海産魚類のタウリン要求とその役割

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「海産魚類のタウリン要求とその役割」

タウリンはタンパク質を構成する成分ではなく、生体内に遊離状態で存在するアミノ酸である。これまで魚類はタウリンを生合成できるため、餌・飼料中にタウリンは必要ないとされてきた。しかしながら近年、海産魚類ではタウリンの合成能が微弱あるいは存在しないため、餌・飼料中にタウリンを要求する可能性が示唆された。本研究は、ブリやマダイなどの産業上重要な海産養殖魚類のタウリンの要求量とその役割を明らかにすることを目的として、タウリンを強化したワムシおよびタウリンを添加した配合飼料を海産魚に給与し、成長や体成分に及ぼす影響について検討した。また、魚粉を一切含まない海産魚用の精製飼料の開発を試みた。

第 1 章では、海産仔稚魚のふ化後の経過日数に伴うタウリン含量の変化を明らかにするとともに、海産仔魚に対するタウリン強化ワムシの効果について検討した。人工採卵・ふ化により得られたふ化仔魚から全長 18cm まで飼育したブリ仔稚魚や、天然で採取した稚魚とともに、人工種苗生産魚および天然魚が摂餌している餌・飼料のタウリン含量を測定した。その結果、天然魚は人工種苗生産魚の 2～4 倍のタウリンを含有すること、ふ化直後にタウリン含量が急激に減少すること、さらに天然魚の餌料（コペポーダ）と比較して人工生産魚に給与する餌料（ワムシ）のタウリン含量は著しく低いことが明らかになった。これらのことから、タウリンはブリ仔魚の成長に重要な役割を担うとともに、種苗生産で使用する餌・飼料へタウリンを強化することにより、健全な種苗が得られる可能性が示唆された（第 1 節）。次に、ワムシへのタウリン強化方法を検討したところ、ワムシ培養水槽へのタウリン添加量および強化時間に比例して、ワムシ中にタウリンが蓄積された。これらのタウリン強化ワムシをふ化直後のマダラ仔魚に給与したところ、仔魚の成長が促進された。以上のことから、タウリンはふ化仔魚の発育に重要な栄養素であることが示唆された（第 2 節）。

第 2 章では、ブリ稚魚および親魚に及ぼす配合飼料中のタウリンの影響について検討した。その結果、タウリンを飼料に添加しブリ稚魚に給与することにより成長促進効果があ

るだけでなく、魚体中のタウリン含量は飼料中のタウリン含量の影響を大きく受けることが明らかになった。さらに遊離アミノ酸組成からタウリンを生合成する酵素活性が低いことが示唆された（第1節）。親魚においては、飼料へタウリンを添加することにより、成熟が促進されるとともに採卵量が増加し、さらに卵の受精率やふ化率が向上することが明らかとなった（第2節）。

第3章では、マダイ稚魚に対する精製飼料中のタウリンの効果を検討した。一般的な養魚用配合飼料には、主なタンパク質源として魚粉が5割以上含まれるため、タウリンの要求性に関する研究には適さなかった。そこで、タンパク質源としてタウリンを含まないカゼインを用いた飼料を新たに開発し、タウリンの添加量を調整した飼料をマダイ稚魚へ給与した。これまで、海産魚はカゼイン飼料の摂餌が劣ると考えられていたが、開発した精製飼料はマダイ稚魚の摂餌が極めて良好であった。低タウリン飼料をマダイ稚魚に給餌すると体色の暗色化や成長停滞などの異常がみられたが、タウリンを添加することにより、これらの症状が改善された。さらに、飼料中のタウリンおよび含硫アミノ酸の1つであるシスチンがマダイ稚魚の摂餌行動に及ぼす影響について検討した結果、タウリン添加区では対照区と比較して、飼料を視認後、餌を飲み込んだ回数（摂餌回数）が有意に高くなること、一方、シスチン添加区では飼料を視認後に餌を飲み込むまでに、1回以上吐き出す回数が有意に高くなることが明らかになった。また、シスチン添加区では魚体中のタウリン含量が増加しないことから、マダイは体内でシスチンをタウリンへ十分量代謝できないことが明らかになった（第1節）。次に、マダイ稚魚におけるタウリン要求量および脂質の消化吸収におけるタウリンの役割について検討した結果、飼料中に約0.5%のタウリンを添加することにより成長が促進されることから、マダイ稚魚は体内で十分量のタウリンを合成できず、マダイ稚魚は飼料中に約0.5%のタウリンを要求量することが明らかになった。また、飼料へのタウリン添加により抱合胆汁酸濃度が上昇するとともに、肝臓中の粗脂肪含量が有意に高い値を示したことから、飼料中のタウリンが脂質の消化吸収に影響を及ぼす可能性が示唆された（第2節）。

以上の結果から、タウリンは数種の海産魚類において重要な栄養素であることが明らかになった。今後、仔魚から稚魚期までの餌・飼料の改善を図ることが可能となるばかりでなく、魚粉に代わるタンパク質原料の積極的な利用と、環境負荷低減飼料の開発に大きく貢献するものと考えられる。さらに、海産魚用精製飼料が開発されたことから、海産魚における微量栄養素の効果および機能解明などの研究に寄与でき、今後の海産魚類用飼料の改善が飛躍的に進展することが期待される。

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緒　言

我が国の漁業生産量は 1988 年を境に急激に減少している。特に遠洋漁業や沖合漁業では、国際的な 200 海里体制の定着及び公海漁業への規制強化や資源状態の悪化により、漁獲量はピーク時の半分以下となっている。このような状況の中で、つくり育てる漁業などの適正な資源管理および増養殖手法により、わが国およびその周辺の水域において持続的に資源を利用する必要性は極めて大きいと考えられる。つくり育てる漁業は、栽培漁業と養殖業に大別される。栽培漁業は、水産動物の減耗が最も激しい卵から仔稚魚の時期を人間の管理下において種苗を生産し、これを天然水域へ放流した上で適切な管理を行い、対象とする水産資源の持続的な利用を図ろうとするものである。一方、養殖業は、人工あるいは天然から入手した種苗を収穫まで人間が管理する方法である。

栽培漁業は、1963 年に重要な水産資源を増やすために、魚介類の種苗生産・放流を中心に瀬戸内海をモデル地域として始められた。その後、約 40 年の間に、対象魚種の拡大や生産尾数の増加などの著しい進歩があり、2000 年には種苗放流対象魚種は約 80 種、マダイ、ヒラメをはじめとする 9 種においては年間 1,000 万尾を超える種苗が放流されている（水産庁・日本栽培漁業協会, 2002)。現在では、このような生産尾数などの「量」から、種苗の形態や行動などの「質」の向上を図ることも求められている。種苗の質については、「健苗性」や「種苗性」の言葉で表わされる（塚本　1990)。健苗性とは、形態的、生理的、および生化学的に健全であることを意味する。一方、種苗性とは、種の特徴的な行動生態とそのための機能が十分に発達した種苗の質と定義されている。ヒラメ稚魚では人工種苗と天然種苗では摂餌行動に明瞭な差がみられ、

ヒラメ稚魚の放流効果を向上させるためには、人工種苗の種苗性について検討する必要あることが報告された（古田, 1998)。天然のヒラメ稚魚は、着底直後から全長100mm前後に至るまで主にアミ類を摂餌することが知られおり（Subiyant et al., 1993)、朴ら(1997)はヒラメ稚魚の健全性の向上を目的に、天然ヒラメ稚魚の主な餌料生物であるアミの栄養価について検討し、アミ類の重要な栄養成分として遊離アミノ酸の有効性を明らかにした。さらに結晶アミノ酸を用いて遊離アミノ酸中のタウリンがヒラメの成長促進の効果があることを明らかにしている（朴ら, 2001)。

タウリンは体構成タンパク質ではなく、生体内に遊離状態で存在するアミノ酸であり、浸透圧調整や神経伝達調整作用などの機能が知られている（Huxtable, 1992)。タウリンは、必須アミノ酸である含硫アミノ酸のメチオニンやシスチンから生合成されることが多くのほ乳類動物で明らかにされている。タウリンの生合成経路として、1) システイン→システインスルフィン酸→ヒポタウリン→タウリン, 2) システイン→システアミン→ヒポタウリン→タウリン, 3) 活性硫酸→システイン酸→タウリンの3経路が推定されている。1) の経路でシステインからタウリンへ代謝される過程の中間代謝物であるシステインスルフィン酸は、システインスルフィン酸脱炭酸酵素（CSD) によってタウリンの前駆物質であるヒポタウリンに脱炭酸される。仔ネコではこのCSDの活性が弱いため、飼料からのタウリン供給が不足すると視覚異常が誘発されること（Knopf et al., 1978）および生殖能力が著しく低下することや生まれた仔ネコに異常がみられることなど、タウリンは必須の栄養素となっている。(Sturman et al., 1985; 1986)。一方、魚類では、ニジマスを用いた飼育試験において飼料中にシスチンを添加すると魚体中のタウリン含量が増加すること(Walton et al., 1982)や、飼料中へのタウリンの添加効果が見られないこと（Yokoyama and Nakazoe, 1992）か

ら、魚類においてタウリンは必要ないとされてきた。このため、魚類におけるタウリンに関する研究は、エキス成分としての研究が多く(Ozawa et al., 1984; 坂口ら, 1988)、増養殖分野への応用をめざした研究はほとんど行われなかった。しかし最近の研究により、タウリン合成に関与する酵素活性は魚種により大きく異なり、肝臓中のCSD 活性はニジマス、ティラピアなどの淡水魚では高く、マダイ、ブリ、ヒラメなどの海水魚では低いことが明らかにされた(Yokoyama et al., 2001; Goto et al., 2001a, b, 2003)。

種苗生産を行うためには、ふ化率やふ化後の仔魚の生残率が高い良質の受精卵を安定的に確保することとともに、ふ化仔魚を効率的に飼育することが極めて重要となる。天然海域での漁獲量の変動などに左右されずに良質の受精卵を安定的に確保するためには、飼育環境下での採卵用の親魚の養成が必要となる。しかしながら、通常の飼育環境下では成熟・産卵が進まない魚種が存在する。このような魚種では、水温や光周期など環境の調節や、各種ホルモンの投与などの人為的な制御などの適切な親魚の養成が必要になる。親魚の養成の中で卵に大きな影響を及ぼすものとして飼育環境の他に、親魚の栄養状態がある (Izquierdo et al., 2001)。従来の親魚養成では、マアジ、サバ類などを主体とした冷凍生餌を給餌する場合が多いが、生餌は漁獲される時期や場所により栄養組成が異なり不安定であることや、病原微生物侵入の懸念などの問題がある。一方、配合飼料は栄養組成の調整が可能であり、さらに生餌に起因する病原微生物の経口感染を未然に防除することが可能となる (虫明ら, 2003)。海産魚類の親魚の栄養要求については、マダイを用いて必須脂肪酸の欠乏により卵質が低下すること(Watanabe et al., 1984a)、さらに飼料原料により卵質が影響を受けることを明らかにした (Watanabe et al., 1984b)。また、ヒラメでは、親魚飼料中の n-3 高度不飽

和脂肪酸(n-3HUFA)，アラキドン酸の要求量が明らかになっている（Furuita et al., 2000; 2003)。ブリでは配合飼料での親魚養成が可能であることが明らかにされ（虫明ら, 1995)、さらに配合飼料中に30mg/kg前後のアスタキサンチンを添加することにより、採卵成績が向上することが明らかにされている（Verakunpiriya et al., 1997)。このように魚種により異なるものの親魚の栄養要求についても、知見が蓄積されつつある。しかしながら、親魚の栄養要求に関する研究には、親魚の飼育には大型の施設が必要なことや、試験期間が長期にわたることなどから、試験を実施するに当たり困難な条件が多くあることから、まだ十分とはいえない。

ふ化仔魚を効率的に飼育するためには、適切な餌料が必要になる。マダイ、ヒラメ、ブリなどの海産魚類は、消化系諸器官がほとんど未分化な形でふ化する(渡辺, 1985)。このため、これらの魚種では、ふ化後約10日ほどは、胃腺の働きなしで、餌・飼料を消化吸収しなければならない。多くの人工飼料の主原料となる魚粉などのタンパク質を十分に消化することが困難なため、実験規模では微粒子飼料の開発が行われているものの（Wang et al., 2004)、完全に生物餌料と置き換わるまでには至っていない。現在でも、多くの魚種で大量培養が可能なワムシが初期仔魚の餌料として用いられている。ワムシを用いた仔魚の飼育は、1960年に汽水産のシオミズツボワムシを普通海水に馴致し、*Chlamydomonus sp.*, *Chlorella sp.*などを餌料として飼育することにより、海水中で容易に増殖することを発見し、さらにこれを餌料として海産仔稚魚の飼育を提唱したこと（伊藤, 1960）に始まる。1965年には日本栽培漁業協会の伯方島事業場においてマダイふ化仔魚の飼育に使用された。海産クロレラを餌料としてワムシの培養を行ったが、ワムシの大量培養が必要になるとそれに見合う海産クロレラの供給が不足することから、入手が容易なパン酵母が使用され始めた。しかしながら、1970

年頃には、パン酵母のみで培養したワムシを仔魚に連続して給与すると摂餌開始7-10日後に仔魚の食欲減退、遊泳緩慢、横転、腹部膨満などの症状が起こり、2-3 日以内に全滅する現状が発生した。渡邉ら(1978)は、これらの原因は海産魚類の必須脂肪酸であるn-3HUFAの欠乏によるものであることを明らかにした。さらに、海産クロレラによるワムシの二次培養（北島ら，1979），イカ肝油を資化させた酵母で生物餌料を培養し強化する間接法（今田ら，1979），イカ肝油などのn-3HUFAを多く含む油脂を卵黄で乳化させ、直接ワムシやアルテミアの培養槽に入れ栄養強化する直接法（Watanabe et al.，1982）を開発した。このようにEFA要求、特にEPAやDHAなどのn-3HUFAの要求量について検討されている（Takeuchi，1997)。しかしながら、ビタミン類などについての検討があるものの、その他の栄養素についてはほとんど検討されていない。

わが国の養殖業は、戦後、順調に成長を続け日本の水産業における重要な地位を占めるに至った。平成15年度の養殖生産量は125万トン、生産額は4,476億円に達し、沿岸漁業に対する養殖の占める割合はそれぞれ約44%、47%となっている（水産年鑑2006)。また養殖業はブリ、カンパチなどの高級魚を一般消費者に比較的身近な存在とするなど、わが国における増養殖業は水産物の安定供給はもとより、高級品生産による経済性追求あるいは伝統的食文化の保全に役立ってきた。しかしながら、近年生産量の増加を目的とした過密養殖や過剰な餌の投与により、過度の有機物負荷が見られるなど、全国的に養殖魚場が悪化してきている。このような状況に適切に対応するために、漁業協同組合等による養殖漁場の改善を促進するための措置及び特定の養殖水産動植物の伝染性疾病のまん延の防止のための措置を講ずることにより、持続的な養殖生産の確保を図り、もって養殖業の発展と水産物の供給の安定に資することを目的

とする「持続的養殖生産確保法」案が提出され、平成11年5月14日可決・成立するなど、生産技術の開発と共に、環境の収容力などにも見合った適正な養殖事業とするため、生物特性と自然特性を考慮した環境保全型養殖業の展開が期待されている。主な海面養殖は、現在では生餌ではなく主要なタンパク質源としてイワシ類を原料とした魚粉を大量に配合した飼料が利用されており、それらの魚粉の大半は南米等からの輸入に依存している。しかし中国における養魚生産量の急増や魚粉生産国における原料となる魚の資源量変動等により、魚粉生産量は不安定かつ供給がひっ迫している状況にある。このため、魚粉に依存しない配合飼料の開発が急務である。なかでも植物性原料は、リンが少なく環境へのリン負荷軽減のためにも養魚飼料への積極的な利用が望まれている。しかし、植物性原料の配合に際しては、アミノ酸バランス、消化性および生理阻害物質の存在などの問題があり(Liener, 1989)、それらを改善するための処置を施さなければならない。これまでに主要な植物性代替タンパク質へメチオニンやリジンなどのアミノ酸を添加することにより、その利用性が大幅に改善されることが明らかになっている (Takagi et al., 2001)。マダイやブリでは魚粉削減飼料を給餌すると、成長の停滞や、肝臓が緑色に変色する「緑肝症」の魚が多発することが報告された(Maita et al., 1997; Takagi et al., 1999; Watanabe et al., 1998)。当初、本原因は胆管に粘液胞子虫が詰まり、肝臓に胆汁が鬱積し緑肝を呈するといわれていたが (Maita et al., 1998)、そのような飼料にタウリンを添加することで症状が軽減することから、飼料中のタウリン濃度が緑肝症に影響を及ぼしていることが示唆された(Goto et al., 2001c)。魚粉の代替原料として有望視されている大豆油粕などにはタウリンがほとんど含まれていないため、飼料中のタウリンが不足する可能性が推察される。

以上のことから、本研究では、海産魚類のタウリン要求と役割を明らかにするために、第1章では、海産仔稚魚のふ化後の経過日数に伴うタウリン含量の変化を明らかにするとともに、海産仔魚に対するタウリン強化ワムシの効果について検討した。人工採卵・ふ化により得られたふ化仔魚から全長18cmまで飼育したブリ仔稚魚や、天然で採取した稚魚とともに、人工種苗生産魚および天然魚が摂餌している餌・飼料のタウリン含量を測定した（第1節)。次に、ワムシへのタウリン強化方法を検討するとともに、異なる濃度でタウリン強化したワムシをふ化直後のマダラ仔魚に給与することにより、タウリンがふ化仔魚に及ぼす影響について検討した（第2節)。

第2章では、ブリ稚魚および親魚に及ぼす配合飼料中のタウリンの影響について検討した。第1節では、0, 0.5, 1.0, 1.5, 2.0%のタウリンを市販飼料に添加しブリ稚魚に6週間給与し、成長をマダイ稚魚の成長や全魚体の遊離アミノ酸組成等を調べた。第2節では、魚粉削減飼料に0, 0.5, 1.0%のタウリンを添加した飼料を産卵前に約5ヶ月間、ブリ親魚へ給与し、親魚の成熟度、採卵量、受精率などを調べた。

第3章では、マダイ稚魚に対する精製飼料中のタウリンの効果を検討した。一般的な養魚用配合飼料には、主なタンパク質源として魚粉が5割以上含まれるため、タウリンの要求性に関する研究には適さなかった。そこで、タンパク質源としてタウリンを含まないカゼインを用いた飼料を新たに開発し、1.0%のタウリンを添加した飼料をマダイ稚魚へ給与した。さらに、飼料中のタウリンおよび含硫アミノ酸の1つであるシスチンがマダイ稚魚の摂餌行動およびアミノ酸代謝に及ぼす影響について検討するため、タウリンを 0, 0.5, 1.0, 2.0%, シスチンを 1.0, 2.0%添加した精製飼料を 6週間給与した。マダイ稚魚の成長や全魚体の遊離アミノ酸組成等を調べるとともに、試験終了後に摂餌行動をビデオ撮影して解析した（第1節)。次に、マダイ稚魚におけ

るタウリン要求量および脂質の消化吸収におけるタウリンの役割を明らかにするために、タウリンを0, 0.1, 0.3, 0.5, 0.7%, タウロコール酸を0.5%添加した精製飼料を6週間給与した（第2節）。

本論文の一部は下記に報告済みである。

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第１章

海産仔稚魚のタウリン含量とタウリン強化ワムシによる給与効果

第１節　人工種苗生産ブリ仔稚魚におけるタウリン含量の変化および天然稚魚との比較　（日本水産学会誌 2003；69，757-762）

人工種苗生産ブリ仔稚魚におけるタウリン含量の変化および天然稚魚との比較

短縮表題　ブリ仔稚魚のタウリン含量の変化

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Changes in the taurine content during the early growth stages of artificially produced yellowtail and a comparison with their wild fish

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人工種苗生産ブリ仔稚魚におけるタウリン含量の変化および天然稚魚との比較

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異なる餌料系列を用いた種苗生産過程におけるブリ仔稚魚および飼育に用いたワムシ、アルテミア、天然コペポーダ、冷凍天然コペポーダ、配合飼料のタウリン含量および天然稚魚におけるタウリン含量との違いを調べた。その結果、人工種苗生産過程におけるブリ仔稚魚のタウリン含量は、餌・飼料中のタウリン含量の影響を受けること、特に開口時までに多くの遊離アミノ酸が減少するのに対して、タウリンは開口後のワムシ給餌期に大きく減少すること、また人工種苗生産稚魚は天然稚魚に比べて、タウリン含量が著しく少ないことが明らかとなった。

キーワード

ブリ, 仔稚魚, 人工種苗, 天然稚魚, タウリン, 遊離アミノ酸

Changes in the taurine content during the early stages of artificially reared yellowtail and a comparison with their wild fish

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This experiment was conducted to investigate the changes in taurine content during the development of artificially produced yellowtail *Seriola quinqeradiata* larvae and juveniles. Besides this, taurine content of wild caught and reared yellowtail was compared. Free amino acids content in larvae and juveniles produced at the Goto Station of Japan Sea-Farming Association and Nagasaki Prefecture Institute of Fisheries (TL, 4.2-186.8 mm) and juveniles caught from the coastal area of Nagasaki Prefecture (TL, 29.7-179.0 mm) were analyzed.

The content of Most of free amino acids such as lysine, leucine, isoleucine, alanine decreased between the fertilized egg stage and open-mouth stage, with the exception of taurine in artificially reared fish. The taurine content in the whole body of the artificially reared larvae decreased rapidly during the rotifer feeding. The amount of taurine in the wild fish was higher compared to the cultured fish. These results reveal that taurine has an important role compared to other free amino acids, suggesting live food and diet used for yellowtail culture would not satisfy taurine requirement of yellowtail.

ブリ　*Seriola quinquerdiata* (Temminck et Schlegel) は、日本の魚類養殖において最も生産量が多く重要な魚種の１つである。ブリの養殖は体長数 cm から 10 数 cm のモジャコと呼ばれる天然の稚魚を採捕したものを用いて行うため種苗の供給は、モジャコ漁に左右され、不安定である。計画的かつ安定的な種苗供給の実現のためには、人工種苗生産技術の確立が必要不可欠であると考えられる。しかしながら、現在の人工種苗生産では生残率が低く、また形態異常の発生など改善しなければならない点も多い。

ブリの栄養要求に関する研究は古くから行われており、稚魚の脂質およびタンパク質の適正量、n−3 高度不飽和脂肪酸要求、[1-2]仔稚魚のドコサヘキサエン酸 (DHA) 要求、[3] 稚魚および幼魚のアミノ酸要求[4-6] などが明らかにされている。

含硫アミノ酸の１つであるタウリンは水産動物の組織に広く分布し、しかも多量に含まれている。その生理作用として、海産生物では浸透圧調節物質として知られている程度で知見は乏しかったが、近年タウリンの魚類に及ぼす影響について研究が進められている。ヒラメ *Paralichthys olivaceus* 稚魚において飼料中のタウリンが稚魚の健全な育成に極めて有効であることが明らかになるとともに、[7] ヒラメ仔稚魚では体内でのタウリン合成に関与するシステイン硫酸脱炭酸酵素 (CSD) の活性が微弱であること、またブリではこの酵素活性がヒラメよりもさらに低いことが明らかにされている。[8] また、マダイおよびヒラメではふ化直後に魚体中のタウリン含量が減少することも明らかにされた。[9]　ブリ稚魚については、これまで、一般組成、無機質含量[10]、脂質組成および脂肪酸組成[11] について飼育魚と天然魚の比較を行ってきたが、タウリンのブリ仔稚魚に対する効果についての研究はこれまで行われていない。そこで本研究では、ブリ仔稚魚に対するタウリンの効果に関する基礎的な知見の集積を目

的に、人工種苗生産過程におけるブリ仔稚魚のタウリン含量の変動を調べるとともに、天然稚魚との比較を行った。

材料および方法

人工種苗は、日本栽培漁業協会五島事業場において生産された種苗（五島産と略記）、および長崎県総合水産試験場において生産された種苗（長崎産と略記）を用いた。五島産は、60m^{3}陸上コンクリート水槽を用い、飼育水温は22℃で飼育した。仔稚魚の給餌に用いた飼料系列をFig.1に示す。給餌したワムシおよびアルテミアの2次強化には、アクアラン（BASFジャパン㈱製）を生物餌料水槽1kL当りそれぞれ100および200g使用した。また、ワムシ給餌期間中は、飼育水にナンノクロロプシスを50万セル／mLとなるように添加した。

長崎産は、100m^{3}陸上コンクリート水槽を用い、飼育水温は22℃で飼育した。給餌したワムシは、午前の給餌にはプラスアクアラン（BASFジャパン㈱製）を生物餌料水槽1kL当り60g、午後の給餌にはマリングロス（日清製油㈱製）を0.5Lとすじこ乳化油（日清サイエンス㈱製）15gを併用して添加し、水温23-24℃で16-20時間の強化を行った。アルテミアは午前の給餌ではDHAセルコ（長瀬産業㈱/INVE）を生物餌料水槽1kL当り600g、午後の給餌にはマリングロスを1Lとすじこ乳化油30gを併用して添加し、水温23-24℃で16-20時間強化した後給餌した。飼育水には、ナンノクロロプシスを50万セル／mLとなるように添加した。仔稚魚および生物餌料のサンプルは、水道水でよく水洗いした後水分をよく拭き取り、湿重量でそれぞれ約2gを採取した。

天然稚魚は、5月24日に長崎県五島列島沖で採集したもの（五島沖採捕）、および5月16日から17日の間に長崎県総合水産試験場・調査船わかづる第2回航海で採集（長崎調査船採捕）し、それぞれサイズ別に分別したものを用いた。

仔稚魚は−20℃で、ワムシおよびアルテミアは−80℃で凍結保存したものを後日分析に供した。

分析方法

供試魚は包丁で細かくした後、遠心粉砕機（長谷川式スーパーファイブレーター ㈱長谷川鉄工所製）を用いてすり潰した。ワムシおよびアルテミアは解凍後、遠心分離機H−200型 国産遠心機社製）を用いて10,000rpm（5590×g）で10分間遠心分離した。これらを、真空凍結乾燥機（共和真空技術RLE-206型）でそれぞれ乾燥させ、乳鉢を用いて細かくすり潰し分析に供した。市販飼料は乳鉢ですり潰し均一にした後分析に供した。試料は遠沈管に入れ、2%スルホサリチル酸を加えホモジナイザーを用いて均一化した後、3000rpm（1509×g）で15分間遠心分離した。さらに2%スルホサリチル酸を加え、上澄み液を50mLに定容したものをアミノ酸自動分析機（Model 8500-A：日立製作所製）を用いて分析した。なお分析はプールサンプルとした。

結　果

餌・飼料の分析結果

人工種苗生産過程で用いた餌・飼料の分析結果をTable 1に示す。餌・飼料中の遊

離アミノ酸の総量は 100ｇ当り、ワムシ・アルテミア・天然コペポーダ・冷凍コペポーダ・配合飼料では、それぞれ 2855mg, 3753mg, 7733mg, 12119mg および 2288mg であった。その中でタウリン含量は 100g 当りそれぞれ 84.7mg、668mg、1182mg、461mg および 439mg であり、ワムシ中のタウリン含量が最も低い値を示していた。

人工種苗生産魚の分析結果

五島産と長崎産の人工種苗生産魚の遊離アミノ酸含量をそれぞれ、Tables 2 および 3 に示す。

魚体中の遊離アミノ酸含量は、五島産では受精卵で 12968mg/100g あったが、日齢 3 の開口では 2908mg/100g であった。その後は、約 3000mg/100g とほぼ一定であった。また長崎産でも同様に、受精卵で 12270mg/100ｇあったものが、日齢 3 では 3578mg/100g まで大きく減少し、両機関でほぼ同様の傾向がみられた。その中で、アルギニン, リジン, ロイシン, イソロイシンなどが日齢１までに特に大きく減少していた。一方、メチオニン, トリプトファンは、ふ化に伴う変化は少なかった。この様な遊離アミノ酸の変化の中で、タウリンのみがやや増加する傾向を示した、すなわち五島産では、受精卵の 410mg/100g から日齢 3 の 626mg/100ｇへ、長崎産でも受精卵の 680mg/100g から日齢 3 の 799mg/100g と増加した。

開口後になると、タウリンの含量が大きく変動した。すなわち、五島産、長崎産ともに、日齢 15～19 (全長:TL, 7～11mm) までに大幅に減少した。その後日齢 23～42 (TL9～28mm) で増加するが、TL40mm を過ぎると減少し、一定となった (Fig. 2)。

天然稚魚の分析結果

天然稚魚の遊離アミノ酸分析結果をTable 4に示す。

天然稚魚は、人工種苗生産魚と比較すると魚体中の遊離アミノ酸含量が多い傾向がみられた。五島沖採捕のものはサイズに関わらず約 3700mg/100ｇとほぼ一定であった。一方、長崎調査船採捕のものではTL30mmで約6000mg/100gあったものが、TL120mmでは五島沖採捕のものとほぼ同様の約4000mg/100gまで減少した。

次に、魚体中のタウリン含量をみると、平均TL30～42mmで、2200～2400mg/100g含まれていたものが成長に伴い減少する傾向を示した(Fig. 2)。先の人工種苗と比較し、天然稚魚はタウリンを同じサイズで2～4倍多く含んでいた。

考 察

今回の分析結果から人工種苗生産過程におけるブリの遊離アミノ酸含量は、日齢3の開口までに大幅に減少することが明らかになった。しかしその変動は、アミノ酸の種類により異なった。すなわち受精卵中700mg/100ｇ以上と多く含まれたアルギニン, リジン, ロイシン, イソロイシンなどは日齢1までに大きく減少した。またフェニルアラニン, チロシンは日齢3の開口までに緩やかに減少した。海産仔魚では遊離アミノ酸が孵化前にはタンパク質合成に、ふ化後はエネルギー源に使用されることが明らかにされている。[12] これらのアミノ酸は、ブリでも同様に卵発生および孵化直後に重要な働きをするのではないかと考えられる。このように各種のアミノ酸が開口までに減少する中、タウリンのみが若干増加した。

開口後の遊離アミノ酸の総量は、長崎産の日齢15で、約2000mg/100gと一旦低い値

を示したが、その後五島、長崎産ともに、約3000mg/100g前後で推移し大きな変動はみられなかった。一方、タウリン含量は大きく変動した。五島産では日齢19で39.4mg/100g、長崎産では日齢15で203 mg/100gとなり、ワムシ給餌期間中は魚体中のタウリン含量が大幅に減少した。その後アルテミアや、天然コペポーダを給餌することにより、五島産では日齢32で738mg/100mg、長崎産では日齢23で1253mg/100gと高くなっている。さらに生物餌料から配合飼料へと給餌した餌・飼料の変化に伴い、五島、長崎産ともに600mg/100g前後となった。ブリでは人工種苗生産過程における魚体中のタウリン含量は、給餌した餌・飼料のタウリン含量に極めて近い値を示すことが明らかになった。つまりブリの仔稚魚は、餌・飼料の影響を大きく受けることから、体内でのタウリンの合成能が低い可能性が考えられる。またブリの魚体中のタウリン含量は、ふ化後20日までに大幅に減少することが明らかになった。海産仔稚魚では必須の栄養素であるDHAが、ふ化後10日前後までに魚体内で著しく減少する[13]ことが明らかになっているが、タウリンでも同様の傾向が確認された。現在、ブリの人工種苗生産過程では開口直後はワムシの単独給餌を行っている。しかし、本研究によりワムシはその他の餌・飼料と比較するとタウリン含量が極めて少ないことが明らかになった。人工種苗生産過程では、ワムシ単用給餌時期に魚体中のタウリン含量が大きく減少していることから、この時期は体内での消費量が供給量を上回り、給餌したワムシのタウリン含量がブリ仔魚のタウリン要求量を満たしていない可能性が示唆される。ブリでは、必須の栄養素であるDHAをワムシに強化することにより高い生残率および活力を示すことが明らかにされている。[14]このようなことから、ワムシへのタウリン強化について検討する必要があると考えられた。

一方、天然稚魚のタウリン含量は、成長に伴い減少することが明らかになった。ブ

リの食性については、TL 約 30mm から魚類の捕食を開始するが、約 60mm までは小型橈脚類や枝角類を、60～70mm を超えると大型のプランクトンを主食とする。以後は魚類への依存度が高まり、TL 約 130mm 以上になると完全に魚食性に転換する。[15] このように、TL30mm から 130mm 前後にかけて食性が大きく変化することが知られている。人工種苗生産過程では、給餌した餌・飼料のタウリン含量に極めて近い値を示す傾向が確認されたことから、天然稚魚の魚体中のタウリン含量が成長するに伴い減少しているのは、捕食生物の変化によることが一因であると考えられる。一方、ブリの人工種苗生産過程では TL 約 30mm 以降は配合飼料のみの給餌を行っている。このため人工種苗のタウリン含量が成長に伴う変化はなく約 700mg/100 g 前後で一定であったのは、飼料中のタウリン含量に大きな変化がなかったためと推察される。天然稚魚と人工種苗生産魚を比較すると、TL30mm 前後の時期には 2 倍以上もの大きな差が認められた。魚体中のタウリン含量の差がどのような影響を及ぼすのかについては明らかではないが、ヒラメでは、タウリン含量の異なる配合飼料を給餌することにより、成長や飼料効率の改善、さらには摂餌行動が機敏になることが報告されている。[16,17] 天然稚魚と人工種苗生産魚のタウリン含量の差が大きい仔稚魚期におけるブリのタウリン要求量やタウリンがブリへ及ぼす影響については今後詳細に検討する必要があろう。

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Table 1. Free amino acid content (mg/100g, dry matter basis) of food used for feeding of yellowtail

	Rotifer	*Artemia*	Wild zooplankton	Frozen zooplankton	Commercial diet
Essential amino acid					
Arginine	274.6	282.6	1251.5	1696.3	136.2
Lysine	182.5	161.1	234.3	817.0	88.5
Histidine	86.3	123.7	68.9	235.2	200.3
Phenylalanine	113.5	74.0	106.1	352.0	53.9
Tyrosine	151.9	113.2	101.7	410.8	37.8
Leucine	139.9	100.2	143.8	667.6	99.0
Isoleucine	87.5	73.2	98.4	401.6	51.6
Methionine	63.7	51.5	74.2	239.6	29.7
Valine	74.3	103.1	142.6	515.4	67.4
Threonine	58.0	83.1	128.2	453.5	49.8
Tryptophan	75.9	35.0	40.6	138.7	16.4
Non-essential amino acid					
Taurine	84.7	668.3	1182.2	461.2	439.0
Alanine	153.7	384.7	492.6	1127.6	137.7
Glycine	108.6	95.8	1750.8	1086.0	67.7
Glutamic acid	282.6	301.4	221.3	832.4	255.6
Serine	110.7	130.0	105.0	466.8	31.9
Aspartic acid	51.8	52.5	56.1	649.9	39.3
Proline	87.7	318.6	755.6	659.9	100.1
Total	2854.8	3753.0	7733.3	12119.1	2288.3

Table 2. Free amino acid content (mg/100g, dry matter basis) of whole body in artificially reared yellowtail (Goto St.)

Day after hatching	Fertilized egg	1day	3day	19day	32day	47day	53day	59day	78day	95day	152day
T.L.(mm)		4.2±0.1	4.6±0.1	10.9±0.6	20.9±2.9	33.0±3.4	42.2±3.3	50.5±5.3	67.3±6.3	95.1±5.9	186.8±17.0
Essential amino acid											
Arginine	880.2	82.7	56.1	237.7	155.1	39.6	43.9	45.8	30.2	36.2	22.0
Lysine	1190.3	148.4	81.7	206.7	149.1	91.5	115.7	116.4	104.6	148.8	89.5
Histidine	416.0	168.1	106.4	102.1	181.5	143.5	278.9	408.9	542.4	766.0	1314.5
Phenylalanine	612.6	521.1	124.8	159.5	84.9	64.9	37.4	67.7	28.0	34.0	18.3
Tyrosine	638.4	438.4	234.2	137.4	77.5	49.9	34.2	49.1	23.1	38.7	14.7
Leucine	1573.9	139.0	73.9	177.9	116.9	48.1	44.4	53.9	33.4	45.0	24.0
Isoleucine	960.8	122.2	46.3	79.6	67.9	32.1	25.9	32.8	22.7	26.8	14.1
Methionine	493.2	129.7	62.4	75.9	55.8	34.8	26.7	29.4	20.8	28.1	11.3
Valine	895.1	176.8	72.0	113.9	97.0	54.6	46.5	57.3	41.5	46.0	30.5
Threonine	647.6	136.9	63.3	103.2	117.8	55.9	54.2	67.8	52.3	59.0	49.2
Tryptophan	164.5	124.7	69.5	84.6	15.4	49.4	12.1	71.8	29.0	6.4	8.3
Non-essential amino acid											
Taurine	410.4	759.6	625.9	39.4	737.5	708.2	589.9	608.8	535.2	547.6	790.7
Alanine	1093.3	180.3	107.9	232.8	292.4	142.4	150.6	168.3	211.5	215.5	119.5
Glycine	265.3	199.4	204.1	92.2	139.7	365.1	322.6	328.3	217.2	248.6	135.1
Glutamic acid	274.0	276.1	255.6	242.7	353.3	224.8	231.2	229.0	268.0	295.4	162.4
Serine	917.6	130.6	125.5	141.4	161.2	100.4	87.1	78.1	87.0	85.8	50.3
Aspartic acid	82.9	84.1	100.5	98.9	75.9	49.0	44.4	41.0	33.4	36.3	8.0
Proline	258.9	126.9	46.1	63.6	103.0	35.7	30.9	34.9	31.3	16.9	19.0
Total	12968.1	4544.5	2908.2	2892.6	3543.0	2711.7	2533.1	2973.1	2711.6	3088.4	3177.6

Table 3. Free amino acid content (mg/100g, dry matter basis) of whole body in artificially reared yellowtail (Nakasaki Pref.)

Day after hatching	Fertilized egg	1day	3day	15day	23day	37day	42day	54day	81day
T.L.(mm)		4.2±0.1	4.5±0.2	7.3±0.5	9.1±1.4	19.3±3.0	28.1±4.8	44.3±6.8	99.6±8.9
Essential amino acid									
Arginine	784.3	63.2	55.3	54.6	48.0	35.3	30.8	25.0	20.0
Lysine	1173.0	136.1	116.5	74.0	76.3	110.4	92.4	156.5	70.0
Histidine	400.8	193.6	159.7	62.2	84.8	155.9	203.4	302.3	787.6
Phenylalanine	586.9	406.4	284.0	51.5	26.8	36.1	38.0	31.2	36.4
Tyrosine	518.8	281.1	254.3	49.0	24.3	37.9	37.1	38.4	40.7
Leucine	1516.3	147.8	113.4	58.1	50.6	52.5	49.6	34.7	39.7
Isoleucine	843.4	116.3	82.8	30.1	25.7	32.5	30.9	24.1	24.6
Methionine	510.0	141.6	104.6	40.1	19.0	23.3	25.5	24.9	25.8
Valine	795.4	166.8	134.3	43.1	44.3	55.6	52.7	28.9	42.7
Threonine	527.2	100.6	88.6	52.9	49.3	48.7	57.4	62.0	61.4
Tryptophan	175.9	114.9	76.0	14.5	14.0	39.9	41.9	50.1	49.6
Non-essential amino acid									
Taurine	680.2	900.6	799.2	203.2	1253.7	1331.8	1268.4	766.6	691.7
Alanine	973.0	175.2	146.8	91.5	124.9	140.2	154.7	178.0	213.8
Glycine	297.1	159.5	180.4	142.4	149.9	238.0	282.8	242.8	190.8
Glutamic acid	114.4	227.1	252.9	285.7	265.4	203.7	222.3	242.4	169.6
Serine	637.7	121.0	128.0	88.6	71.5	67.0	68.6	93.4	74.2
Aspartic acid	22.1	64.8	24.5	106.0	61.1	40.0	53.0	38.6	16.2
Proline	247.5	73.4	72.1	37.6	33.8	189.0	43.7	33.9	66.9
Total	12269.8	4201.2	3578.1	1992.6	2832.0	3240.9	3159.2	2801.7	3119.9

Table 4. Free amino acid content(mg/100g, dry matter basis) of whole body in wild caught yellowtail

	Goto area					Nagasaki area			
T.L.(mm)	41.5±7.5	71.0±4.6	92.7±4.1	137.3±4.4	179.0±1.4	29.7±2.2	61.5±3.4	92.0±4.2	123.2±6.7
Essential amino acid									
Arginine	45.2	30.1	30.3	45.3	37.6	209.0	134.6	109.7	32.1
Lysine	102.1	76.4	80.0	123.5	133.8	222.9	161.8	158.6	164.5
Histidine	211.6	452.2	612.4	897.6	1110.0	264.7	511.1	710.4	915.7
Phenylalanine	38.2	24.0	28.0	44.4	28.7	140.4	97.6	74.7	22.6
Tyrosine	34.2	21.4	22.2	44.0	23.7	129.7	91.2	71.5	20.2
Leucine	56.4	39.9	42.6	62.1	48.2	211.8	141.2	120.4	41.5
Isoleucine	34.8	22.7	24.0	33.7	23.9	116.2	76.8	63.1	22.8
Methionine	28.0	22.5	23.1	31.2	18.0	98.0	67.7	57.4	23.6
Valine	54.4	39.7	42.5	51.6	37.2	148.1	96.9	80.7	37.5
Threonine	66.5	51.0	51.0	55.5	50.3	164.0	122.3	120.9	75.3
Tryptophan	10.8	8.3	8.9	14.4	9.9	35.9	37.9	18.2	0.0
Non-essential amino acid									
Taurine	2199.0	1937.7	1981.6	1703.7	1477.1	2384.7	2190.4	1913.9	1815.3
Alanine	165.4	133.0	150.4	170.7	148.2	350.9	268.6	237.8	190.9
Glycine	152.3	131.8	141.9	112.8	79.0	200.6	163.5	148.1	102.7
Glutamic acid	189.2	140.2	155.0	151.8	127.2	304.4	244.6	207.6	137.6
Serine	65.2	41.6	44.7	50.3	48.1	189.3	125.4	108.8	40.9
Aspartic acid	36.5	24.4	23.7	32.0	28.8	145.0	84.6	73.3	21.7
Proline	41.4	28.4	30.2	56.0	71.9	141.0	101.8	106.5	83.7
Total	3799.0	3421.9	3716.1	3967.6	3724.3	5969.7	5133.8	4739.0	3987.3

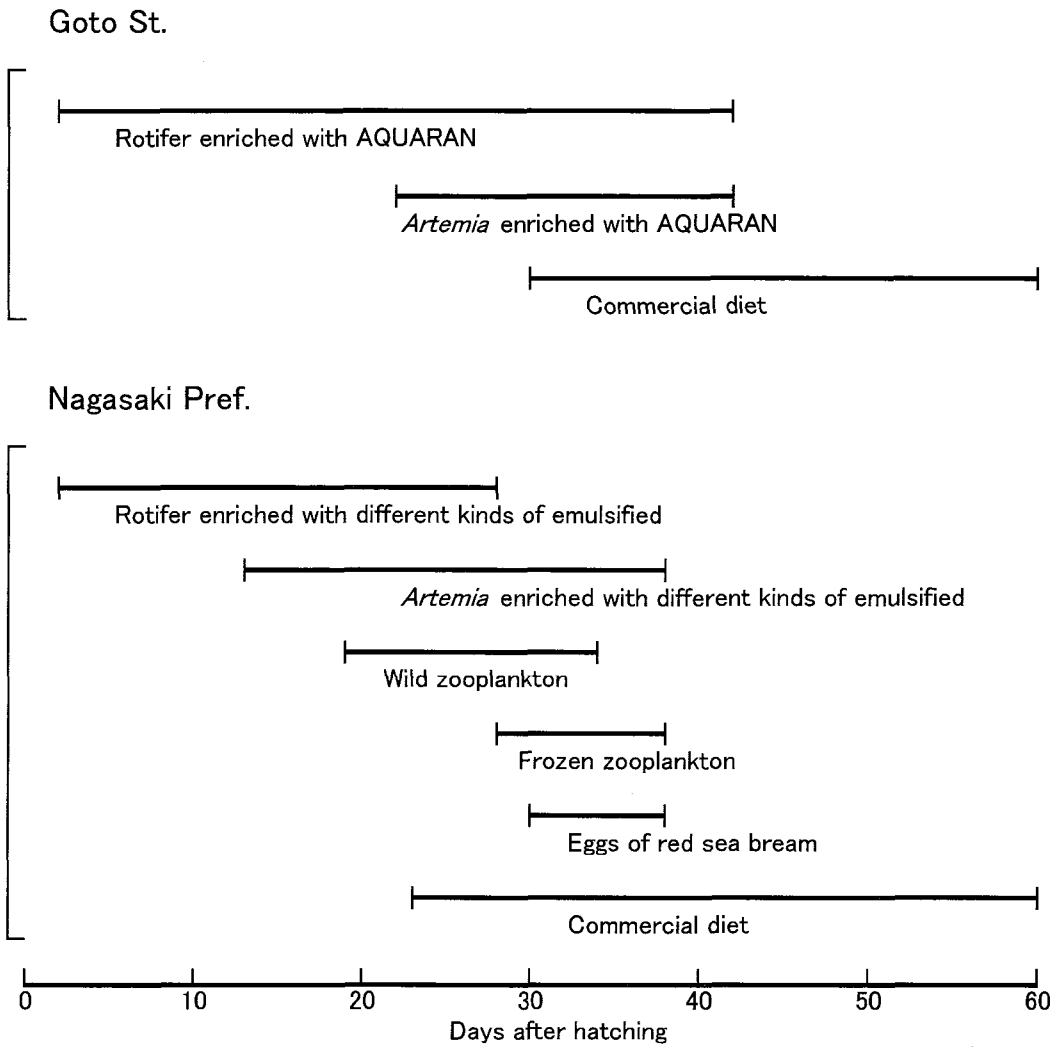

Fig.1. Feeding schedule of artificially reared yellowtail.

第1章

海産仔稚魚のタウリン含量とタウリン強化ワムシによる給与効果

第2節　マダラ仔魚の成長および体組成に及ぼすタウリン強化ワムシの効果

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Effect of Feeding Rotifers Enriched with Taurine on Growth Performance and Body Composition of Pacific Cod Larvae *Gadus macrocephalus*

Effect of taurine on Pacific cod larvae

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Abstract: The present study was conducted to investigate the effect of taurine on the growth performance and body composition of larval Pacific cod *Gadus macrocephalus* by feeding rotifers enriched with different levels of taurine. Rotifers enriched with three different kinds of enrichment materials (*Nannochloropsis*, freshwater type of Chlorella, and shark egg) and with or without 400 (mg/*l*) taurine were given to larvae (TL = 4.3mm, 0 DAH) for 20-day in Exp. Ⅰ. Rotifers enriched with 0, 400, 800 and 1200 (mg/*l*) taurine were given to larvae (TL = 4.3mm, 0 DAH) for 25-day in Exp. Ⅱ. In case of highest, we succeeded in the enrichment of rotifers contained 6 times higher level of taurine compared with the group to without taurine supplementation. Larval growth was significantly improved in fish fed rotifers enriched with taurine. Taurine content in the whole body of the fish fed taurine enriched rotifers was much higher than those with no taurine supplement. The growth performance was improved significantly ($P < 0.05$) with each increase in the level of taurine enrichment. Taurine content in the whole body proportionally increased with the taurine level in the rotifers. These results suggest that taurine enrichment of rotifers is effective to improve the growth in cod larvae, and indicate that cod larvae are dependent on dietary taurine to maintain the body taurine pool.

Key words: *Gadus macrocephalus*; Growth; Taurine; Rotifer

So far, it has been shown that taurine is present in various tissues of fishes (Ozawa et al. 1984) and several studies have indicated that there are interspecific differences in the pathway and capacity of taurine biosynthesis in fish (Goto et al. 2001a; Goto et al. 2003). For example, the enzyme activity of cysteinesulfinate decarboxylase which is the rate limiting step to synthesize taurine in Japanese flounder is a half of that of the rainbow trout and no activity was found in yellowtail and bluefin tuna (Yokoyama et al. 2001). Rainbow trout can synthesize hypotaurine and taurine from cystine (Yokoyama and Nakazoe 1992; Yokoyama and Nakazoe 1998), but the juvenile flounder are unable to use dietary cystine for taurine biosynthesis (Park et al. 2002). These results suggested that juvenile flounder lack of the capacity to convert cystine to taurine. The juvenile flounder fed the diets supplemented with taurine showed improved growth and taurine content of the whole body proportionally increased with the increase in the dietary taurine level (Park et al. 2002; Kim et al. 2003). These observations were also found to be true in other fish, such as red sea bream and sea bass (Chen et al. 2004; Martinez et al. 2004). This suggests that these juvenile marine fish are dependent on the dietary taurine to maintain the body taurine pool. It is therefore clear that taurine is an essential element for larval and juvenile marine fish.

Pacific cod (*Gadus macrocephalus*) is a commercially important species in Japan. It's marked decrease, about 20% at the most of catches in Ishikawa Prefecture (Morioka et al. 1998). We need to establish and develop of techniques for mass production and mass release of the juveniles of this species. Some studies regarding the dietary essential fatty acids requirements of cod larvae have been conducted. Dietary docosahexaenoic acid has been shown to influence the growth, survival and vitality of larval cod (Feng et al. 1995). Its requirement, based on survival and vitality, were reported to be approximately

1% and 1.6-2.1% on a dry matter basis in rotifers and *Artemia*, respectively (Takeuchi et al. 1994; Feng et al. 1996). However, there is no information on amino acid requirements of this species. Therefore, this study investigated the effect of rotifers enriched with taurine on growth and body composition of larval cod.

MATERIALS AND METHODS

Feeding of rotifer

Brachionus plicatilis (Obama strain), so-called L type rotifers, were cultured with the seawater on 15℃ and 20psu, feed with *Nannochloropsis* and yeast. Rotifers were stocked into 30L tanks at a density of 200 individuals/*ml*, and enriched for 22h with dilution of seawater on 26psu, and water temperature of 14℃. In Exp. I , rotifers were enriched with: 1) Frozen *Nannochloropsis* sp., 2) commercial shark eggs extract (Plus Aquaran; BASF Japan, Ltd.), or 3) freshwater type of Chlorella (Super fresh Chlorella V12; Chlorella Kogyo Co., Ltd) and each of these three enrichment materials were added 4×10^7cell/mL, 4.8g and 24mL, respectively. At the same time, they were supplemented with either 0 or 400 mg/*l*, taurine supplement (Aquaplus ET; MarubeniNisshin Feed Co, Ltd.) respectively. In Exp. II , rotifer enriched with 24mL freshwater type of Chlorella (Super fresh Chlorella V12; Chlorella Kogyo Co., Ltd) and 0, 400, 800 or 1200 mg/*l* taurine supplement (Aquaplus ET; MarubeniNisshin Feed Co, Ltd.), simultaneously.

Rearing of larvae

Details of the rearing conditions for Expts. I and II are shown in Table 1. The total length of each initial fish (0 DAH) was approximately 4.3 mm in Expts. I and II . The

fish were divided into six groups in duplicate in Expt. I , and four groups in triplicate in Expt. II . Each tank held approximate 10,000 fish in a 500l polycarbonate tank (water volume 500*l*). Fish were fed rotifers enriched with various materials for 20 and 25 days in Expts. I and II , respectively. Fifty fish were harvested every 5 days after feeding experiment for measurement of the total length from all groups. Phytoplankton were added (suspended) to the larvae rearing tanks with $50\sim100\times10^4$ cells/*ml* of Frozen *Nannochloropsis*. Samples of rotifers used for each tank were frozen after being washed with freshwater. At the end of experiments, samples of fish were taken from each treatment, frozen immediately. To minimize the influence of the ingested preys on the biochemical composition, fish were starved for 24h, prior to sampling. Samples were stored at –80℃ until analysis.

Chemical analysis

Lipids were extracted by the chloroform-methanol (2:1,v/v) method (Folch et al.1957). Crude lipids were saponified by using one mL of 50% KOH in 15mL ethanol and heated for 40 min at 80℃. The saponifiable matter was then esterified by using 6.7% BF_3 in methanol and heated for 20 min at 80℃ (Morrison and Smith 1964). Fatty acid methyl esters were diluted in hexane (20 mg/m*l*) and analyzed by gas liquid chromatography (GC-14B Shimadzu, Kyoto, Japan) equipped with Supelcowax-10 fused silica capillary column (30 m×0.32 mm×0.25 ㎛ film thickness) (SUPELCO, Bellefonte, USA). Helium was used as the carrier gas and the pressure was adjusted to 100 kPa. Temperatures in the column, injection port, and detector were adjusted to 205, 250 and 250℃, respectively. Fatty acid methyl esters were identified by comparing the retention times against the standards. Free amino acids were homogenized with 2%

sulfosalicylic acid and centrifuged at 1,230×g for 15 min. Free amino acid levels were determined individually with an automatic amino acid analyzer (JLC-500/v JEOL, Tokyo, Japan.).

Statistical analysis

All data were statistically analyzed by one-way ANOVA and Tukey's test. Probability values less than 0.05 were considered significant. When two groups were compared, data were analyzed using Student's *t*-test.

Results

Growth and survival of fish

Table 2 shows the results of the feeding trials of larval cod in Expts. I and II. The fish fed rotifers enriched with taurine showed significantly higher growth than those fed rotifers without taurine enrichment in Expt. I. Moreover, the growth of the larvae was effectively improved by elevation of the taurine levels in Expt. II. The results of survival rate did not reflect the taurine enrichment in Expts. I and II.

Fatty acid composition of rotifers and larvae

Tables 3 and 4 show the levels of lipid and major fatty acids in samples of rotifers and larvae in Exp. I. There was no difference in the fatty acids profiles in rotifers and cod larvae irrespective of enrichment with or without taurine. The fatty acid composition of larvae reflected only that of the rotifers. Thus, the fatty acid composition of larvae was not influenced by the enrichment of taurine.

Taurine content of rotifers and larvae

Taurine content of rotifers and larvae are shown in Figs. 1, 2 and 3. Taurine content of rotifers enriched with or without taurine in Exp. I were, 335-447 and 94-139 (mg/100g), respectively (Fig.1). The taurine content in rotifers in Exp. II were increased from 103 to 630 mg/100 g with the taurine enrichment (Fig.3).

The taurine content of larvae in the taurine enrichment group ranged from 400 to 730 mg/100 g, whereas those of no supplemental group in Expt. I ranged from 230 to 280 mg/100 g (Fig.2). Taurine content in larvae in Expt. II was proportionally increased from 118 to 946 mg/100 g with the taurine level of rotifers (Fig.3).

Discussion

It has been well demonstrated that many marine fishes require dietary n-3 highly unsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid as essential fatty acids. These fatty acids are greatly affect growth, survival rate and vitality (Watanabe 1993; Takeuchi 1997). However, regardless of the concentration of essential fatty acids in rotifer and larvae, cod larval growth of the taurine supplemented group was significantly higher than that of the group with no taurine supplementation ($P < 0.05$) in the present study. These observations are the same as that of other fish, such as Japanese flounder, red sea bream and chum salmon (Sakaguchi et al. 1988; Kim et al. 2003; Chen et al. 2004). This result implies that taurine enrichment of rotifers is related to improvement of the growth in cod larvae.

The taurine content of larval cod in the taurine enrichment group were much higher ($P < 0.05$) than the group without taurine supplementation in Expt. I . Taurine contents have been found to be stable during the yolk-sac and mouth opening stage (Fyhn and

Serigstad 1987; Fyhn 1989; Ronnestad et al. 1994). In seed production, taurine content in larval red sea bream and yellowtail decreased in fish larvae after conversion to exogenous feeding (Takeuchi et al. 2001; Matsunari et al. 2003). At the start of exogenous feeding, the larval digestive system is not yet fully developed reviewed by Ronnestad et al., 1999. Furthermore, the digestive enzymes (trypsin-like enzymes, pepsin-like enzymes and amylase) of Pacific cod are very low (Kawai 2001). Larvae that develop gastric digestion during metamorphosis initially assimilate free amino acids more efficiently than amino acids in a polymerized form (Rust et al. 1993; Ronnestad et al. 2000). Therefore, it can be consider that taurine is absorbed more efficiently than more complex nutrients.

Fish larvae have rapid growth compared with that of adult fish, thus larvae have a larger amino acid requirement to maintain both the appropriate concentration in the tissues necessary to obtain an optimal growth rate and amino acid utilization (Tacon and Cowey 1985). It has been found that free amino acid levels of marine invertebrate such as copepods which are food items of larvae in the natural environment has approximate 2 times higher of that in *Artemia* (Helland et al. 2003). Taurine has an important role in osmoregulation in marine invertebrate (Allen and Garrett 1971). *Artemia* and especially rotifers, which are used as live feeds as the initial form of nutrition for many aquacultured fish and invertebrate species, contain markedly lower levels of taurine compared to wild copepods (Conceicao et al. 1997; Oie et al. 1997; Aragao et al. 2004). Few studies have focused on modulation of the protein and amino acid contents and compositions of such prey items to improve their nutritional value for marine fish larvae. *Atremia* can successfully be enriched with free methionine (Tonheim et al. 2000). In case of highest, we succeeded in the enrichment of rotifers contained 6 times higher

level of taurine compared with the group to without taurine supplementation in this study.

It has been suggested that the concentration of free amino acids in animal tissues can be used as a sensitive index to determine the adequate amount of dietary amino acids and to quantify the amino acid requirements of animals (Pion 1976). The whole body essential amino acid profile has been reflected by amino acid requirement in fish (Cowey and Walton 1989) and free amino acid compositions are affected by dietary quality (Kaushik and Luquent 1980). Thus, the tissue free amino acids levels in fish may be useful to estimate the amino acid requirement. In this study, the taurine content in cod larvae proportionally increased with the taurine level in rotifers in Expt. II. More research effort is needed to clarify the requirement level of taurine for cod larvae. Some reports have demonstrated the effect of low-taurine diets in mammals. For example, a taurine-depleted diet does not support normal growth in infant monkeys (Hayes et al. 1980). Cats fed low-taurine diets develop retinal degeneration; similar results have been reported in rats (Hayes et al. 1975; Hageman and Schmidt 1987). In fish, Japanese flounder fed a low taurine diet showed abnormal behavior (Takeuchi 2001) and low taurine diet induces the occurrence of green liver in red sea bream (Goto et al. 2001b).

The survival rates of Expt. II were markedly lower than those of Expt. I. The cause of the higher mortality in fish of Expt. II is unclear. However it seems that the difference might depend on quality of eggs or larvae. Egg quality is a major factor for successful mass production of marine fish (Kjorsvil et al. 1990). Comparing Expt. I to Expt. II, free amino acids (data not shown) content of initial fish in Expt. I were higher than those of Expt. II. It has been shown that free amino acids are predominantly

used as metabolic fuel, and body protein synthesis until the fish larvae convert to exogenous feeding (Ronnestad et al. 1999), and the levels of free amino acids are related to egg viability (Lahnsteiner and Patarnello 2004). Therefore, it is assumed that the difference of the amino acid levels of eggs and larvae were influenced the mortality.

It has been shown that the taurine has an important role in lipid digestion as bile acid conjugate (Haslewood 1967). Bile acids stimulate lipolysis in the gut and esterification of fatty acids. Taurine deficiency also causes significant changes in the liver lipid content and fatty acid distribution in cat liver (Cantafora et al. 1991). In addition, preterm infants fed an addition of taurine to formula feed showed improvement in the absorption of fat especially saturated fatty acids (Galeano et al. 1987). In this study, lipid contents and fatty acid composition of cod were not affected by taurine contents in rotifers. However, adult red sea bream fed substitute protein diets (low-taurine diets) had showed reduced plasma triglyceride and cholesterol levels (Goto et al. 2001b). These observations indicate that taurine also plays an important role in fat digestion via its conjugation with bile acids in fish. Further studies to analyze the effect of the lipid absorption on dietary taurine are required.

The results of the present study suggest that the rotifers supplemented with taurine improved the growth of cod larvae. However, the suitable level of the taurine for cod remains undetermined. More research effort is needed to determine the requirement level of taurine for cod larvae and to clarify the physiological role of taurine in cod.

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マダラ仔魚の成長および体組成に及ぼすタウリン強化ワムシの効果

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タウリンを強化したワムシを用いて、マダラ仔魚に及ぼすタウリンの影響を調べた。実験1では平均全長4.3mmの仔魚にタウリンを400mg/Lの濃度で強化したワムシ及びタウリン無強化のワムシを20日間給餌した。この結果、タウリン強化ワムシは仔魚に対し優れた成長効果を示すとともに、魚体内へのタウリン蓄積量の増加がみられた。実験2では平均全長4.3mmの仔魚に、タウリンを0,400,800,1200mg/*l*の濃度で強化したワムシを25日間給餌した。成長および魚体中のタウリン含量は、タウリン強化量に比例して改善および増加した。これらの結果から、マダラ仔魚へのタウリン強化ワムシ給餌による成長促進が明らかになった。

Figures

Fig. 1. Taurine content of rotifers feeding to Pacific cod *Gadus macrocephalus* larvae in Expt. Ⅰ. Na, Frozen *Nannochloropsis*; Na＋Tau, Frozen *Nannochloropsis*＋400mg/L Taurine; PAQ, Plus Aquaran; PAQ＋Tau, Plus Aquaran＋400mg/L Taurine; SV12, Super fresh Chlorella V12; SV12＋Tau, Super fresh Chlorella＋400mg/L Taurine. Data connected with lines and vertical lines indicate a significant difference (Student's *t*-test, $P < 0.05$) and standard error, respectively. Values are means ± S. D. (n = 4).

Fig. 2. Taurine content of Pacific cod *Gadus macrocephalus* larvae in Expt. Ⅰ. Na, Frozen *Nannochloropsis*; Na＋Tau, Frozen *Nannochloropsis*＋400 mg/*l* Taurine; PAQ, Plus Aquaran; PAQ＋Tau, Plus Aquaran＋400 mg/*l* Taurine; SV12, Super fresh Chlorella V12; SV12＋Tau, Super fresh Chlorella V12＋400 mg/*l* Taurine. Data connected with lines and vertical lines indicate a significant difference (Student's *t*-test, $P < 0.05$) and standard error, respectively. Values are means ± S. D. (n = 2).

Fig. 3. Taurine content of rotifers and Pacific cod *Gadus macrocephalus* larvae in Expt. Ⅱ. SV12, Super fresh Chlorella V12; SV12＋400, Super fresh Chlorella V12＋400 mg/*l* Taurine, SV12＋800, Super fresh Chlorella V12＋800 mg/*l* Taurine, SV12＋1200, Super fresh Chlorella V12＋1200 mg/*l* Taurine. Significant differences (Tukey's test $P < 0.05$) between dietary groups are indicated with different alphabet letters. Values are means ± S. D. (n = 3).

Table 1. Feeding and some of the larvae rearing condition in the experiment of Pacific cod *Gadus macrocephalus*.

	Experiments	
	I	II
Initial larave size	4.28 mm	4.26 mm
Tank capacity	500*l*	500*l*
Number of fish*1	7500-9730 per tank	8684-12368 per tank
Water temperature	7.0-9.1℃	7.0-11.1℃
Circulation rate	100%/day	100%/day
Rotifer density	2 rotifers/m*l*	2 rotifers/m*l*
Feeding period	20 days	25 days

*1 The number of larvea were estimated that sampled counted three point of each rearing tanks.

Table 2. Effect of feeding rotifers enriched with taurine on growth and survival rate of Pacific cod Gadus macrocephalus larvae in Expt. I and II.

Group	Trial period (day)	Initial Total length[*1] (mm)	Final Total length[*2] (mm)	Final Survival rate (%)
Expt. I	20			
Na[*5]			6.7 ± 0.3 [*3]	67.9
Na+Tau[*5]			6.9 ± 0.4 [*3]	52.7
PAQ[*5]		4.3 ± 0.2	6.5 ± 0.4 [*3]	45.2
PAQ+Tau[*5]			6.7 ± 0.4 [*3]	55.8
SV12[*5]			6.5 ± 0.3 [*3]	50.0
SV12+Tau[*5]			6.7 ± 0.3 [*3]	70.0
Expt. II	25			
SV12[*6]			7.3 ± 0.4[a*4]	5.1
SV12+Tau400[*6]			7.9 ± 0.5[b]	10.2
SV12+Tau800[*6]		4.3 ± 0.1	8.1 ± 0.5[b]	11.4
SV12+Tau1200[*6]			8.3 ± 0.4[c]	9.7

*1 Mean ± standard deviation n = 50.
*2 Mean ± standard deviation of duplicate groups of 50 fish each in Expt. I (n = 2), triplicate groups of 50 fish each in Expts. II (n = 3).
*3 Data connected with lines are significantly different (Student's t-test $P < 0.05$)
*4 Significant differences (Duncan's test $P < 0.05$) between dietary groups are indicated with different alphabet letters.
*5 Na; Frozen *Nannochloropsis*, Na+Tau; Frozen *Nannochloropsis* +400 mg/*l* Taurine, PAQ; Plus Aquaran, PAQ+Tau; Plus Aquaran+400 mg/*l* Taurine, SV12; Super fresh Chlorella V12, SV12+Tau; Super fresh Chlorella V12+400 mg/*l* Taurine
*6 SV12; Super fresh Chlorella V12, SV12+Tau400; Super fresh Chlorella V12+400 mg/*l* Taurine, SV12+Tau800; Super fresh Chlorella V12+800 mg/*l* Taurine, SV12+Tau1200; Super fresh Chlorella V12+1200 mg/*l* Taurine

Table 3. Crude lipid and major fatty acid contents in rotifers used for feeding trials in Expt. I (g/100 g, dry weight)[*1]

Fatty acid	Na[*2]	Na+Tau[*2]	PAQ[*2]	PAQ+Tau[*2]	SV12[*2]	SV12+Tau[*2]
18:1	1.53	1.27	3.01	3.02	1.03	0.87
18:2n-6	2.39	2.18	1.83	1.85	3.64	2.92
18:3n-3	0.17	0.12	0.17	0.18	1.52	1.34
20:4n-6	0.53	0.43	0.53	0.59	0.18	0.19
20:3n-3	0.02	0.02	0.02	0.03	0.10	0.08
20:4n-3	0.04	0.03	0.12	0.12	0.06	0.06
20:5n-3	2.43	2.11	1.79	1.89	0.91	0.92
22:5n-3	0.29	0.22	0.56	0.59	0.35	0.35
22:6n-3	nd[*3]	nd	1.13	1.26	0.76	0.67
Crude lipid	17.2	15.3	17.6	18.5	16.4	14.8

*1 Values are means (n = 2)

*2 Na; Frozen *Nannochloropsis*, Na + Tau; Frozen *Nannochloropsis* +400 mg/*l* Taurine,
PAQ; Plus Aquaran, PAQ + Tau; Plus Aquaran+400 mg/*l* Taurine,
SV12; Super fresh Chlorella V12, SV12 + Tau; Super fresh Chlorella V12+400 mg/*l* Taurine

*3 nd = not detected.

Table 4. Crude lipid and major fatty acid content of whole body in cod larvae in Expt. I (g/100 g, dry weight)[*1]

Fatty acid	Initial	Final					
		Na[*2]	Na+Tau[*2]	PAQ[*2]	PAQ+Tau[*2]	SV12[*2]	SV12+Tau[*2]
18:1	2.13	1.13	1.04	1.24	1.64	0.83	0.96
18:2n-6	0.08	0.86	0.89	0.67	0.72	1.12	1.33
18:3n-3	0.03	0.06	0.07	0.07	0.06	0.35	0.40
20:4n-6	0.35	0.67	0.62	0.58	0.68	0.40	0.46
20:3n-3	0.01	0.02	0.01	0.01	0.01	0.05	0.06
20:4n-3	0.04	0.04	0.04	0.07	0.08	0.05	0.06
20:5n-3	2.88	2.68	2.93	2.41	2.14	1.81	2.19
22:5n-3	0.19	0.46	0.38	0.38	0.48	0.33	0.39
22:6n-3	3.50	1.02	0.64	1.26	1.91	1.39	1.46
Crude lipid	16.9	13.3	13.3	14.5	13.9	12.6	14.2

*1 Values are means (n = 2)

*2 Na; Frozen *Nannochloropsis*, Na + Tau; Frozen *Nannochloropsis* +400 mg/*l* Taurine,
PAQ; Plus Aquaran, PAQ + Tau; Plus Aquaran+400 mg/*l* Taurine,
SV12; Super fresh Chlorella V12, SV12 + Tau; Super fresh Chlorella V12+400 mg/*l* Taurine

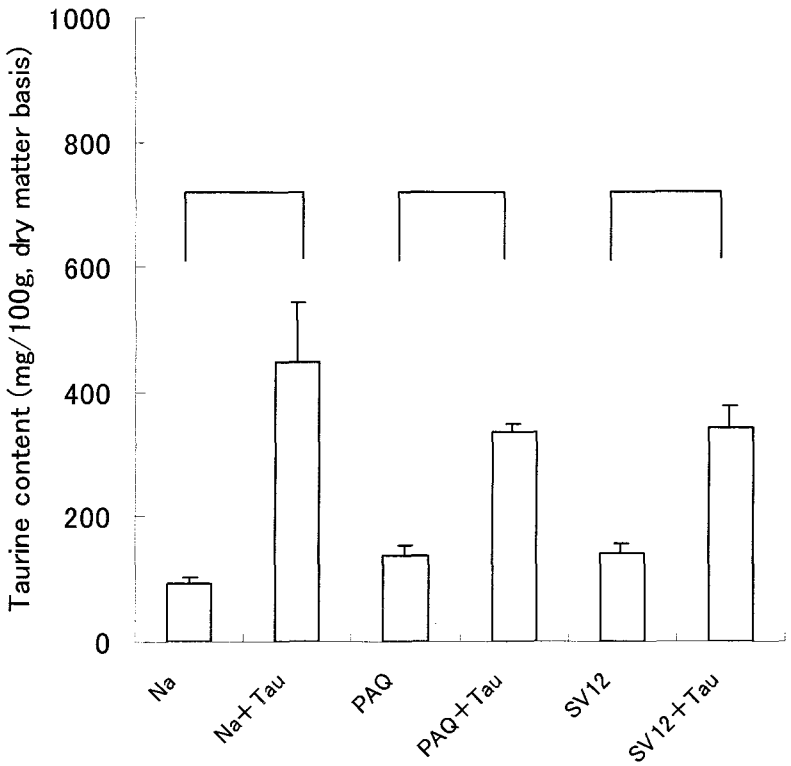
Taurine content (mg/100g, dry matter basis)
1000
800
600
400
200
0
Na
Na+Tau
PAQ
PAQ+Tau
SV12
SV12+Tau

Fig. 1

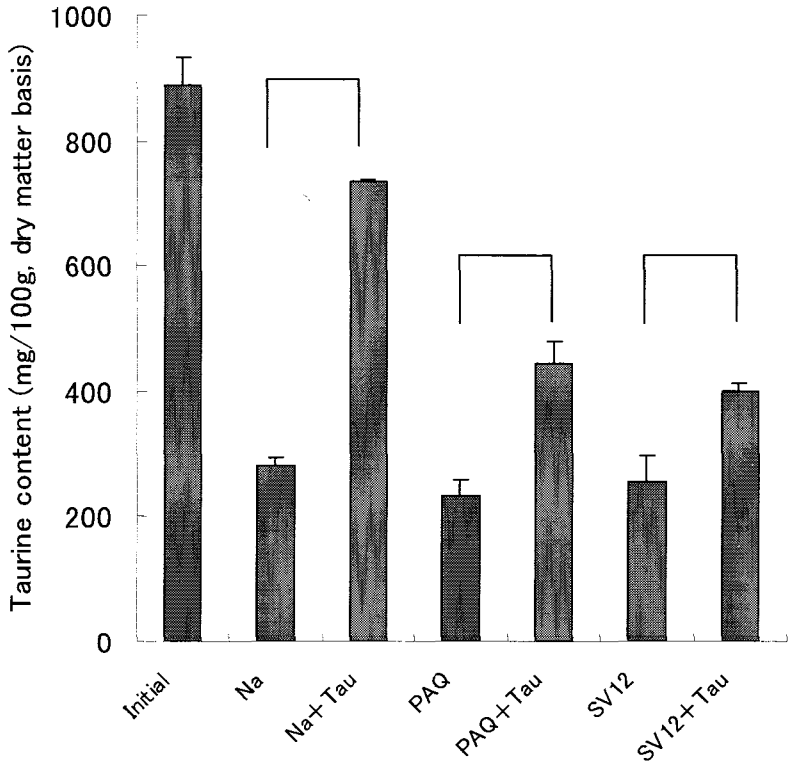
Taurine content (mg/100g, dry matter basis)
1000
800
600
400
200
0
Initial
Na
Na+Tau
PAQ
PAQ+Tau
SV12
SV12+Tau

Fig. 2

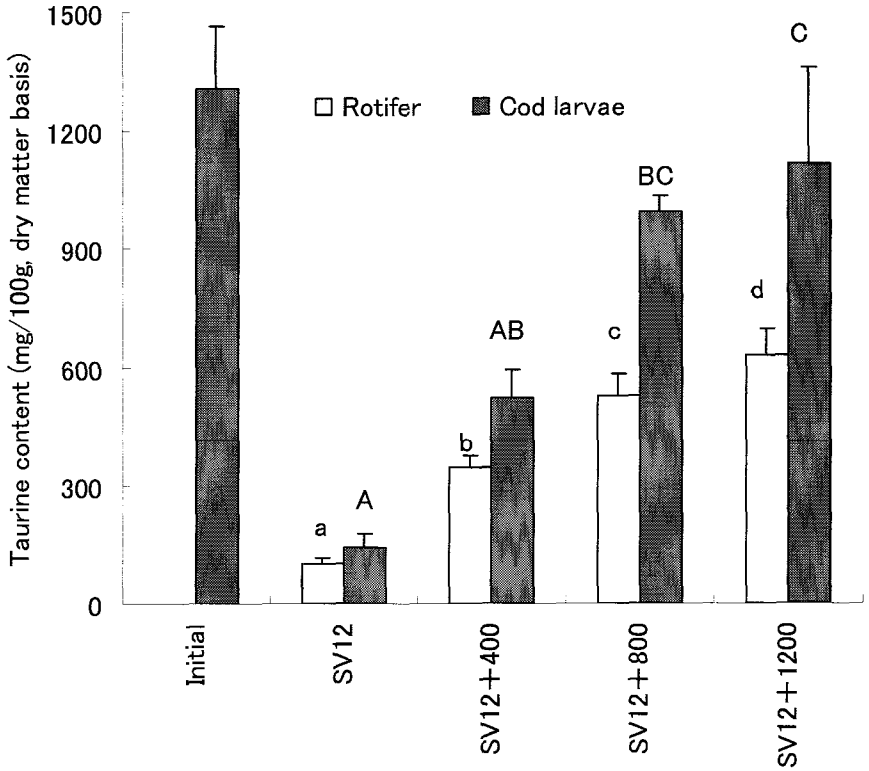
Taurine content (mg/100g, dry matter basis)
1500
1200
900
600
300
0
Rotifer
Cod larvae
a
A
b
AB
c
BC
d
C
Initial
SV12
SV12+400
SV12+800
SV12+1200

Fig. 3

第2章

ブリ用配合飼料へのタウリン添加効果

第1節　飼料中のタウリンがブリ稚魚へ及ぼす影響

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Effect of dietary taurine supplementation on growth performance of yellowtail juveniles *Seriola quinqueradiata*

Effect of taurine on yellowtail juveniles

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ABSTRACT

The effect of taurine on growth of yellowtail juveniles *Seriola quinqueradiata* was investigated by a feeding experiment of diets containing various levels of taurine. Test diets supplemented with 0, 0.5, 1.0, 1.5 and 2.0% of taurine were prepared. These diets were fed to yellowtail juveniles with an initial mean body weight of 0.5g for 6 weeks.

Supplementation of taurine in the diet of yellowtail improved their growth performance significantly ($p<0.05$) over the initial 3-week period. The fish fed the taurine supplemented diet improved in percent gain and feed efficiency over both 3- and 6-weeks. Taurine content in the muscle proportionally increased with the dietary taurine level. The fish fed without supplemented taurine diet showed higher contents of serine in the muscle. With the each increase in the inclusion level of taurine content in the diet, the concentration of serine in the muscle decreased. The cystathionine content in the muscle of each group was unchanged. These results of present study suggest that the supplementation of taurine in the diet not only improves the growth but also affects the sulfur amino acid metabolism of yellowtail juveniles.

Key Words growth, juvenile, metabolism, serine, *Seriola Quinqueradiata*, taurine

ブリ稚魚に対するタウリンの影響

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市販飼料にタウリンを 0, 0.5, 1.0, 1.5, 2.0%添加した飼料を、平均体重 0.5g のブリ稚魚に 6 週間給餌し、ブリ稚魚に及ぼすタウリンの影響について検討した。3 週間目では 1.5%以上添加した区で成長が有意に優れた。タウリン添加区では、3 および 6 週間目ともに増重率が改善した。筋肉中のタウリン含量は飼料中のタウリン含量に比例して増加した。市販飼料区では筋肉中のセリンが増加した。ブリ稚魚では飼料中へのタウリン添加により成長が改善されること、さらに含硫アミノ酸代謝に影響を及ぼすことが明らかになった。

INTRODUCTION

Taurine has been implicated to play roles in bile salt synthesis, osmoregulation, modulation of neurotransmitters and hormone release, antioxidation in mammals.[1] Taurine is also known to be present in various tissues of marine fishes at considerably high concentrations.[2, 3] Although taurine is a well known constituent in fish, there is little information available on its applications to aquaculture. In teleost fishes, taurine is known to be the sole amino acid that conjugates with cholic acid to produce bile salts. [4]

Recent investigations have indicated that taurine is an essential element for some kinds of larval and juvenile marine finfish.[5, 6] For example, supplementation of taurine in the diet improves the growth of the juvenile Japanese flounder[7, 8] and taurine enrichment of rotifers is effective to improve the growth and survival ability in red sea bream larvae.[9]

Yellowtail *Seriola quinqueradiata* is one of the most economically important species for aquaculture in Japan. Many reports on the quantitative and qualitative requirements of amino acids for this species have been published.[10-12] However, the influence of taurine enrichment of diets for yellowtail juveniles is unknown. Previous research has shown that the amount of taurine in wild fish is higher compared to the cultured fish. These results suggest that the live food and the diets used for culture do not satisfy the taurine requirement for yellowtail.[13] The objective of this study is to investigate the effect of dietary taurine supplementation on growth of the yellowtail juveniles

MATERIALS AND METHODS

Diet and fish rearing

Table 1 shows the composition of the experimental diets and their proximate and taurine contents. These diets are prepared to extruded type by Nippon Formula Feed Mfg Co., Ltd. Except for taurine and cellulose content, other ingredient are all the same values. Dietary protein and lipid levels were about 53% and 11%, respectively. Taurine contents in diets 1 to 5 were 391, 860, 1297, 1792 and 2298mg/100g, respectively.

Yellowtail juveniles used in this study were obtained from the Japan Sea-Farming Association, Goto Station. The average weight of the fish at the beginning of the experiment was 0.5g. The fish were divided into 10 groups each of 100 individuals, and each group was assigned to one of the five experimental diets, giving two replicates for each of the five diets. Each of the 10 groups was placed in a 500L polycarbonate tank. Each rearing trial was conducted in a flow-through system (3000mL/min) with natural seawater, and the water temperature was controlled at 22℃. Each experimental diet was given to satiation three times a day throughout the 6-week feeding experiment. After the 3-week feeding trial, the fish were randomly weighed (41-50 fish from each tank) and sampled so finally were remain for future experimental sampling 50 individuals. The remaining fish were fed their respective diets for more 3 weeks. In the end of feeding trial, the fish were weighted and sampled and stored at −20℃ until chemical analyses.

Chemical analysis

The diet and muscle were homogenized with 2% sulfosalicylic acid and centrifuged at 2,300×g for 15 min. The free amino acid levels were determined individually with an automatic amino acid analyzer (model L-8500A; Hitachi, Tokyo, Japan).

Statistical analysis

Data were analyzed statistically by using a one-way ANOVA and Tukey's multiple range test. Probability values less than 0.05 were considered to be significant.

RESULTS

Feeding results

Results of the feeding experiments are shown in Table 2. The 3-week average body weight of yellowtail juveniles was effectively improved by elevating the taurine levels to 1.0% in the diet ($p<0.05$). However, further elevation of the taurine levels to 1.5 and 2.0% did not result in further improvement. Percent gain of 3-week period of the fish fed 1.5 and 2.0% taurine supplemented diets were significantly higher ($p<0.05$) than no supplemented. There was no significant difference in growth, percent gain and feed efficiency over the 6- week period.

Accumulation of free amino acids in yellowtail juveniles

Taurine, histidine, serine and cystathionine in the muscle are shown in Fig.1. Both over 3- and 6-week periods, taurine contents in the muscle increased as the dietary taurine level. Serine contents of the muscle in the fish fed no taurine supplemented diet were much higher than those of the fish fed the taurine-supplemented diets. Cystathionine contents in the muscle were not influenced by the dietary taurine level. In comparison between the 3-week and 6-week periods, the taurine contents in the muscle decreased in all groups in 6-week. These tendencies are clearer in the taurine supplemented groups. In all groups the histidine contents increased with the growth of fish.

DISCUSSION

In the present study, supplementation of taurine to the diet of yellowtail juveniles improved their growth performance over the 3-week period, but over 6-week period growth performance was not significantly related to the taurine supplementation in the diet. These results suggest that taurine has an important role during the early juvenile stage. A similar observation was made for the Japanese flounder.[8]

It has been known that taurine and histidine account for a large proportion of the free amino acids in the muscle of red meat fish. With growth, a reduction of taurine and an increase of histidine contents in white muscle was found in the present study and similar results have been observed in milkfish,[14] and wild yellowtail.[13] Histidine content of juvenile was not affected by dietary taurine level. The most prominent free amino acid in the muscle of adult yellowtail is histidine, which accounted for 90% of total free amino acids and is nearly 20 times higher than the level of taurine.[15] Such high levels of histidine in the white muscle of fish may play a role in buffering capacity for maintaining the intracellular pH of skeletal muscle during burst of swimming.[16] Taurine content in the muscle of the fish fed taurine supplemented diets were much higher than those with taurine non-supplemented diet. The concentration of free amino acids in animal tissues can be used as a sensitive index to determine the adequacy of dietary amino acids and to quantify the amino acid requirements of the animals.[17] A relationship between content of essential amino acid in tissues and dietary requirements has been suggested.[18] In addition, free amino acid compositions are affected by dietary protein quality.[19] Thus, the tissue free amino acid levels in fish may be useful to determine the amino acid requirement. In the present study, for taurine supplemented group from 1.0 to 2.0%, taurine content in muscle were approximately 1800mg/100g in 3-week and

1000 mg/100g in 6-week. In addition, the taurine content in whole body of wild yellowtail juvenile were decreased from 2200 to 1480mg/100g with growth.[13] These results imply that the requirement of juvenile yellowtail for taurine is more than 1000 mg/100g in dry basis of diet and decreases with growth.

The major pathway for taurine synthesis from cysteine in mammals involves the oxygenation of cysteine to cysteinesulfinate, followed by decarboxylation to hypotaurine and then to taurine.[20] However, De La Rosa and Stipanuk suggested that the differences in hepatic cysteinesulfinic acid decarboxylase (EC4.1.1.29) activity account for the observed differences in taurine synthesis.[21] Cat lack considerable amount of the hepatic activity of this enzyme. Taurine is an essential dietary amino acid for cat, which fed low-taurine diets develop retinal degeneration.[22] In fish species, Yokoyama *et al.*[6] reported that cysteinesulfinate decarboxylase activity in Japanese flounder showed a half the activity of rainbow trout and no activity was found for yellowtail fingerling and adult. The rainbow trout can synthesize hypotaurine and taurine from cysteine[23-26] although, juvenile flounder are unable to use dietary cystine for taurine biosynthesis.[7] It is believed that the pathway for taurine synthesis in fish is different among the species.[27-29] Japanese flounder that were fed low taurine diets show increased cystathionine content in tissues.[7] Cystathionine is an intermediate product in the trans-sulfuration pathway from methionine to cysteine. It is supposed that the accumulated cystathionine of those fish may be used for taurine biosynthesis.[8] In this study, the cystathionine content in muscle were not increased in taurine non-supplemented group. It is suggested that in yellowtail juveniles the enzymes activities of intermediate metabolism from methionine to cystathionine are absent or markedly low. In addition, the yellowtail juveniles fed the non-supplemented diet

showed higher serine contents than yellowtail juveniles fed a taurine-supplemented diet. Serine is one of the raw materials for cystathionine biosynthesis in mammals.[30] The relationship between the level of serine in the tissues and dietary taurine level remains to be investigated with yellowtail.

The results of the present study suggest that the diet supplemented with taurine improved the growth of juvenile yellowtail. More research is needed on sulfur amino acid metabolism and to clarify the physiological role of taurine in yellowtail.

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Figures

Fig. 1. Taurine, histidine, serine, and cystathionine contents in the muscle of yellowtail juveniles fed different levels of taurine supplemented diet for 3and 6weeks.

Values without a common superscript letter are significantly different ($P<0.05$).

Data are mean±standard deviation of duplicate groups in each treatment.

Table 1 Composition of the experimental diet for yellowtail (g/100g diet)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	65.1	65.1	65.1	65.1	65.1
Krill meal	15	15	15	15	15
Squid meal	10	10	10	10	10
$Ca(H_2PO_4)_2.H_2O$	1.0	1.0	1.0	1.0	1.0
Vitamin C calcium	0.5	0.5	0.5	0.5	0.5
Choline chloride	1.0	1.0	1.0	1.0	1.0
Betaine	1.0	1.0	1.0	1.0	1.0
Synthetic taurine	0.0	0.5	1.0	1.5	2.0
Cellulose	2.0	1.5	1.0	0.5	0.0
Beer yeast	2.0	2.0	2.0	2.0	2.0
Fe(peptide)	0.01	0.01	0.01	0.01	0.01
Bile powder	0.1	0.1	0.1	0.1	0.1
Vitamin mixture	1.5	1.5	1.5	1.5	1.5
Mineral mixture	0.8	0.8	0.8	0.8	0.8
Starch	4.00	4.00	4.00	4.00	4.00
Squid oil	3.50	3.50	3.50	3.50	3.50
DHA oil (EPA,6.3%; DHA,27.0%)	3.50	3.50	3.50	3.50	3.50
Total	111.0	111.0	111.0	111.0	111.0
Analytical contents(dry matter basis)					
Taurine (mg/100g)	391	860	1297	1792	2298
Crude protein(%)	52.4	52.7	52.5	53.6	54.5
Crude lipid(%)	10.9	11.0	11.0	11.1	11.3

Table 2 Results of the 6 weeks feeding trial for yellowtail

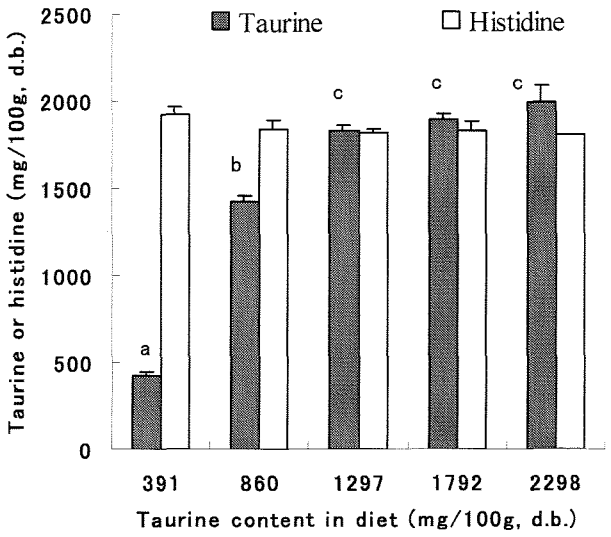
Diet no.	Taurine content in diet (mg/100g)	Initial[*1]	3 weeks				6 weeks			
		Body weight (g)[*2]	Body weight (g)[*2]	Percent gain	Feed efficiency	Survival rate (%)	Body weight (g)[*3]	Percent gain	Feed efficiency	Survival rate (%)
1	391		9.5±1.9[a*4]	1641[a]	1.49	95.5	42.1±5.5	7659	1.48	95.5
2	860		10.0±2.0[ab]	1735[ab]	1.52	92.5	42.6±6.4	7747	1.47	92.5
3	1297	0.5±0.1	10.5±1.9[b]	1831[ab]	1.55	94.0	42.6±6.5	7746	1.47	94.0
4	1792		10.8±2.2[b]	1896[b]	1.55	94.0	44.0±6.6	8002	1.49	94.0
5	2298		10.8±1.7[b]	1881[b]	1.64	97.5	44.3±6.1	8062	1.49	97.5

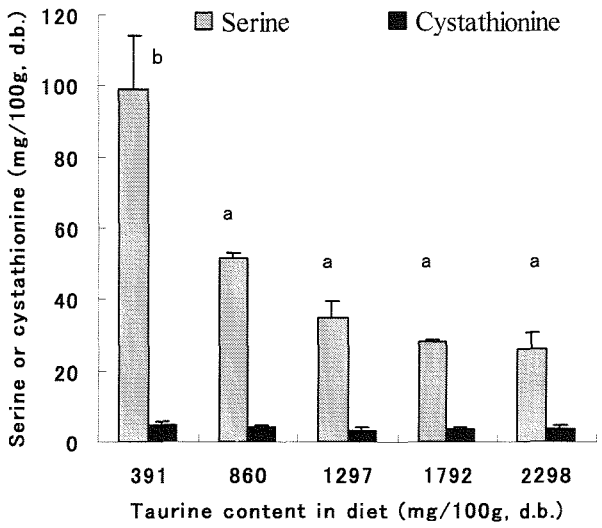
*1:Mean±standard deviation (n=100).

*2 Mean±standard deviation of duplicate groups of 41-50 fish

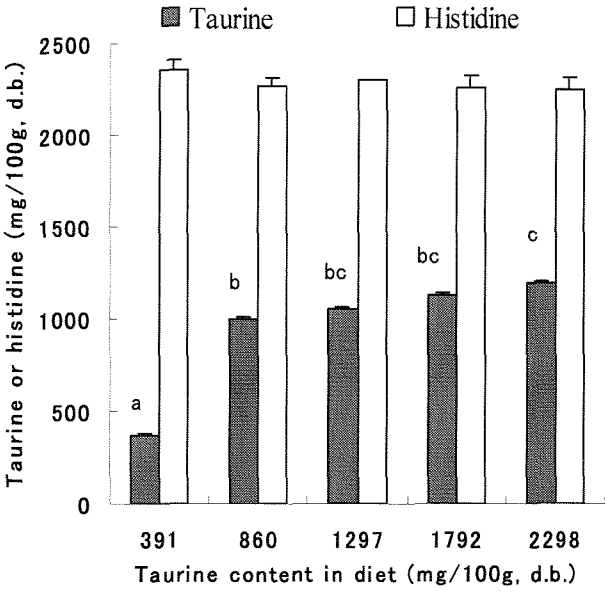
*3 Mean±standard deviation of duplicate groups of 50 fish

*4: Significant differences (Tukey's multiple range test) between dietary groups are indicated with different alphabet letters.


Taurine
Histidine
Taurine or histidine (mg/100g, d.b.)
2500
2000
1500
1000
500
0
a
b
c
c
c
391
860
1297
1792
2298
Taurine content in diet (mg/100g, d.b.)


Serine
Cystathionine
Serine or cystathionine (mg/100g, d.b.)
120
100
80
60
40
20
0
b
a
a
a
a
391
860
1297
1792
2298
Taurine content in diet (mg/100g, d.b.)

6 week


Taurine
Histidine
Taurine or histidine (mg/100g, d.b.)
2500
2000
1500
1000
500
0
a
b
bc
bc
c
391
860
1297
1792
2298
Taurine content in diet (mg/100g, d.b.)

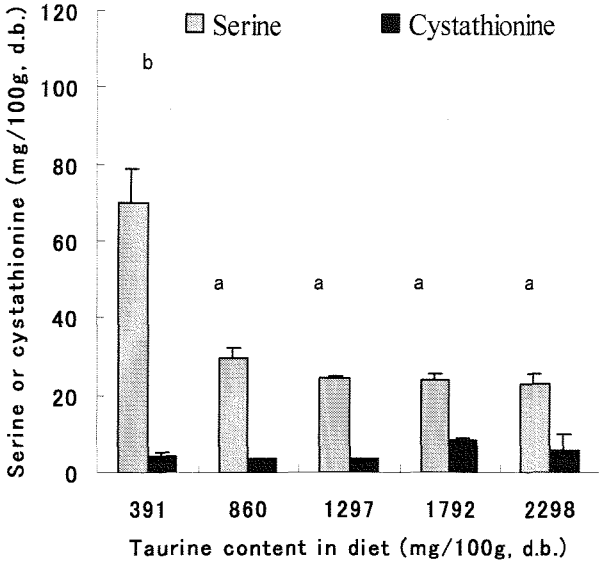

Serine
Cystathionine
Serine or cystathionine (mg/100g, d.b.)
120
100
80
60
40
20
0
b
a
a
a
a
391
860
1297
1792
2298
Taurine content in diet (mg/100g, d.b.)

Fig.1

第2章

ブリ用配合飼料へのタウリン添加効果

第2節　ブリ親魚の産卵に及ぼすタウリン添加飼料の影響

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Effects of taurine levels in broodstock diet on the reproductive performance of yellowtail *Seriola quinqueradiata*

Effect of taurine on yellowtail broodstock

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ABSTRACT

The effect of dietary taurine was investigated on reproductive performance in the yellowtail *Seriola quinqueradiata*. Two-year-old fish of average body weight of 6.1 kg fed on the diets containing three levels of taurine (T-0%, 0.5% or 1.0%) for 5 months prior to spawning. For spawning investigations, fish were induced to maturity by human chorionic gonadotropin injection (600 IU/kg-fish) and artificially inseminated.

The oocyte growth improved significantly ($p<0.05$) with the increase of dietary taurine. The collection of eggs from females reared on the T-0% diet was not successful. The success rate of spawning for females fed on T-0.5 and 1.0% diets was one out of 6 and 6 out of 7, respectively. The taurine level of the liver and serum in the T-0% diet group was much lower than that in the T-0.5 and 1.0% diets group ($p<0.05$). The fish fed T-0% diet showed higher contents of serine in the liver and serum. The taurine content of the ovary was not significantly different among the different dietary treatments. These results indicated that taurine has a positive effect on the improvement of spawning performance of yellowtail.

Keywords broodstock, reproductive performance, *Seriola quinqueradiata*, taurine, yellowtail

INTRODUCTION

Yellowtail is one of the most important fish in Japanese aquaculture. The total production of yellowtail was about 106,000 tons in 2003. However, fingerlings of yellowtail known as mojako are still entirely dependent on the collection of wild fish.[1] Researchers have strived to establish and develop of techniques for mass production of the juveniles of this species. One of the most important aspects in seed production is the production of fertilized eggs, which yield larvae with high survival and growth rate. It has been reported that the nutritional composition and quality of broodstock diets greatly influence egg and larval quality.[2] A number of studies on the broodstock nutritional requirements of this species have been conducted. At the start of these studies, the yellowtail broodstock were fed raw fish. Dry pellets are ideal for broodstock management because their composition can be controlled and they are able to avoid the risk of disease transmission from the raw fish. Mushiake *et al.* reported that dry pellets were effective for broodstock management of yellowtail.[3] Verakunpiriya *et al.* revealed the effects of astaxanthin levels on the spawning performance, and they suggested that the optimal supplemental level was around 30 ppm (mg/kg diet) for yellowtail broodstock.[4]

Taurine is one of the most abundant free amino acids in fish and mammalian tissues. Taurine has been implicated to play roles in osmoregulation, modulation of neurotransmitters, hormone release, antioxidation, modulation of cellular calcium levels and conjugation with bile acids in mammals.[5] Some studies have established taurine to be essential for reproduction in mammals. Female cats fed the taurine-free diet have poor reproductive performance such as fetal abortion and stillbirth, on the other hand, the cat fed taurine supplemented diet throughout gestation usually experienced no

difficulties in completing normal pregnancies.[6, 7] Mice with a disruption of the taurine transporter gene leads to reduced fertility.[8] Previous research has shown that the amount of taurine in yellowtail is affected by the dietary taurine contents.[9] In addition, juvenile yellowtail fed diets supplemented with taurine showed improved growth and taurine content of the whole body increased proportionally with the increase in dietary taurine level.[10] So far no experiment was conducted to influence the taurine broodstock diet on the reproductive performance of yellowtail. In this study, we investigated the effect of different dietary taurine levels in the broodstock diet on the spawning success of yellowtail.

MATERIALS AND METHODS

Broodstock

Two-year-old yellowtail having average body weight of 6.1 kg and fork length of 66 cm were used. Five months before the spawning manipulation, the fish were split into three groups each of 14 fish (ratio of male: female, 5:9, 8:6, 7:7 for T-0%, T-0.5% and T-1.0%, respectively) and the respective experimental diets were given. Each group was separately stocked into 5m×5m×5m floating net cages belonging to the Goto Station of National Center for Stock Enhancement in Nagasaki Prefecture. Water temperature ranged from 13.5 to 18.7℃ (Fig.1). They were fed to near satiation for three times a week, from December 2001 to May 2002.

Experimental diets

Table 1 shows the composition of the experimental diets and their proximate and taurine contents. Fish meal contains approximately 500-700 mg/100g dry basis of taurine, in

contrast plants protein such as soybean meal and corn gluten included a trace amount of taurine.[11] In order to formulate low taurine diet, 35% of soybean meal and corn gluten meal were used for protein source. Except for taurine and wheat flour content, other ingredients did not vary among the diets. These diets were prepared as an extrude type by Nippon Formula Feed Mfg. Co., Ltd., (Kanagawa, Japan). The experimental diet was supplemented at the levels of 0, 0.5, and 1.0% taurine are designated as T-0%, T-0.5% and T-1.0%, respectively. Dietary protein and lipid levels were about 43% and 24%, respectively. Taurine contents in diet, T-0%, T-0.5% and T-1.0% were 174, 730 and 1234mg/100g, respectively.

Examinations of ovarian maturation

Examinations of the maturation state of ovaries were conducted on the 0, 79, 108 and 145th days. A piece of ovarian tissue was sampled by inserting a cannula into the genital pore of females individually. The diameters of 50 sampled eggs per fish were measured under a stereoscopic microscope and the mean was calculated.

Spawning manipulation

The fish were injected with HCG (human chorionic gonadotropin) at a dosage of 600 IU/kg-fish and they were immediately transferred and stocked separately into $90m^3$ indoor spawning concrete tanks on land at 19℃ for 48h. After 48h of the HCG injection, milt was pooled from male broodfish of each test diet group and then preserved in an ice-cooler box, and ovulating eggs of individual female broodfish of each test diet group were obtained by abdominal stripping and they were fertilized separately with spermatozoa from the same group by the dry method as described by

Mushiake *et al.*[12] After the spawning, the liver and oocytes of female fish were stored at –20℃ until extraction of free amino acids (FAAs) from the tissues.

Investigation of spawning and the evaluation of egg quality

The eggs were transferred to appropriate measuring cylinders and the volumes of floating and negatively buoyant eggs were measured. The fertilization rate, abnormality rate, egg diameter, and oil-globule diameter of buoyant eggs were determined, using a profile-projector at the morula stage.

Chemical analysis

The extraction of FAAs from diets, liver, and oocyte tissue was carried out by homogenization with 2% sulfosalicylic acid and centrifugation at 2,300 ×g for 15 min. The plasma samples were deproteinized by the addition of an equal volume of 10% sulfosalicylic acid, and centrifuged at 10,000 rpm for 10 min at 5℃ as described by Yamamoto *et al.*[13] Free amino acid levels were determined individually with an automatic amino acid analyzer (model L-8500A; Hitachi, Tokyo, Japan).

Statistical analysis

Data were analyzed using one-way ANOVA and Tukey's multiple range test. All statistical analyses were conducted by using the SPSS 11.0 microcomputer software package (SPSS, Chicago, IL, USA).

RESULTS

Spawning and egg quality

Figure 1 shows changes in oocyte diameter with time. The mean oocyte diameter was

distributed 128.5 μ m at the start of each experiment. The mean oocyte diameters on the 145th day were 311.8, 438.6 and 545.1 μ m for T-0%, T-0.5% and T-1.0%, respectively. Table 2 shows the results of spawning. The eggs of females reared on the T-0% diet were not successfully collected. The success of spawning on females fed on T-0.5% and T-1.0% diets, were one out of 6 and 6 out of 7, respectively. The buoyancy, fertilization and hatching rates of the broodstock fed the T-1.0% diet were higher than those of T-0.5% diet.

Chemical analysis

Figure 2 shows the taurine, serine and cystathionine levels of the liver, serum and ovary in female broodstock at the end of the experiment. The taurine content of the liver and serum of the group fed T-0% diet was significantly lower than that of the other two groups. The serine levels of liver and serum of the fish fed T-0% diet were significantly higher than those of the other two groups. The levels of taurine, serine and cystathionine of oocytes were independent of the dietary treatments.

DISCUSSION

The present study showed that the ovarian maturation was accelerated significantly (p<0.05) with increased dietary taurine. Therefore, poor maturation in yellowtail was induced by T-0% or T-0.5% diet. This result provides the first evidence of the importance of the role of dietary taurine in reproductive performance in yellowtail. The ovary of the yellowtail is reported to mature at water temperature ranged from 14 to 19℃, and to be accelerated more rapidly in higher temperature.[14] Although the oocyte diameter did not show any difference until 108th day an increase of oocyte diameter

was accelated from 108 to 145th day. These differences might be related to the elevated water temperature (Fig.1).

The percentage of buoyant eggs and fertilized eggs are important parameters for evaluating the egg quality of marine fish. A deficiency of essential fatty acids in the broodstock diet for red sea bream *Pagrus major* decreased the percentage of buoyant eggs and the hatchability.[15] Japanese flounder *Paralichthys olivaceus* fed a low n-3 HUFA diet show a lower percentage of buoyant eggs than those fed a high amount of n-3 HUFA diet.[16] Yellowtail fed a diet supplemented with approximately 30 mg/kg diet astaxanthin improves the egg quality and the final number of normal larvae.[4] The percentage of buoyant eggs, fertilization eggs and hatching rate of the broodstock fed the T-1.0% diet were higher than those of T-0.5% diet. These results indicate that the egg qualities were improved with the addition of at least 1.0% of taurine to the diet of yellowtail broodstock. In other word, the optimal level of yellowtail broodstock for taurine is more than 1000 mg/100g in dry basis of low content of fish meal diet.

The surviving kittens from taurine-depleted mothers have been reported to have a reduced growth rate and a number of neurological abnormalities.[9] In the present study, the relation between larval quality and dietary taurine was not investigated, however further research on larval quality is being carried out.

The effects of dietary sulfur amino acid on the taurine accumulation in tissues of fish are known in rainbow trout *Oncorhynchus mykiss* and Japanese flounder. The taurine levels in the liver and plasma increased as the levels of sulfur-containing amino acids increased in diet, suggesting a considerable synthesis of taurine from sulfur amino acid in rainbow trout.[17-19] These observations are in accordance with the results found in Atlantic salmon *Salmo salar* L.[20] However, juvenile flounder are unable to use dietary

cystine for taurine biosynthesis.[21] The taurine levels of the whole body and tissues increased with the increase in dietary taurine. In addition, the supplementation of taurine in the diet improves the growth of the Japanese flounder. These results indicate that juvenile Japanese flounder require taurine in their diets.[22] The taurine content of the liver and serum of fish fed T-0.5 and T-1.0% diets were much higher ($p<0.05$) than that of T-0% diet. These observations are in agreement with the results from juvenile yellowtail.[10, 23] Moreover, the hepatic activities for taurine biosynthesizing enzymes in yellowtail were quite low irrespective of the dietary taurine levels.[23] These data may indicate that yellowtail are not able to synthesize taurine effectively.

In this study, significantly higher serine concentrations of the broodstock fed the T-0% diet were found compared to those fed T-0.5 and T-1.0% diets. This result confirms previous investigations in juvenile yellowtail.[7] Serine is one of the important materials in the trans-sulfuration pathway from homocysteine to cystathionine in mammals.[24] Japanese flounder fed low taurine diets show increased cystathionine contents in tissues.[22] In contrast, the cystathionine levels of yellowtail were not affected by the dietary treatments. There is a possibility that the enzyume activities during intermediate metabolism from homocysteine to cystathionine are absent or markedly lower in yellowtail. Cystathionine β -synthase catalyzes the condensation of homocysteine and serine to cystathionine in an irreversible reaction. The trans-sulfuration of homocysteine and cysteine is catalyzed by cystathionine β -synthase and cystathionine γ -lyase.[25] Some studies have reported that the cystathionine γ -lyase activity apparently occurs in the liver of fish and differs among species,[26,27] but the cystathionine β -synthase activity is not clear in fish. Further experiments are necessary to clarify the disrtribution of cystathionine β -synthase

activity in fish.

In conclusion, this study shows that the dietary taurine levels in the broodstock diet affects the spawning success of yellowtail. Yellowtail fed high level taurine diets had significantly higher oocyte growth than that fed low taurine diet. However, the suitable level of the taurine for yellowtail broodstock remains undetermined. More research is needed to determine a suitable level of the taurine for yellowtail broodstock diet and clarify the physiological role of taurine in yellowtail.

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Figures

Fig. 1. Water temperature (upper panel) and the changes of mean oocyte diameter (lower panel) during maturation of yellowtail.

Significant differences (Tukey's test $p < 0.05$) between dietary groups are indicated with different alphabet letters. T-0 results represent mean ± S.D. (n=5); T-0.5 and T-1.0, results represent mean ± S.D. (n=7).

Fig. 2. Taurine, serine, and cystathionine contents in the liver, oocyte and serum of yellowtail broodstock fed different levels of taurine supplemental diet. T-0 Results represent mean ± S.D. (n=5); T-0.5 and T-1.0, results represent mean ± S.D. (n=7). Significant differences (Tukey's test $p < 0.05$) between dietary groups are indicated with different alphabet letters.

Table 1 Composition of the experimental diet for yellowtail (g/100g diet)

Ingredient (%)	T-0	T-0.5	T-1.0
Wheat flour	2.00	1.37	0.73
Tapioca starch	8.00	8.00	8.00
Soybean meal	24.00	24.00	24.00
Corn gluten meal	11.00	11.00	11.00
Fish meal	40.00	40.00	40.00
Yeast	10.00	10.00	10.00
$Ca(H_2PO_4)_2.H_2O$	2.00	2.00	2.00
Betaine	0.15	0.15	0.15
Vitamin E	0.02	0.02	0.02
Choline chloride	0.80	0.80	0.80
APM[1]	0.03	0.03	0.03
Marigold	0.20	0.20	0.20
Yeast extract	0.50	0.50	0.50
Synthetic taurine	0.00	0.64	1.27
Vitamin mixture	1.00	1.00	1.00
Mineral mixture	0.30	0.30	0.30
Feed oil	18.90	18.90	18.90
Palm olein	8.10	8.10	8.10
Total	127.00	127.00	127.00
Analytical contents (dry matter basis)			
Taurine (mg/100g)	174	730	1234
Crude protein (%)	43.2	43.7	43.5
Crude lipid (%)	24.7	24.9	25.2

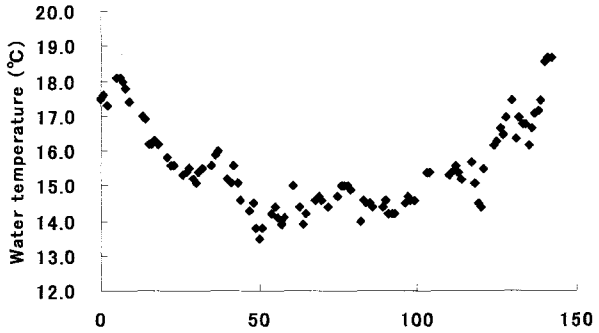
[1] Ascorbyl-2-P/Mg (Hospitan C, Showa Denko K K.).

Table 2 Results of artificial insemination and egg collection in yellowtail fed different levels of taurine diets

Diet		T-0	T-0.5	T-1.0[1]
Spawning success		0/5	1/7	6/7
Total eggs collected	($\times 10^3$)	-	28.0	106.2 ± 82.1
Buoyant eggs	($\times 10^3$)	-	14.0	81.7 ± 60.2
Buoyancy rate	(%)	-	50.0	79.4 ± 11.3
Fertilization eggs	($\times 10^3$)	-	4.1	64.1 ± 55.3
Fertilization rate	(%)	-	29.0	67.8 ± 22.0
Egg diameter	(μm)	-	1151.0	1105.7 ± 22.9
Oil droplet diameter	(μm)	-	274.6	283.2 ± 16.3
Abnormal eggs[2]	(%)	-	100	75.8 ± 24.3
Hatching rate	(%)	-	6.1	33.0 ± 29.8

[1] Results represent means and S.D. (n=6).

[2] Egg with more than one oil-globule.


Water temperature (℃)
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
0
50
100
150

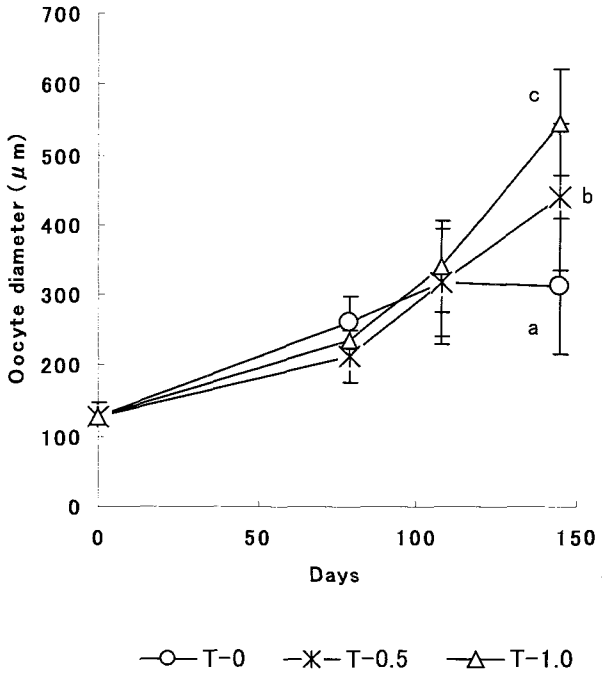

Oocyte diameter (μm)
700
600
500
400
300
200
100
0
0
50
100
150
Days
c
b
a
T-0
T-0.5
T-1.0

Fig.1

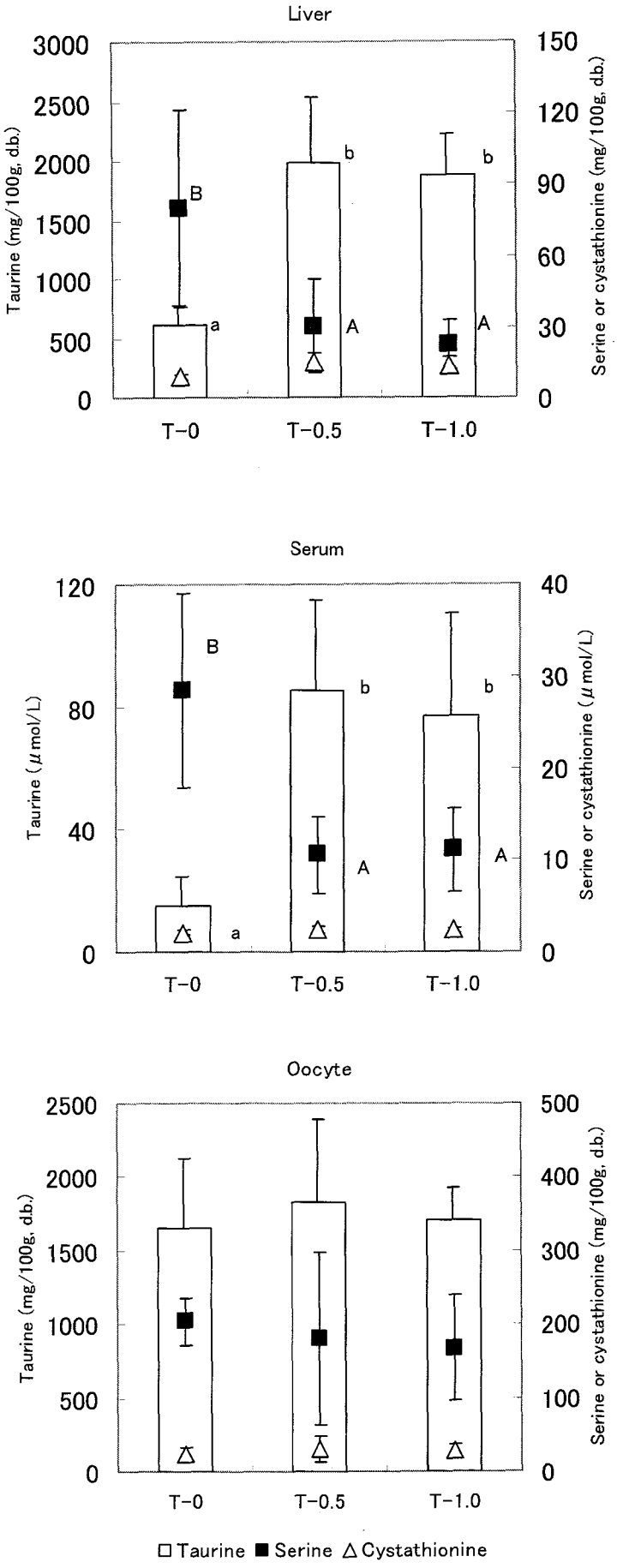

Liver
Taurine (mg/100g, d.b.)
Serine or cystathionine (mg/100g, d.b.)
3000
2500
2000
1500
1000
500
0
150
120
90
60
30
0
B
a
b
A
b
A
T-0
T-0.5
T-1.0
Serum
Taurine (μmol/L)
Serine or cystathionine (μmol/L)
120
80
40
0
40
30
20
10
0
B
a
b
A
b
A
T-0
T-0.5
T-1.0
Oocyte
Taurine (mg/100g, d.b.)
Serine or cystathionine (mg/100g, d.b.)
2500
2000
1500
1000
500
0
500
400
300
200
100
0
T-0
T-0.5
T-1.0
□ Taurine ■ Serine △ Cystathionine

Fig.2

第3章

マダイ稚魚に対するタウリン添加精製飼料の影響

第1節　飼料中のタウリンおよびシスチンがマダイ稚魚の摂餌行動に及ぼす影響

Effect of dietary taurine and cysteine levels on growth performance and feeding behavior of red sea bream *Pagrus major*

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INTRODUCTION

In acuacluture, fish meal have been used as a basic ingredient in commercial diet for fish. The increasing demand, price and world supply fluctuations of fish-meal has emphasized the need to look for alternative protein sources. Many studies have been conducted to use of alternative proteins as a replacement for fishmeal in many kinds of fish. The red sea bream fed SPC-based diets were not efficient in maintaining normal growth and revealed symptoms of green liver (Takagi et al., 1999). Similarly, the juvenile of yellowtail fed the non-fish meal diets was appeared green liver (Maita et al., 1997; Watanabe et al., 1998). Recent investigations have indicated that the low hepatic taurine level was one of the probable factors responsible for the occurrence of green liver in red sea bream fed substitute protein diets (Goto et al., 2001c). Moreover, the green liver syndrome and inferior growth performance of red sea bream and yellowtail fed diets without fishmeal were evaluated in relation to dietary taurine concentration (Takagi et al., 2005; 2006).

Taurine is one of the most abundant free amino acids in many tissues, but it is not incorporated into protein. Taurine had already been implicated to play an important role in numerous physiological functions including: bile salt synthesis, osmoregulation, modulation of neurotransmitters, membrance stabilization, and antioxidation in mammals (Huxtable, 1992). The major pathway for taurine synthesis from cysteine in mammals involves the oxygenation of cysteine to cysteinesulfinate, followed by decarboxylation to hypotaurine and then to taurine. The activities of hepatic cysteinesulfinic acid decarboxylase needed for the enzyme-catalyed step in taurine biosynthesis were differences by species, age and sex (Worden and Stipanuk, 1985). Several studies have indicated that there are interspecific differences in the pathway and

capacity of taurine biosynthesis in fish (Goto et al. 2001a; Goto et al. 2003). The rainbow trout have the capacity to synthesize taurine from cysteine (Yokoyama et al. 1997). The enzyme activity of cysteinesulfinate decarboxylase which is the rate limiting step to synthesize taurine in red sea bream and Japanese flounder is a half of that of the rainbow trout (Yokoyama et al. 2001). The juvenile flounder fed the diets supplemented with taurine showed improved growth and taurine content of the whole body proportionally increased with the increase in the dietary taurine level. (Park et al. 2002; Kim et al. 2003). These results indicate that taurine requirements could vary with species.

In the present study, the feeding experiments were conducted to low fishmeal diets formulated with casein. We investigated the effect of taurine deficiency and the effect of dietary taurine on the growth, body composition and feeding behavior of red sea bream juveniles.

MATERIALS AND METHODS

Diet

The composition of the experimental diets I and II and their proximate and taurine contents in Tables 1 and 2, respectively. The experimental diets were based on casein, and gelatin as the main protein source. The experimental diet was supplemented at the levels of 0 and 1.0 % taurine in Experiment I , and 0, 0.5, 1.0, 2.0 % taurine and 1.0, 2.0% cysteine in Experiment II . All ingredients were well mixed together with distilled water, mixed to make a mash, pelleted with a press machine, and then dried for 24h in a freeze-dryer (Nissei Co., Ltd., Tokyo, Japan)

Fish and feeding

In Experiment Ⅰ, red sea bream juveniles used in this study were obtained from the Fisheries Research Division, Mie, Prefectural Science and Technology Promotion Center, Owase Station. The average weight of the fish at the beginning of the experiment was 2.3g. The fish were divided into 4 groups each of 30 individuals, and each group was assigned to one of the two experimental diets, giving two replicates for each of the two diets. In Experiment Ⅱ, red sea bream juveniles used in this study were obtained from the Fish Nursery Center of Kinki University. Susami, Japan. The average weight of the fish at the beginning of the experiment was 2.5g. The fish were divided into 12 groups each of 30 individuals, and each group was assigned to one of the two experimental diets, giving two replicates for each of the two diets. Each of the groups was placed in a 100L polycarbonate tank. Each rearing trial was conducted in a flow-through system (3000mL/min) with natural seawater, and the water temperature was controlled at 20℃. Each experimental diet was given to satiation three times a day throughout the 6-week feeding experiment. In the end of feeding trial, the fish were weighted and sampled and stored at -20℃ until chemical analyses.

Chemical analysis

The extraction of FAAs from diets, and whole body tissue was carried out by homogenization with 2% sulfosalicylic acid and centrifugation at 2,300 ×g for 15 min. free amino acid levels were determined individually with an automatic amino acid analyzer (JLC-500/v JEOL, Tokyo, Japan).

Feeding behavior observation

At the end of the feeding trial, three larvae from each of the experimental tanks were placed in a transparent 60L square tank to observe the feeding behavior. Fish feeding behavior was recorded for about 10 min for three times using a CCD camera (Matsushita Electric Industrial Co., ltd., Osaka, Japan). The video recordings were analyzed the number of times in ingestions and handlings. Feeding behavior of red sea bream can be broken into classes of activities: the rate of ingestion and handling. Ingestion was expressed as the ingestion times of fish/ 5 min. Handling was expressed as the handling times fish/ 5 min.

Statistical analysis

Statistical analyzed of growth performances, free amino acid concentrations and the feeding behavior are compared by one-way ANOVA and Tukey's test. When two groups were compared, data were analyzed using Student's *t*-test. For all statistical analyses, a SPSS 11.0 microcomputer software package (SPSS, Chicago, IL, USA) was used. In all statistical testing, differences at $P<0.05$ were considered as significant.

RESULTS

Feeding results

Results of the feeding experiments are shown in Table 2. In Experiment Ⅰ, supplementation of taurine in the diet of red sea bream improved their growth performance significantly ($p<0.05$). The fish fed the 1.0% taurine supplemental diet showed significant effects on weight gain, specific growth rate, and feed efficiency than the fish fed control diet ($p<0.05$). In Experiment Ⅱ, fish fed the taurine supplemental

diet had significantly higher SGR and WG, and feed efficiency. However, no significant treatment effects were found the fish fed the cysteine supplemental diet.

Accumulation of free amino acids in red sea bream juveniles

Taurine, serine and cystathionine in the muscle are shown in Fig.1. In Experiment Ⅰ, taurine content in the whole body of taurine-free group was decreased only 4.0% of that of taurine group. Serine contents of the whole body in the fish fed no taurine free diet were much higher than those of the fish fed the taurine-supplemented diet. The levels of cystathionine of the whole body were independent of the dietary treatments. In Experiment Ⅱ, taurine content of the whole body increased significantly with increasing dietary tuarine levels, while was independent of dietary cystine levels. The whole body of fish fed control diet show the lowest taurine level, while fish fed Tau 2.0% diet showed the highest value.

Feeding behavior

The ingestion times were significantly higher in fish fed the 1.0 and 2.0% taurine diets than those of control diet. The handling/ingestion rate was significantly higher in fish fed the 1.0 and 2.0% cystine diets than those of control diet.

DISCUSSION

In the present study, the red sea bream fed the taurine supplemental diet was significantly higher growth performance than that of no taurine supplementation ($p<0.05$). There observations are similar to other report (Park et al., 2002; Kim et al., 2005; Matsunari et al., 2005). The results indicate that taurine is an important element

for growth of juvenile red sea bream, and is able to utilize crystalline taurine.

The whole body taurine content of taurine supplement group is higher than in the no supplement group in Experiments I and II. Takagi et al., reported the hepatic taurine levels increased with dietary taurine levels. The taurine level of red sea bream were easily affected the taurine content in the diets.

In Experiment I, the tissue serine level of the fish fed taurine-free diet was significantly higher than those of taurine diet. Moreover, the dietary taurine level did not affect the cystathionine contents of tissues in red sea bream. The juvenile flounder fed the low taurine diet are increased in cystathionine. Hence, juvenile flounder are able to metabolized cystathionine (Kim et al., 2003). These observations attempted that there are low capacity of cystathionine biosynthesis in red sea bream.

Several studies have indicated that there are interspecific differences in the capacity of taurine biosynthesis from cystine in fish. The taurine levels in the liver and plasma increased as the levels of not only taurine but also cystine increased in diet, suggesting a considerable synthesis of taurine from cystine in rainbow trout (Walton et al. 1982; Yokoyama and Nakazoe., 1998). In contrast, juvenile flounder are unable to use dietary cystine for taurine biosynthesis, the taurine levels of the whole body and tissues increased only with the increase in dietary taurine (Park et al. 2002). Taurine contents of the fish were independent of dietary cystine levels. The taurine biosynthetic enzyme activities of the red sea bream were very low. These data may indicate that red sea bream are not able to synthesize taurine effectively.

Some studies on the alternative protein have reported that the free or low fish meal diets have poor palatability. The reduction in diet palatability usually results in decrease in feed intake. In this study, the times of ingestion were significantly higher in fish fed

the 1.0 and 2.0% taurine diets than those of control diet. The sea bass were free to access the different levels of taurine diets selected more actively the 0.2 and 0.3% than the 0 and 0.1% taurine-supplemented diets. The amount of taurine 0.2 and 0.3% supplemented diet feed delivered from self-feeders were significantly increased (Martinez et al., 2004). These results may indicate that the taurine improves the palatability of experimental diet. Although the extract of marine worm glycine, alanine, lysine, valine, glutamic acid and arginine, act as strong chemical stimulants, taurine was judged to be a weak stimulant for red sea bream (Fuke et al, 1981). Taurine in marbled rockfish have adverse effects in palatability having been reported (Takaoka et al., 1990). These results indicate that taurine do not act as chemical stimulants. And the increase of feed intake of the fish fed taurine supplemental diet is cased by another reason.

The results of the present study suggest that the diet supplemented with taurine improved the growth of red sea bream. More research is needed on sulfur amino acid metabolism and to clarify the physiological role of taurine in red sea bream.

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Figures

Fig. 1. Taurine, serine, and cystathionine contents in the whole body of red sea bream juveniles fed different levels of taurine supplemented diet for 6 weeks in Experiment I.

Values without a common superscript letter are significantly different ($P<0.05$).

Data are mean±standard deviation of duplicate groups in each treatment.

Table 1 Composition of the experimental diet for red sea bream in Experiment I

	Control	Taurine
Fish meal	10.0	10.0
Casein	42.0	42.0
Gelatin	10.0	10.0
α-Starch	7.0	7.0
Feed oil	12.0	12.0
Soybean lecithin	3.0	3.0
Vitamin mix*1	2.0	2.0
Mineral mix*2	5.0	5.0
Chromic chloride	0.9	0.9
VE(50%)	0.1	0.1
Ascorbic acid calcium	0.2	0.2
Cellulose	7.3	6.3
Feeding stimulant*3	0.5	0.5
Taurine	0.0	1.0
	100.0	100.0
Analytical contents(dry matter basis)		
Taurine (mg/100g)	24.0	924.0
Crude protein(%)	57.4	58.2
Crude lipid(%)	14.8	15.5

*1Vitamin mixture ingredient (mg/118g): Vitamin B_1 900 mg, Vitamin B_2 1500 mg, Vitamin B_6 600mg, Vitamin B_{12} 1.5 mg, Niacin 6×10^3 mg, Ca-pantotenate 1500 mg, Inositol 30×10^3 mg, Biotine 90 mg, Folic acid 225 mg, *p*-Aminobenzoic acid 750 mg, Vitamin K_3 750 mg, Vitamin A 600000 IU, Vitamin D_3 600000 IU.

*2 Mineral mixture ingredients (g/100g): NaCl 1.0 g, $MgSO_4\cdot7H_2O$ 15.0 g, $NaH_2PO_4\cdot2H_2O$ 25.0 g, KH_2PO_4 32.0 g, $Ca(H_2PO_4)_2\cdot H_2O$32.0g, Fe-citrate 2.5 g, Ca-lactate 3.5 g, Trace element mixture 1.0 g, Cellulose 13.0 g.

Trace element mixture ingredients (mg/100mg): $ZnSO_4\cdot7H_2O$ 35.3mg, $MnSO_4\cdot4H_2O$ 17.5mg, $CuSO_4\cdot5H_2O$ 3.1mg, $AlCl_3\cdot6H_2O$ 1.5mg, KIO_3 0.3mg, $CoCl_2\cdot6H_2O$ 0.1mg, Cellulose 42.2 mg.

*3 Proline, 354; Alanine, 232; Inosine 5'-monophosphate, 414. (mg/g)

Table 2 Composition of the experimental diet for red sea bream in Experiment II

	Control	Tau-0.5	Tau-1.0	Tau-2.0	Cys-1.0	Cys-2.0
Casein	51.0	51.0	51.0	51.0	51.0	51.0
Gelatin	11.0	11.0	11.0	11.0	11.0	11.0
α-Starch	7.0	7.0	7.0	7.0	7.0	7.0
Feed oil	5.0	5.0	5.0	5.0	5.0	5.0
Cuttlefish lecithin	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin mix*1	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix*2	5.0	5.0	5.0	5.0	5.0	5.0
Chromic chloride	0.9	0.9	0.9	0.9	0.9	0.9
VE(50%)	0.1	0.1	0.1	0.1	0.1	0.1
Ascorbic acid calcium	0.2	0.2	0.2	0.2	0.2	0.2
Cellulose	7.3	6.8	6.3	5.3	6.3	6.3
Feeding stimulant*3	0.5	0.5	0.5	0.5	0.5	0.5
Taurine	0.0	0.5	1.0	2.0		
Cystine					1.0	2.0
Analytical contents(dry matter basis)						
Taurine (mg/100g)	5.3	422.0	993.4	1598.4	6.5	5.0
Cystine (mg/100g)	0.0	0.0	0.0	0.0	994.5	1895.2
Crude protein(%)	61.2	61.3	61.7	62.6	62.0	62.3
Crude lipid(%)	15.4	15.4	15.3	15.4	15.5	15.4

*1Vitamin mixture ingredient (mg/118g): Vitamin B_1 900 mg, Vitamin B_2 1500 mg, Vitamin B_6 600mg, Vitamin B_{12} 1.5 mg, Niacin 6×10^3 mg, Ca-pantotenate 1500 mg, Inositol 30×10^3 mg, Biotine 90 mg, Folic acid 225 mg, *p*-Aminobenzoic acid 750 mg, Vitamin K_3 750 mg, Vitamin A 600000 IU, Vitamin D_3 600000 IU.

*2 Mineral mixture ingredients (g/100g): NaCl 1.0 g, $MgSO_4\cdot7H_2O$ 15.0 g, $NaH_2PO_4\cdot2H_2O$ 25.0 g, KH_2PO_4 32.0 g, $Ca(H_2PO_4)_2\cdot H_2O$32.0g, Fe-citrate 2.5 g, Ca-lactate 3.5 g, Trace element mixture 1.0 g, Cellulose 13.0 g.

Trace element mixture ingredients (mg/100mg): $ZnSO_4\cdot7H_2O$ 35.3mg, $MnSO_4\cdot4H_2O$ 17.5mg, $CuSO_4\cdot5H_2O$ 3.1mg, $AlCl_3\cdot6H_2O$ 1.5mg, KIO_3 0.3mg, $CoCl_2\cdot6H_2O$ 0.1mg, Cellulose 42.2 mg.

*3 Proline, 354; Alanine, 232; Inosine 5'-monophosphate, 414 (mg/g).

Table 3 Results of the 6-week feeding trail in Experiments I and II

	Intitial BW (g)	Final BW (g)	Specific growth rate[*1]	Weight gain[*2]	Feed efficiency[*3]	Mortality
Experiment I						
Control	2.3 ± 0.0	6.9 ± 0.3 ┐[*4]	2.5 ┐[*]	194.2 ┐[*]	74.9 ┐[*]	0
Taurine	2.3 ± 0.0	11.9 ± 0.3 ┘	3.8 ┘	408.3 ┘	92.4 ┘	0
Experiment II						
Control	2.5 ± 0.2	6.6 ± 2.2[a*5]	2.3[a]	165.0[a]	91.5[a]	1.7
Tau-0.5	2.5 ± 0.3	15.7 ± 3.3[b]	4.4[b]	529.2[b]	137.0[b]	1.7
Tau-1.0	2.5 ± 0.2	16.5 ± 2.8[b]	4.5[b]	559.8[b]	123.9[b]	0
Tau-2.0	2.5 ± 0.3	17.1 ± 3.0[b]	4.6[b]	581.0[b]	126.2[b]	0
Cys-1.0	2.5 ± 0.3	7.1 ± 2.3[a]	2.5[a]	181.5[a]	94.8[a]	1.7
Cys-2.0	2.5 ± 0.3	7.2 ± 2.3[a]	2.5[a]	187.9[a]	98.4[a]	6.7

*1 Specific growth rate = 100×(ln(final BW)-ln(initial BW))/days.
*2 Weight gain = 100× (final BW-initial BW) /initial BW.
*3 Feed efficiency = 100 ×(final BW-initial BW)/DM intake.
*4 Data connected with lines are significantly different (Student's t-test $P < 0.05$)
*5 Significant differences (Tukey's test $P < 0.05$) between dietary groups are indicated with different alphabet letters.

Table 4 Taurine, serine and cystahionine content in the whole body of juvenile red sea bream in Experiment Ⅱ (mg/100g, d.b.)

	Control	Tau-0.5	Tau-1.0	Tau-2.0	Cys-1.0	Cys-2.0
Taurine	74.3 ± 8.1	413.2 ± 41.7	894.2 ± 101.2	1584.4 ± 76.2	79.1 ± 15.6	76.4 ± 9.8
Serine	77.4 ± 27.3	44.8 ± 9.3	31.0 ± 4.7	20.6 ± 2.7	93.3 ± 12.8	76.1 ± 28.3
Cystathionine	7.8 ± 2.4	14.9 ± 3.6	11.8 ± 1.8	9.6 ± 1.5	9.3 ± 3.4	15.1 ± 5.5

Table 5 Effects of dietary amino acid components on the feeding behavior of red sea bream

	Behavioral traits (frequency fish^{-1} 5 minutes^{-1})		
	Ingestion	Handling	Handling/Ingestion (%)
Control	13.7 ± 3.2 ^{bc*1}	2.3 ± 1.2	16.3 ± 5.4 ^{b}
Tau-0.5	28.3 ± 5.1 ^{ab}	2.3 ± 0.6	8.2 ± 0.7 ^{b}
Tau-1.0	31.3 ± 8.7 ^{a}	1.7 ± 2.1	4.6 ± 4.9 ^{b}
Tau-2.0	33.3 ± 4.5 ^{a}	2.0 ± 2.0	5.8 ± 6.1 ^{b}
Cys-1.0	13.7 ± 7.2 ^{c}	6.7 ± 4.7	46.0 ± 11.1 ^{a}
Cys-2.0	10.3 ± 0.6 ^{c}	5.7 ± 1.5	54.8 ± 15.0 ^{a}

*1 Significant differences (Tukey's test $P < 0.05$) between dietary groups are indicated with different alphabet letters.

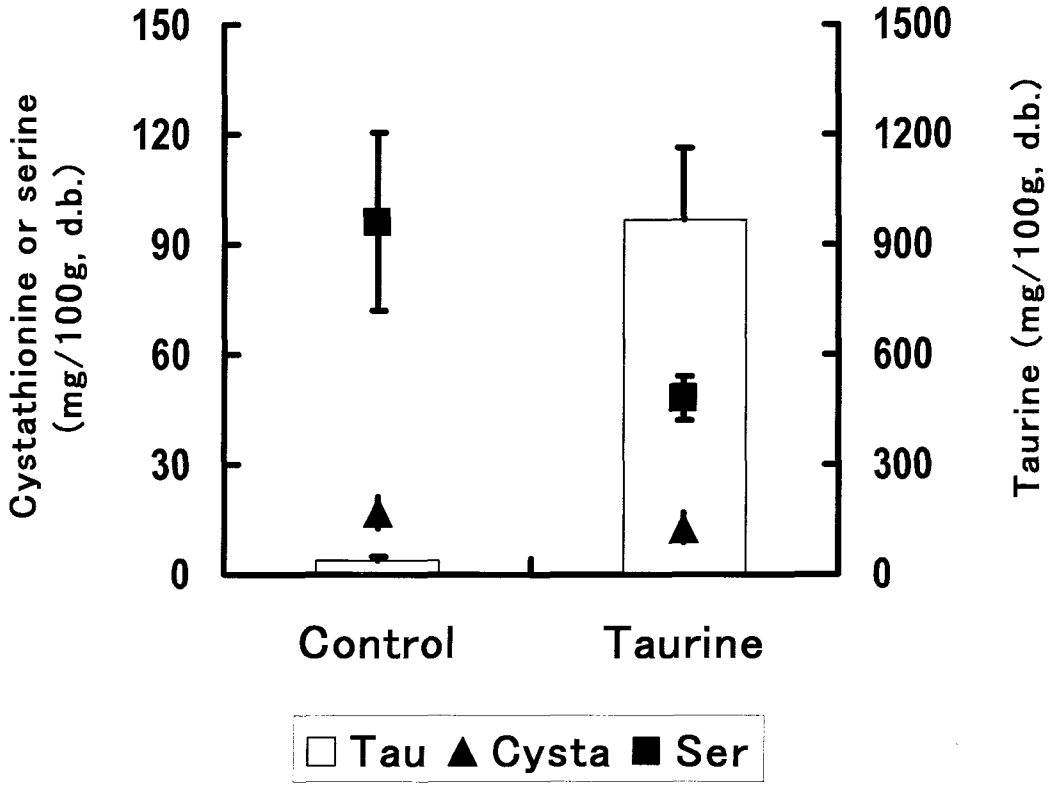
Cystathionine or serine (mg/100g, d.b.)
150
120
90
60
30
0
1500
1200
900
600
300
0
Taurine (mg/100g, d.b.)
Control
Taurine
□ Tau ▲ Cysta ■ Ser

Fig.1

第3章

マダイ稚魚に対するタウリン添加精製飼料の影響

第2節 マダイ稚魚のタウリン要求量および胆汁酸への影響

Optimal dietary taurine level for growth of juvenile red sea bream *Pagrus major*

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INTRODUCTION

Clarification of the requirement of dietary amino acid in fish is necessary in order to formulate efficient and economical diets. Recent investigations of amino acid have shown that taurine is an important element in several fish species, such as Japanese flounder, yellowtail and red sea bream which are important marine culture species in Japan (Takeuchi et al. 2001; Kim et al. 2003; Matsunari et al. 2005). However, the requirement of juvenile red sea bream for taurine is not yet clarified.

It has been known that a major biochemical reaction of taurine is the conjugation of bile acids in the liver (Danielsson, 1963). Cholesterol is metabolized to bile acid which is secreted into the small intestine to promote lipid absorption. Several studies have demonstrated that dietary tauirne showed a notable cholesterol-lowering effect in hyprercholesterolemic rats fed a high chesterol diet (Tsuji et al., 1979; Masuda and Horisaki, 1986). A few studies have been investigated the effect of taurine on the metabolism of lipids in fish. The major bile salts of red sea bream were both cholyltaurine (C-tau) and chenodeoxycholyltaurine (CDC-tau) (Goto et al., 1996).

The present investigation is undertaken to clarify the effect of taurine and (C-taru) on the liver lipid levels, and determine the optimum dietary taurine requirement for juvenile red sea bream.

MATERIALS AND METHODS

Diet

Table 1 shows the composition of the experimental diets and their proximate and taurine contents. The experimental diets were based on casein and gelatin as the main protein source. The experimental diet was supplemented at the levels of 0, 0.1, 0.3, 0.5, 0.7 %

taurine and 0.5% (C-tau). All ingredients were well mixed together with distilled water, mixed to make a mash, pelleted with a press machine, and then dried for 24h in a freeze-dryer (Nissei Co., Ltd., Tokyo, Japan). Dietary protein and lipid levels were about 58% and 15%, respectively. Taurine contents in diets were 5.4, 102.4, 292.0, 472.5, 653.3, and 6.3mg/100g, respectively.

Fish and feeding

Red sea bream juveniles used in this study were obtained from the Fish Nursery Center of Kinki University, Susami, Japan. The average weight of the fish at the beginning of the experiment was 0.5g. The fish were divided into 12 groups each of 40 individuals, and each group was assigned to one of the six experimental diets, giving two replicates for each of the diet. The fish were reared on a commercial feed (Marubeni Nisshin Feed, Tokyo, Japan), three times daily to apparent satiation until used for feeding trials. Each of the 12 groups was placed in a 100L polycarbonate tank. Each rearing trial was conducted in a flow-through system (3000mL/min) with natural seawater, and the water temperature was controlled at 20℃. Each experimental diet was given to satiation three times a day throughout the 6-week feeding experiment. In the end of feeding trial, the fish were weighted and stored at −20℃ until chemical analyses. The liver and gallbladder used for and free amino acid and bile acid analysis were stored at −80℃ until chemical analyses.

Chemical analysis

The extraction of free amino acids in diet, whole body and liver (n=3) were homogenized with 2% sulfosalicylic acid and centrifuged at 2,300×g for 15 min. Free

amino acid levels were determined individually with an automatic amino acid analyzer (JLC-500/v JEOL, Tokyo, Japan). Lipids were extracted by the chloroform-methanol (2:1,v/v) method (Folch et al., 1957). The C-tau and CDC-tau contents were extracted according to methods described by Cantafora et al., 1987. The gall blabber bile were extracted isopropanol (1:9, v/v) for 10 min and centrifuged at 500g for 10 min. The isopropanol extracts were dissolved in the mobile phase and the solution was filtered through a DISMIC-3 0.5 μ m filter (Advantec, CA, U.S.A.), and were measured by high performance liquid chromatography (HPLC) using a pump (LC-10AS, Shimadzu Corp., Kyoto, Japan) attached to a UV-VIS spectrophotometric detector (SPD-10AUV; Shimadzu Corp.) with a packed column (Shimpack CLC-ODS 6mmi.d. × 150mm; Shimadzu Corp), with methanol/ 20mM phosphate buffer/ water/ acetonitrile (150:50:20:20) as the mobile phase.

Statistical analysis

Statistical analyzed of growth performances, free amino acid concentrations were carried out using one-way ANOVA and Tukey's test. When two groups were compared, data were analyzed using Student's *t*-test. For all statistical analyses, a SPSS 11.0 microcomputer software package (SPSS, Chicago, IL, USA) was used. In all statistical testing, differences at $P<0.05$ were considered as significant.

RESULTS

Feeding results

Results of the feeding experiments are shown in Table 2. The fish weights were the highest in fish fed the 0.5% taurine diet and the lowest in those fed the control diet. The

specific growth rate increased with increasing dietary taurine level up to 0.3% and remained nearly the same thereafter. The feed efficiency was significantly higher in fish fed taurine and C-tau than those fed the control diet.

Accumulation of free amino acids in red sea bream juveniles

Taurine content in the whole body and liver of juvenile red sea bream are shown in Table 3. Taurine content of the whole body increased significantly with increasing dietary tuarine levels, while was independent of dietary C-tau level. The livers of fish fed taurine from 0.1 to 0.7% were significantly higher than those fed control and C-tau diet.

Bile salt composition of the gallbladder bile in red sea bream juveniles

The concentrations of bile acids in the gallbladder bile are shown in Table 4.

The total content, concentration and composition of C-tau of bile salt of the gallbladder bile increased with increasing dietary taurine and C-tau levels. The composition of CDC-tau in the gallbladder bile increased with increasing taurine levels, while was independent of C-tau levels.

Crude lipid of the liver in red sea bream juveniles

Crude lipid content in the liver of juvenile red sea bream shown in Fig.1. The crude lipid levels increased with increasing dietary taurine level up to 0.3% and then plateaued, while was independent of C-tau levels.

DISCUSSION

The fish fed supplementation taurine diet improved to growth and feed efficiency. The fish weights increased with increasing dietary taurine level up to 0.5% and remained nearly the same thereafter. Results of the previous study indicated that the growth and feed efficiency were not significantly different from values for the 0.5% to 2.0% taurine levels in diet. The optimal dietary taurine requirement for juvenile red sea bream was estimated to be 0.5% in diet. The taurine requirement of Japanese flounder is 1.5-2.0% in the diet (Park et al., 2002). The taurine has an important role only during the juvenile period of Japanese flounder, not the fingerling period (Kim et al., 2003). These results indicate that taurine requirements could vary with species. More research is needed to clarify the requirement of the different stages of red sea bream.

Taurine is an essential dietary nutrient for cats because of the low level of hepatic cysteinesulfinate decarboxylase activity. The cats fed a taurine-depleted diet are unable to maintain normal tissue levels of taurine and results in retinal degeneration, greatly increased reproductive wastage (Knopf et al., 1978). The kittens fed the low taurine diet showed plasma and retinal pools of taurine are largely depleted compared with that of the taurine supplemented diet, the taurine conjugation of bile acids is only moderately affected (Rabin et al., 1976; Sturman et al., 1978). These results indicate that the amount of taurine conjugated to bile acids was unchanged by the taurine depletion. The total content and concentration of bile salt of the gallbladder bile increased with increasing dietary taurine and C-tau levels. This observation is the same as that of other stage (Takagi et al., 2002). These results indicate that the amount of taurine conjugated to bile salts in red sea bream were easily affected by the dietary taurine depletion.

Taurine is conjugated with bile salts and has an important role in excretion of hepatic

biliverdin into bile (Sakai et al.,1985), and fat digestion via its conjugation with bile acids (Iijima et al., 1998). Atlantic salmon fed a C-tau supplementation diet showed nearly a 20% increase in the astaxanthin blood levels (Olsen et al., 2005). In this study, crude lipid contents in the livers of fish fed taurine were significantly higher than those fed control diet. These observations indicate that taurine also plays an important role in fat digestion via its conjugation with bile acids in red sea bream.

The results of the present study suggest that the optimal dietary taurine requirement for juvenile red sea bream was estimated to be 0.5% in diet. More research is needed on lipid metabolism and to clarify the physiological role of taurine in red sea bream.

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Figures

Fig. 1. Crude lipid contents in the liver of juveniles red sea bream fed different levels of taurine and C-tau supplemented diet.

Values without a common superscript letter are significantly different ($P<0.05$).

Data are mean±standard deviation of duplicate groups in each treatment.

Table 1 Composition of the experimental diet for red sea bream

	Control	Tau-0.1	Tau-0.3	Tau-0.5	Tau-0.7	C-tau0.5
Casein	51.0	51.0	51.0	51.0	51.0	51.0
Gelatin	11.0	11.0	11.0	11.0	11.0	11.0
α-Starch	7.0	7.0	7.0	7.0	7.0	7.0
Feed oil	5.0	5.0	5.0	5.0	5.0	5.0
Cuttlefish lecithin	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin mix[*1]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix[*2]	5.0	5.0	5.0	5.0	5.0	5.0
Chromic chloride	0.9	0.9	0.9	0.9	0.9	0.9
VE(50%)	0.1	0.1	0.1	0.1	0.1	0.1
Ascorbic acid calcium	0.2	0.2	0.2	0.2	0.2	0.2
Cellulose	7.3	6.8	6.3	5.3	6.3	6.3
Feeding stimulant[*3]	0.5	0.5	0.5	0.5	0.5	0.5
Taurine	0.0	0.1	0.3	0.5	0.7	
Taurocholic acid						0.5
Analytical contents(dry matter basis)						
Taurine (mg/100g)	5.4	102.4	292.0	472.5	653.3	6.3
Crude protein(%)	59.5	59.7	59.6	59.7	60.2	59.1
Crude lipid(%)	15.2	15.0	15.2	15.1	15.3	15.1

*1Vitamin mixture ingredient (mg/118g): Vitamin B_1 900 mg, Vitamin B_2 1500 mg, Vitamin B_6 600mg, Vitamin B_{12} 1.5 mg, Niacin 6×10^3 mg, Ca-pantotenate 1500 mg, Inositol 30×10^3 mg, Biotine 90 mg, Folic acid 225 mg, *p*-Aminobenzoic acid 750 mg, Vitamin K_3 750 mg, Vitamin A 600000 IU, Vitamin D_3 600000 IU.

*2 Mineral mixture ingredients (g/100g): NaCl 1.0 g, $MgSO_4\cdot7H_2O$ 15.0 g, $NaH_2PO_4\cdot2H_2O$ 25.0 g, KH_2PO_4 32.0 g, $Ca(H_2PO_4)_2\cdot H_2O$32.0g, Fe-citrate 2.5 g, Ca-lactate 3.5 g, Trace element mixture 1.0 g, Cellulose 13.0 g.

Trace element mixture ingredients (mg/100mg): $ZnSO_4\cdot7H_2O$ 35.3mg, $MnSO_4\cdot4H_2O$ 17.5mg, $CuSO_4\cdot5H_2O$ 3.1mg, $AlCl_3\cdot6H_2O$ 1.5mg, KIO_3 0.3mg, $CoCl_2\cdot6H_2O$ 0.1mg, Cellulose 42.2 mg.

*3 Proline, 354; Alanine, 232; Inosine 5'-monophosphate, 414 (mg/g).

Table 2 Results of the 6-week feeding trail

	Intitial BW (g)	Final BW (g)	Specific growth rate*1	Weight gain*2	Feed efficiency*3
Control	4.7 ± 0.9	9.1 ± 3.4[a*4]	1.6	95.3	80.0
Tau-0.1	4.8 ± 0.9	15.2 ± 4.2[b]	2.7	217.3	119.0
Tau-0.3	4.7 ± 0.7	20.7 ± 4.7[c]	3.5	343.7	138.1
Tau-0.5	4.7 ± 0.7	23.2 ± 4.9[c]	3.8	389.4	137.8
Tau-0.7	4.7 ± 0.9	22.1 ± 4.4[c]	3.7	368.4	135.5
C-tau0.5	4.7 ± 1.1	13.8 ± 3.4[b]	2.6	192.2	131.9

*1 Specific growth rate = 100×(ln(final BW)-ln(initial BW))/days.
*2 Weight gain = 100×(final BW-initial BW)/initial BW.
*3 Feed efficiency = 100 ×(final BW-initial BW)/DM intake.
*5 Significant differences (Tukey's test $P < 0.05$) between dietary groups are indicated with different alphabet letters.

Table 3 Taurine content in the whole body and liver of juvenile red sea bream (mg/100g, d.b.)

	Control	Tau-0.1	Tau-0.3	Tau-0.5	Tau-0.7	C-tau0.5
Whole body	60.7 ± 14.7	203.4 ± 19.9	271.2 ± 16.4	524.2 ± 15.2	746.0 ± 32.1	33.1 ± 7.8
Liver	7.4 ± 0.6	301.8 ± 37.7	332.9 ± 146.4	386.5 ± 22.8	470.9 ± 175.2	17.4 ± 12.5

Table 4 Biliary bile salt concentration of red sea bream

	Control	Tau-0.1	Tau-0.3	Tau-0.5	Tau-0.7	C-tau0.5
Total content (mg/fish)	52.8 ± 8.9	89.1 ± 24.0	111.2 ± 44.0	108.1 ± 24.8	97.8 ± 17.7	70.7 ± 15.9
Concentration (mM)	9.4 ± 3.9	26.4 ± 3.3	30.8 ± 4.5	35.4 ± 3.8	30.3 ± 4.5	19.1 ± 1.7
Composition (%)						
C-tau (%)	98.2	84.0	83.0	82.3	79.0	98.8
CDC-tau (%)	1.8	16.0	17.0	17.7	21.0	1.2

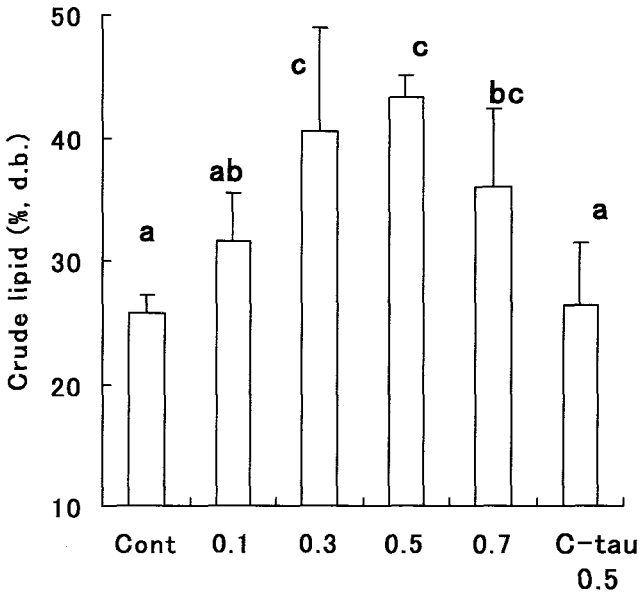

Fig.1

総　括

本研究では、タウリンの要求量とその役割を明らかにすることを目的に、生育段階の異なる海産魚について、生体内における消化吸収機能が十分に整っていない仔魚期、栄養素に対する要求性が最も高い稚魚期および再生産のための成熟・産卵期における飼・餌料のタウリン含量と、供試験魚の成長や飼料効率、生体のタウリンを含むアミノ酸含量との関係を検討した。

まず第1章では、成長に伴うブリ魚体中のタウリン含量の変化を調べた。ふ化後日数経過に伴うブリ全魚体中のタウリン含量の変化を調べるため、人工採卵・ふ化により得られたふ化仔魚から180mmまで飼育した仔稚魚、天然で採取した稚魚とともに、餌生物や配合飼料などの餌・飼料中のタウリン含量を測定した。その結果、天然魚は人工種苗生産魚の2～4倍のタウリンを含有すること、ふ化直後にタウリン含量が急激に減少することおよび天然魚の餌料（コペポーダ）と比較して人工生産魚に給与する餌料（ワムシ）のタウリン含量は著しく低いことを明らかにした。魚体中のタウリンがふ化後に急激に減少する傾向は、ヒラメふ化仔魚においてもみとめられている（Takeuchi et al., 2001）。このような現象はふ化後に、魚体中の海産仔稚魚で必須の栄養素であるDHAと同様の傾向（Takeuchi, 1997）であることから、タウリンはふ化仔魚の発生に重要な役割を担うとともに、種苗生産で使用する飼餌料へタウリンを強化することにより、健全な種苗が得られる可能性が示唆された。また、種苗生産に用いられている餌・飼料のタウリン含量を比較した結果、ふ化直後に給与するワムシのタウリン含量が、その他の餌・飼料と比較して著しく低いことが明かになった（1章1節）。

高橋ら（2005）がワムシへのタウリン強化方法を検討したところ、ワムシ培養水槽

へのタウリン添加量および強化時間に比例して、ワムシ中にタウリンが蓄積されるなど、水溶性物質であるタウリンをワムシに直接強化する方法の開発に成功した。1章2節では、これらのタウリン強化ワムシをふ化直後のマダラ仔魚に給餌して、タウリンの効果を検討した。その結果、タウリンは成長の促進を図る上で重要な栄養素であることが示唆された。本法を用いてマダイ（陳ら, 2004)、ヒラメ（陳ら, 2005)、クロマグロ仔魚（高木, 2005）に適用したところ同様の傾向がみられている。さらにタウリン強化ワムシを摂餌したマダイ仔魚は飢餓耐性が有意に優れることが報告された。このように、タウリンは海産ふ化仔魚の生残率の向上および成長の促進を図る上で重要な栄養素であることが示唆された。

1章1節の研究では人工種苗生産されたブリ稚魚中のタウリン含量が低かったことから、市販のブリ稚魚用飼料へタウリンを濃度を変えて添加して検討した結果、タウリン添加飼料をブリ稚魚へ給餌すると成長促進に有効であるばかりでなく、魚体中のタウリン含量は飼料中のタウリン含量の影響を大きく受けること、また筋肉中のタウリン含量は、1章1節の天然魚の魚体中のタウリン含量の変動と同様に成長に伴い減少することが明らかになった。次に、含硫アミノ酸の代謝について検討するため、筋肉中のセリン、シスタチオニン含量を測定した結果、ブリ稚魚ではタウリン無添加の飼料を給餌した場合、セリン含量は増加するが、シスタチオニン含量は変化しなかったことから、メチオニンからタウリンを合成する際の酵素反応の中でシスタチオニン合成酵素の能力が低いことが示唆された。このことからブリ稚魚が飼料中にタウリンを要求することが示唆された(2章1節)。

人工種苗生産で健康な仔稚魚を生産するためには、ふ化率やふ化後の生残率が優れた卵を親魚から得ることが重要になるが、タウリンが親魚の成熟に及ぼす影響につい

て検討された報告はない。そこで、ブリ親魚にタウリン含量の異なる飼料を給餌し、産卵成績に及ぼす影響を検討した結果、飼料へタウリンを添加することにより、成熟の促進と採卵量の増加とともに、卵の受精率やふ化率が向上することが明らかとなった(2章2節)。

第3章では、海産魚類におけるタウリンの役割を検討するために、マダイ稚魚を用いて実験を行った。一般的な養魚用配合飼料には、主なタンパク質源としてタウリンを多く含む魚粉が5割程度含まれるため、魚類におけるタウリンの要求性に関する研究には適さなかった。これまでのタウリン要求性に関する研究では、魚粉をエタノールにより簡易洗浄処理し、さらに飼料への配合に際しては、必須アミノ酸含量を満たすために結晶アミノ酸を添加する必要があった。本実験では、タンパク質源として、タウリンを含まないカゼインを用いた飼料を新たに開発した。これまで、海産魚はカゼイン飼料の摂餌が劣ると考えられていたが、開発した精製飼料はマダイ稚魚の摂餌が極めて良好であった。低タウリン飼料をマダイ稚魚に給餌すると体色が暗色化することや、成長が停滞するなどの異常がみられたが、低タウリン飼料へタウリンを添加することにより、これらの症状が改善された。次に、飼料中のタウリンおよび含硫アミノ酸の１つであるシスチンがマダイ稚魚の摂餌行動および脂質吸収に及ぼす影響について検討した結果、タウリン添加区では対照区と比較して、飼料を視認後、餌を飲み込んだ回数（摂餌回数）が有意に高くなること、一方、シスチン添加区では飼料を視認後に餌を飲み込むまでに、1回以上吐き出す回数が有意に高くなり、飼料中のアミノ酸がマダイの摂餌行動に影響を及ぼすことが明らかになった。また、魚体中の遊離アミノ酸を分析した結果、シスチン添加区では魚体中のタウリン含量が増加しないこと、対照区およびシスチン添加区では、魚体中のタウリン含量の減少がみられたことから、

マダイではタウリンを合成するための酵素活性が微弱あるいは欠損し、体内でシスチンをタウリンへ代謝できないことが明らかになった（3章1節）。

次にマダイ稚魚におけるタウリン要求量および脂質の消化吸収におけるタウリンの役割について検討した結果、タウリン添加区の平均体重はその他の試験区と比較して有意に大きく、中でも0.5%添加区が最も大きかったことから、マダイ稚魚は精製飼料中に約 0.5%のタウリンを添加することによりマダイ稚魚の成長が促進されることが明らかになった。また、飼料へのタウリン添加により抱合胆汁酸濃度が上昇するとともに、肝臓中の粗脂肪含量が有意に高い値を示したことから、飼料中のタウリンが脂質の消化吸収に影響を及ぼす可能性が示唆された（3 章 2 節）。

以上の結果から、タウリンは数種の海産魚類において重要な栄養素であることが明らかになった。今後、仔魚から稚魚期までこのタウリンを用いることにより餌・飼料の改善を図ることが可能となるばかりでなく、魚粉に代わるタンパク質原料の積極的な利用と、環境負荷低減飼料の開発に大きく貢献するものと考えられる。また、海産魚用精製飼料が開発されたことから、海産魚における微量栄養素の効果および機能解明などの研究に寄与でき、今後の海産魚類用飼料の改善が飛躍的に進展することが期待される。本研究では検討していない当歳魚などへのタウリンの影響については、マダイ、ブリでは魚粉削減飼料にタウリンを添加することにより成長改善に加え、緑肝症の予防に効果があることが明らかにされている（Takagi et al., 2005; 2006）。飼料は、「飼料の安全性の確保および品質の改善に関する法律」（飼料安全法）により管理されている。現在、タウリンは飼料安全法の中で飼料添加物の指定を受けていないため、養魚飼料へ添加することができない。飼料添加物は、酸化防止剤などの飼料の品質の保持、ビタミン類などの栄養素の補給あるいは消化酵素などの栄養素の利用促進の、

いずれかの目的でないと指定されない。今後は海産魚類の必須栄養素としてタウリンを位置付け、魚粉削減飼料への添加を検討する必要がある。

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