of lipase in both the pancreatic tissue and intestinal digesta of the tuna fed artificial feeds were higher than those of fish fed raw fish under the analytical condition with taurocholate being supplemented to the reaction medium. Our preliminary result in another study shows that total bile acid concentrations in the intestinal digesta of Pacific bluefin tuna fed an artificial feed (14 ± 15 μ mol/g DM) tended to be lower compared to fish fed raw fish (83 ± 31 μ mol/g DM) ($n$ = 2, $P$ = 0.10, Yamamoto et al. unpubl. data). These findings suggest that Pacific bluefin tuna fed an artificial feed might secrete less bile juice from the gallbladder, resulting in less activation of pancreatic lipase, lower lipid digestibility and body lipid deposition. In addition, since the tuna fed AF were unable to obtain enough energy from dietary lipid, they consumed dietary protein as an additional energy source, which caused an insufficient histidine supply to the muscle

free amino acid pool and an increase in the anserine and carnosine synthesis and deposition. Biswas et al. (2016) reported that growth and lipid retention in juvenile Pacific bluefin tuna decreased as the dietary lipid level increased.

In conclusion, food-induced differences between Pacific bluefin tuna fed raw fish and artificial feeds were observed in the free amino acid pools in the liver and dorsal white muscle. In the liver, the differences in the taurine concentrations were considered to be due to the differences in the dietary taurine contents (raw fish < starter feeds). The tuna fed the artificial feeds deposited less lipid in the liver and muscle than fish fed the raw fish, although the lipid contents of both foods were similar, which could be attributable to the lowered lipid digestibility from the artificial feeds. As the result of insufficient exogenous digestible lipid supply, the tuna fed the artificial feeds might have used much protein as an energy source, and increased the concentrations of anserine and carnosine in the muscle free pool to compensate for the reduced histidine supply from dietary protein for the maintenance of the muscle proton buffering capacity. These finding will contribute to the improvement of the quality of artificial tuna feeds, especially by means of optimization of dietary lipid utilization.

## Acknowledgements

The authors are grateful to the staff of Nagasaki Prefectural Institute of Fisheries for donating juvenile tuna for the digestibility trial, and to Dr. S. Satoh, Tokyo University of Marine Science and Technology, for his kind approval to use the fecal collection tanks. This work was supported by Grant-in-Aid from Ministry of Agriculture, Forestry and Fisheries of Japan (Strategic Program on Agricultural Research and Innovation, JPJ007159).

## References

Abe, H. (2000) Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. *Biochemistry* (*Moscow*), **65**, 757-765.

Aguado, F., F. J. Martinez and B. Garcia-Garcia (2004) In vivo total nitrogen and total phosphorus digestibility in Atlantic bluefin tuna (*Thunnus thynnus thynnus* Linnaeus, 1758) under industrially intensive fattening conditions in southeast Spain Mediterranean coastal waters. *Aquacult. Nutr.*, **10**, 413-419.

Biswas, A., M. Nakajima, T. Nakao, O. Takaoka and K. Takii (2016) Determination of suitable protein and lipid levels in diets for Pacific bluefin tuna, *Thunnus orientalis* at grow-out stage. *Aquacult. Sci.*, **64**, 281-288.

Cashion, T., F. Le Manach, D. Zeller and D. Pauly (2017) Most fish destined for fishmeal production are food-grade fish. *Fish Fish.*, **18**, 837-844.

Dabrowski, K. and H. Guderley (2002) Intermediary metabolism. In *"Fish Nutrition, Third Edition"* (ed. by J.E. Halver, R.W. Hardy). Academic Press, California, pp. 309-365.

Daniel, N. (2018) A review on replacing fish meal in aqua feeds using plant protein sources. *Int. J. Fish. Aquatic Studies*, **6**, 164-179.

Food and Agriculture Organization (2020) The State of World Fisheries and Aquaculture 2020. Rome (http://doi.org/10.4060/ca9229en).

Hagey, L. R., P. R. Møller, A. F. Hofmann and M. D. Krasowski (2010) Diversity of bile salts in fish and amphibians: evolution of a complex biochemical pathway. *Physiol. Biochem. Zool.*, **83**, 308-321.

Hirasawa, Y., T. Murai, T. Akiyama and T. Nose (1984) Influence of rearing period on free ninhydrin reactive substances in the white muscle, dark muscle and liver of bluefin tuna, *Thunnus thynnus orientalis*. *Bull. Natl. Res. Inst. Aquacult.*, **5**, 27-32 (in Japanese with English abstract).

Kondo, F., T. Iwai, C. Miura, J. Sakata, T. Ohta, A. Ido, S. Irie, K. Okamatsu, K. Kakumasa and T. Miura (2016) Analysis of feeding effects of EP on growth and digestion in cultured bluefin tuna. *Nippon Suisan Gakkaishi*, **82**, 923-933 (in Japanese with English abstract).

Matsunari, H., H. Furuita, T. Yamamoto, S.-K. Kim, Y. Sakakura and T. Takeuchi (2008a) Effect of dietary taurine and cystine on growth performance of juvenile red sea bream *Pagrus major*. *Aquaculture*, **274**, 142-147.

Matsunari, H., T. Yamamoto, S.-K. Kim, T. Goto and T. Takeuchi (2008b) Optimum dietary taurine level in casein-based diet for juvenile red sea bream *Pagrus major*. *Fish. Sci.*, **74**, 347-353.

Mourente, G. and D. R. Tocher (2009) Tuna nutrition and feeds: current status and future perspectives. *Rev. Fish. Sci.*, **17**, 373-390.

Murai, T., Y. Hirasawa, T. Akiyama and T. Nose (1982) Free ninhydrin reactive substances in the white muscle, dark muscle, and liver of cultured and wild bluefin tuna juvenile *Thunnus thynnus orientalis*. *Nippon Suisan Gakkaishi*, **48**, 1633-1637 (in Japanese with English abstract).

Murashita, K., H. Hashimoto, T. Takashi, T. Eba, K. Kumon, H. Matsunari, S. Soma, H. Oku, H. Furuita, H. Yoshinaga and T. Yamamoto (2021)

Characterization of digestive physiology in Pacific bluefin tuna *Thunnus orientalis* juvenile fed a raw fish and a commercial diet. *Aquaculture*, doi: 10.1016/j.aquaculture.2021.736562.

Ottolenghi, F. (2008) Capture-based aquaculture of bluefin tuna. In "*Capture-based aquaculture, Global overview*" (ed. by A. Lovatelli and P. F. Holthus). FAO Fisheries Technical Paper 508, FAO, Rome, pp. 169-182.

Qi, G., A. Qinghui, K. Mai, W. Xu, Z. Liufu, B. Yun and H. Zhou (2012) Effects of dietary taurine supplementation to a casein-based diet on growth performance and taurine distribution in two sizes of juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture*, **358-359**, 122-128.

Rust, M. B. (2002) Nutritional physiology. In "*Fish Nutrition, Third Edition*" (ed. by J. E. Halver, R. W. Hardy). Academic Press, California, pp. 367-452.

Salze, G. P. and D. A. Davis (2015) Taurine: a critical nutrient for future fish feeds. *Aquaculture*, **437**, 215-229.

Shearer, K. D. (1994) Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*, **119**, 63-88.

Suyama, M. and Y. Yoshizawa (1973) Free amino acid composition of the skeletal muscle of migratory fish. *Nippon Suisan Gakkaishi*, **39**, 1339-1343 (in Japanese with English abstract).

Takii, K., M. Seoka, M. Izumi, H. Hosokawa, S. Shimeno, M. Ukawa and J. Kohbara (2007) Apparent digestibility coefficient and energy partition of juvenile Pacific bluefin tuna, *Thunnus orientalis* and chub mackerel, *Scomber japonicus*. *Aquacult. Sci*., **55**, 571-577.

Watanabe, T., V. Viyakarn, H. Kimura, K. Ogawa, N. Okamoto and N. Iso (1992) Utilization of soybean meal as a protein source in a newly developed soft-dry pellet for yellowtail. *Nippon Suisan Gakkaishi*, **58**, 1761-1773.

Wilson, R. P. (2002) Amino acids and proteins. In "*Fish Nutrition, Third Edition*" (ed. by J. E. Halver, R. W. Hardy). Academic Press, California, pp. 143-179.

Yamamoto, T., H. Matsunari, T. Sugita, H. Furuita, T. Masumoto, Y. Iwashita, S. Amano and N. Suzuki (2012) Optimization of the supplemental essential amino acids to a fish meal-free diet based on fermented soybean meal for rainbow trout *Oncorhynchus mykiss*. *Fish. Sci*., **78**, 359-366.

Yamamoto, T., H. Matsunari, H. Oku, K. Murashita, H. Yoshinaga and H. Furuita (2019) Supplemental effects of histidine to fishmeal-based and low-fishmeal diets on the growth performance and tissue free amino acid levels of fingerling yellowtail *Seriola quinqueradiata*. *Aquacult. Sci*., **67**, 367-375.

Yamamoto, T., H. Matsunari, H. Furuita, M. Miura, R. Ozawa, T. Okazaki, H. Oku, K. Murashita and H. Yoshinaga (2020) First feeding a plant-based diet to rainbow trout *Oncorhynchus mykiss* fry. *Aquacult. Sci*., **68**, 211-220.

# 生餌および配合飼料で養成したクロマグロ幼魚の肝臓および背側普通筋における遊離アミノ酸組成の違い

山本剛史・橋本　博・村下幸司・松成宏之・高志利宣・江場岳史・久門一紀

クロマグロ用配合飼料の栄養価改善に資するため，生餌および市販飼料を給餌した幼魚の肝臓および普通筋の遊離アミノ酸組成等を比較した。生け簀に収容した人工種苗（初期体重 2 g）に7月末から翌年3月中旬まで飽食給餌し，約1ヶ月間隔でサンプリングして分析に供した。肝臓のタウリンは9月または10月にかけて増加し，飼餌料のタウリン含量を反映して配合区で高かった。一方，他のアミノ酸は11月にかけて減少した。筋肉ではヒスチジンが優占し，徐々に増加する傾向が見られたが，生餌区でやや高かった。一方，アンセリンやカルノシンは配合区に多く，ヒスチジンと同様に増加した。また，肝臓や筋肉の粗脂肪は生餌区で高く，水分は配合区で高かった。なお，配合区の体重あたりの給餌率は生餌区と同等以上であった。以上の結果から，配合飼料を摂取したクロマグロでは脂質の利用性が劣ることによりタンパク質がエネルギー源として利用されている可能性が示唆された。