PROGRAMA DE FORMACIÓN DE RECURSOS HUMANOS PARA LA INNOVACIÓN

APOYO FINANCIERO PARA LA REALIZACIÓN DE ACTIVIDADES DE FORMACIÓN VENTANILLA ABIERTA

FORMULARIO DE POSTULACIÓN E INSTRUCTIVO

MAYO DE 2006

PROGRAMA DE FORMACIÓN DE RECURSOS HUMANOS
PARA LA INNOVACIÓN

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FOLIO DE BASES		CÓDIGO (uso interno)	FIA-FR-V-2006-1-)-004

1. ANTECEDENTES GENERALES DE LA PROPUESTA

NOMBRE DE LA PROPUESTA

Debe corresponder a una frase clara y breve que exprese el tema principal de la actividad.

CURSO EN FISIOLOGIA NUTRICIONAL DE PECES

TIPO O MODALIDAD DE FORMACIÓN

Indicar si es un curso corto de especialización o programa de pasantía, u otro tipo de actividad de formación.

X	Curso corto de especialización		Pasantía		Otro, ¿cuál?		

LUGAR DONDE SE REALIZARÁ LA ACTIVIDAD

Indicar el nombre de la ciudad o localidad, comuna, provincia y región donde se realizará la actividad. En caso de haber más de un lugar, listarlos todos.

V	Región	Valparaíso	Provincia	Valparaíso	Comuna	Valparaíso	Localidad/Ciudad

AREAS O SECTORES

Indicar si la actividad se formación se inserta en el área Agrícola, Pecuario Forestal, Dulceacuícola o Gestión.

X	Agrícola		Pecuario		Forestal	X	Dulceacuícola

RUBRO (S)

Indicar el o los rubros en los cuales se insertan los objetivos de la propuesta (ovinos de carne, frutales, hortalizas, berries o frutal menor, flores, etc.)

Peces, cultivo de cereales, leguminosas.

TEMAS (S) Indicar el tema en el cual se inserta el objetivo de la propuesta. Para identificar el tema, se sugiere tomar como referencia el listado entregado en el Anexo 2 del documento "Bases de Postulación".
NUTRICION ANIMAL

ENTIDAD RESPONSABLE QUE REALIZARÁ LA ACTIVIDAD

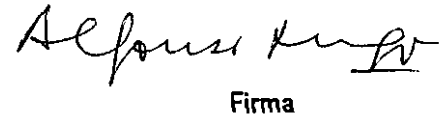
Indicar el nombre completo de la entidad, RUT, teléfono, dirección postal, comuna, ciudad, región, fax, e-mail, web y cuenta bancaria (N°, tipo y banco)

Nombre: Pontificia Universidad Católica de Valparaiso
RUT:
Identificación cuenta bancaria:
Dirección comercial: Av. Brasil 2950
Ciudad/Región: Valparaíso/ Valparaíso
Fono: 32-273000
Fax:32-273000
Correo electrónico:

REPRESENTANTE LEGAL DE LA ENTIDAD RESPONSABLE

Indicar nombre, RUT, profesión, cargo, dirección, comuna, ciudad, región, fono, fax, E-mail, del Representante Legal de la Entidad Responsable. *Esta información deberá además ir acompañada de su firma.*

Nombre: Alfonso Muga Naredo
Cargo en la Entidad Responsable: Rector
RUT:
Dirección: Av. Brasil 2950
Ciudad/Región: Valparaíso/ Valparaíso
Fono: 32-273000
Fax: 32-273000
Correo electrónico: rector@ucv.cl



Firma

TIPO DE ENTIDAD RESPONSABLE

Se debe indicar si se trata de una universidad, institución de investigación, organización o asociación de productores (pequeños o medianos-grandes), asociación gremial o empresa productiva y/o de procesamiento, entre otras.

UNIVERSIDAD

NATURALEZA ENTIDAD RESPONSABLE

Indicar si la Entidad Responsable corresponde a una entidad pública o privada.

[X] Pública [ ] Privada

COORDINADOR DE LA PROPUESTA

El coordinador es una persona designada por la Entidad Responsable encargada del cumplimiento de la propuesta, su administración e informes finales.

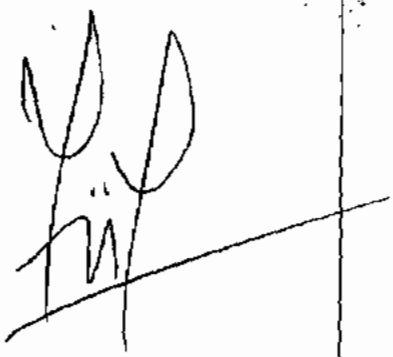
Indicar su nombre, RUT, profesión, especialidad, cargo, dirección (laboral), comuna, ciudad, región, fono, celular, fax, E-mail. En relación a la especialidad y/o cargo, podrán mencionarse entre otras actividades o funciones la de docente, investigador, empresario, productor, técnico, consultor, etc.

Esta información deberá ir acompañada de su firma y además de completar la ficha en **Anexo 1** se deberá adjuntar su *curriculum vitae* completo y una carta (fax o correo electrónico) de respaldo que exprese su compromiso de participar en las actividades previstas

Nombre: Gabriel Yany González
Cargo en la Entidad Responsable: Docente
RUT:
Dirección: Av. Altamirano 1480, Of. 204
Ciudad/Región: Valparaíso/ V región
Fono:32-273444
Fax: 32-274263
Correo electrónico: gyany@ucv.cl

Firma

Profesor CO – Coordinador
Nombre: Mª Isabel Toledo Donoso
Cargo en la Entidad Responsable: Docente
RUT
Dirección: Av. Altamirano 1480, Of. 204
Ciudad/Región: Valparaíso/ V región
Fono:32-273444
Fax: 32-274263
Correo electrónico: itoledo@ucv.cl



ENTIDAD ASOCIADA (1)

Indicar el nombre de el o los organismos o instituciones (nacionales o extranjeras) que actuarán como asociados en la realización de la actividad de formación. Deberá indicarse el nombre completo de la entidad, RUT, teléfono, dirección postal, comuna, ciudad, región, fax, e-mail, web y cuenta bancaria (N°, tipo y banco).

Deberá también especificarse el tipo de entidad a la cual corresponde, es decir si es una universidad, institución de investigación, organización o asociación de productores (pequeños o medianos-grandes), asociación gremial o empresa productiva y/o de procesamiento, entre otras. Además, deberá indicarse si corresponde a una entidad pública o privada.

Nombre:
RUT: **NO APLICA**
Dirección:
Fono:
Fax:
Correo electrónico:
Tipo de Entidad:
Naturaleza de la Entidad:

REPRESENTANTE LEGAL DE LA ENTIDAD ASOCIADA (1)

Indicar el nombre del representante legal, RUT, profesión, cargo, dirección, comuna, ciudad, región, fono, fax, E-mail.

Nombre:

Cargo en la Entidad Asociada:
RUT: **NO APLICA**
Dirección:
Fono:
Fax:
Correo electrónico:

Firma

FECHA DE INICIO Y TÉRMINO DEL PROGRAMA DE ACTIVIDADES

Indicar las fechas de inicio y término del proceso completo de organización, difusión y realización de la actividad de formación.

Inicio:	**13 de Junio de 2006**	**Término:**	**24 de agosto de 2006**

ESTRUCTURA DE FINANCIAMIENTO

Indicar el costo total de la iniciativa, el cual deberá incluir los aportes de contraparte, el financiamiento solicitado a FIA y otros aportes (si los hay) indicados en el formulario de postulación, en **la Sección 15.1**. "Cuadro resumen y procedencia de aportes de contraparte".

Además se debe especificar el financiamiento solicitado a FIA y el aporte de contraparte, y el porcentaje que dicho monto representa respecto al costo total de la iniciativa. Los valores que se indiquen en esta sección deben ser iguales a los presentados en la **Sección 15** del formulario de postulación "Costos totales y estructura de financiamiento de la propuesta".

COSTO TOTAL DE LA PROPUESTA		**$4.114.756**	
FINANCIAMIENTO SOLICITADO A FIA	**$**	**1.728.965**	**42.1 %**
APORTE DE CONTRAPARTE	**$**	**2.385.791**	**57.9 %**

2. RESUMEN Y JUSTIFICACIÓN DE LA PROPUESTA

En esta sección deberá incluirse un breve planteamiento y definición del problema y del nivel de desarrollo actual de la materia a la que se refiere la actividad, según el criterio, percepción y conocimientos del postulante.

Luego, se debe desarrollar la concepción teórica y el enfoque en el que se enmarca la propuesta y que conduce a su desarrollo. Todo ello se debe expresar, finalmente, en los contenidos y los énfasis con los cuales se propone trabajar a través de las distintas materias a impartir, lo cual debe ser presentado en esta sección del formulario.

Así también se deberá hacer una descripción breve de la propuesta en su conjunto, indicando aquellos aspectos que permitan tener una visión global de ella, por ejemplo, su objetivo principal, a qué tipo de asistentes va dirigida, contenidos, metodología de trabajo, entre otros.

Esta propuesta se presenta a FIA con el objeto de solicitar financiamiento para la realización de un curso internacional dictado por el Dr. Sungchul Charles Bai, de la Universidad de Punkyon, Busan , Corea, consultor internacional y experto en alimentación , nutrición y fisiología de peces para dictar un curso en *Fisiología nutricional en peces.*

La nutrición de peces es un área técnica de alta relevancia en la salmonicultura por los impactos ambientales, económicos y sociales que se originan producto de los alimentos no consumidos y materia orgánica proveniente del metabolismo de los peces. Los desafíos actuales para la investigación y desarrollo en el tema de la nutrición y alimentación de peces, se orientan a la optimización de formulaciones de alimento que cumplan las exigencias nutricionales de los peces y que a su vez sean económicamente amigables con el medio ambiente.

De acuerdo a las conclusiones del reciente Taller *"Relevancia de los Estudios Nutricionales para el Cultivo de Especies Acuícolas: Fortalezas y Debilidades de la Investigación Científica y Tecnológica"*, realizado el 27 y 28 de febrero del año en curso, se concluyó , que para diversificar la acuicultura , es preciso disponer del conocimiento técnico para el desarrollo de dietas artificiales que cumplan con los requerimientos físicos y nutricionales de las nuevas especies, tengan buena aceptación , sean altamente digestibles y posean una baja lixiviación de nutrientes.

Actualmente la investigación sobre este tema en nuestro país, se ha avocado al estudio de reemplazos de materias primas como la harina y aceite de pescado. Es así que en este contexto, FIA apoyo, entre el 2001 y 2004, la ejecución del proyecto FIA *"Diversificación del uso del lupino, utilizándolo como fuente proteica alternativa en la alimentación de la salmonicultura"*, cuyos resultados permitieron validar científicamente el reemplazo de harina de pescado por harina de lupino hasta un 15% en las formulaciones de alimentos para la salmonicultura.

Complementariamente , es necesario desarrollar investigaciones que permitan entender cuales son los cambios fisiológicos que se producirían en los peces cuando se realizan otras sustituciones, como el reemplazo del aceite de pescado dietario por aceite de origen vegetal, cuales serían las variaciones en la digestibilidad de los alimentos y sus efectos en el medio. En otros países estos estudios ya están siendo desarrollados con aceites vegetales locales, en Chile es preciso conducirlos a la menor brevedad para entregar las respuestas requeridas por el sector acuicultor. Para ello, es importante actualizar y profundizar los conocimientos en nutrición y formulación de alimentos para definir óptimamente las estrategias nutricionales y ambientales, que maximicen los beneficios productivos, frente a la sustitución de materias primas tradicionalmente utilizadas en la elaboración de los alimentos.

Estos temas serán cubiertos en el curso, lo cual permitirá contribuir con la formación de capacidades técnicas en el área de la nutrición de peces, como lo son los futuros doctorandos del Programa de Doctorado en Acuicultura integrado por tres Universidades: Pontifica Universidad Católica de Valparaíso, la Universidad Católica del Norte y la Universidad de Chile, académicos e investigadores de Universidades, Institutos de Investigación, profesionales que laboran en las distintas empresas ligadas directa e indirectamente con la acuicultura y el sector agrícola. El curso taller también dará la oportunidad a que los profesionales asistentes de 20 a 30 empresas , puedan exponer sus experiencias , problemas y discutir alternativas de solución directamente con el expositor.

Nota: esta sección se puede extender como máximo en 3 páginas.

ANTECEDENTES DE LA ENTIDAD QUE REALIZARÁ LA ACTIVIDAD Y DE LAS ENTIDADES ASOCIADAS

En esta sección se deberá incluir la información esencial que permita evaluar la conveniencia de que la entidad postulante y la o las entidades asociadas realicen la actividad, indicando su trayectoria en el tema, capacidades de gestión y profesionales, vinculación con el sector al cual está dirigida la actividad de formación o capacitación, alianzas con otras entidades, fuentes de información que posee o que utilizará, entre otros. Señalar además cómo esta actividad se enmarca en las tareas y programas que desarrolla la entidad postulante y la o las entidades asociadas.

3.1. ANTECEDENTES DE LA ENTIDAD RESPONSABLE

(Adjuntar antecedentes adicionales en el Anexo N°2)

La Escuela de Ciencias del Mar de la Universidad Católica de Valparaíso cuenta con académicos pioneros y líderes en la investigación de área de acuicultura, y de profesionales especialistas en esta área. Posee centros de investigación localizados en Dalcahue, Castro y Río Blanco, Los Andes.

En sus 50 años de trayectoria se han desarrollado una serie de proyectos de Investigación y Desarrollo (I&D) y de Transferencia Tecnológica dirigidos al sector salmonicultor.

En la actualidad la Escuela de Ciencias del Mar ha formado una alianza estratégica con la Universidad de Chile y la Universidad Católica del Norte para crear el primer programa de Doctorado en Acuicultura , cuyo objetivo es la formación de capacidades de investigación para el fortalecimiento y entrega de soluciones reales al sector acuicultor.

La Escuela de Ciencias del Mar se destaca por las tesis dirigidas al sector acuicultor, libros y revistas. Algunas de estas publicaciones son:

- **Libros**

Toledo, M., E. von Baer, G. Olivares, P. Soto, A. Manríquez, C. Harrison, D. Mex, F. Garrido, 2004. Lupino dulce: leguminosa en la producción de alimento para salmónidos. Pontificia Universidad Católica de Valparaíso. Valparaíso. 41 p.

Yañez, E. 2003. Actividad Pesquera y de Acuicultura en Chile. Editorial Universitaria. Valparaíso. 444 p.

Arana, P., A. Guerrero, M. Ahumada, M. Tapia. 2001. Normativa Pesquera Chilena. Editorial Universitaria. Valparaíso. 265 p.

- **Revista.**

Investigaciones Marinas UCV es una revista de publicación anual que considera contribuciones científicas originales en Ciencias del Mar, dando preferencia a los trabajos realizados en el Pacífico Sudoriental.)

- **Tesis**

Algunas tesis desarrolladas en el tema:

AHUMADA QUEZADA, CARLOS ALBERTO, 1996. Efectos de dietas con distinta relación proteína/energía en el vaciamiento gástrico de trucha arcoiris (*Oncorhynchus mykiss*). 65 p. Ilus. Prof. Guía: María Isabel Toledo D.

CAJAS RAMIREZ, DANIELA ANDREA, 1997. Efecto de tres niveles distintos de proteína en la dieta sobre índices reproductivos de hembras trucha arcoiris (*Oncorhynchus mykiss*). 33 p. Ilus. Prof. Guía: María Isabel Toledo D.

CAMILO LEON, JORGE HERNAN, 1998. Evaluación de la larva de mosca como fuente proteica alternativa en la alimentación de juveniles de trucha arcoiris (*Oncorhynchus mykiss*). 50 p. Ilus. Prof. Guía: María Isabel Toledo D.

CASTAÑER SARIEGO, CLAUDIO, 1998. Evaluación de la tasa de crecimiento de peces juveniles de trucha arcoiris (*Oncorhynchus mykiss*) alimentados con dietas conteniendo distintos niveles de potasio. 30 p. Ilus. Prof. Guía: Gabriel Yany G.

FIGUEROA GUAJARDO, FABRIZZIO RENE, 1998. Validación de funciones matemáticas que definen patrones de alimentación en trucha arcoiris (*Oncorhynchus mykiss*). 49 p. Ilus. Prof. Guía: María Isabel Toledo D.

SILVA CABRERA, MARCELO, 1998. Efecto del tamaño de las raciones diarias de alimento en la eficiencia reproductiva de trucha arcoiris (*Oncorhynchus mykiss*). 62 p. Ilus. Prof. Guía: María Isabel Toledo D.

GALENO DUBRAVCIC, DINKO ANDRES, 1999. Evaluación del efecto de fotoperíodo v/s temperatura de cultivo en el vaciamiento gástrico de juveniles de trucha arcoiris (*Oncorhynchus mykiss*). 45 p. Ilus.

MUGA HAUG, CLAUDIA, 1999. Efecto del origen de los pigmentos tiempos de suministro en los índices reproductivos, calidad de las ovas y posteriores etapas de incubación y alevinaje de trucha arcoiris (*Oncorhynchus mykiss*). 59 p. Ilus. Prof. Guía: María Isabel Toledo D.

DELAVEAU CONLEY, MYLÉNE MARIE. 2001. Diseño y dimensionamiento de un sistema de remoción de desechos orgánicos para ser implementado en balsas-jaulas. Prof. Guía María Isabel Toledo.

CASTILLO SEPÚLVEDA, JUAN LUIS. 2001. Evaluación de la prefactibilidad técnica-económica de cultivo de carpa (*Cyprinus Carpio*) utilizando agua proveniente del sistema de refrigeración de una central termoeléctrica. Prof. Guía María Isabel Toledo.

VALDIVIA BELLIDO, MARIO CÉSAR. 2002. Prefactibilidad técnico-económica para el cultivo del puye (*Galaxias maculatus*) a escala comercial, en la IX región. Prof. Guía María Isabel Toledo.

MÜNDNICH WIEGOLD, KARIN ELIZABETH. 2003. Impacto de la introducción de tecnología de incubación de ovas de Salmón del Atlántico (*Salmo salar*) a bajas temperaturas. Prof. Guía Gabriel Yany.

MORALES JEREZ, LILIANA RACHEL. 2004. Comparación de la eficiencia de distintos tratamientos antifúngicos en ovas de trucha arco iris (Oncorhynchus mykiss) en un establecimiento de producción industrial.

SOTO AZOCAR, PAULA ANDREA. 2004. Utilización de harina de lupino (*Lupinus albus)* en formulaciones de alimento para juveniles de trucha arcoiris (*Oncorhynchus mykiss)*. 54 p. Prof. Guía María Isabel Toledo.

MANRIQUEZ LAGOS, ALEX FELIPE. (En desarrollo). Utilización de macroalgas enriquecidas proteicamente en la alimentación de trucha arcoiris (*O. mykiss*).

OLIVARES CANTILLANO, GERMAN. (En desarrollo). Utilización del lupino dulce (L.albus) en la alimentación de salmón coho (*O. kisutch*).

3.2. ANTECEDENTES DE LA(S) ENTIDAD(ES) ASOCIADA(S)
(Adjuntar antecedentes adicionales en el Anexo N°3)

NO APLICA

CARACTERÍSTICAS DE LA RELACIÓN ENTRE LA ENTIDAD RESPONSABLE Y LA(S) ENTIDAD(ES) ASOCIADA(S). (Sólo completar si la Entidad Responsable se presenta asociada con otras *Entidades*)

En esta sección se debe entregar información sobre la relación que se establece entre la Entidad Responsable y la(s) Entidad(es) Asociada(s). Esta información debe entregar los antecedentes necesarios para comprender la naturaleza, características y mecanismos mediante los cuales se concreta la relación entre las entidades.

Asi mismo, en esta sección se debe describir en forma detallada qué forma o qué mecanismo de apoyo se utilizará y los contenidos temáticos en los cuales se trabajará en forma conjunta o serán complementados a través de dichos mecanismos

NO APLICA

OBJETIVOS DE LA PROPUESTA

5.1. OBJETIVO GENERAL

Indicar lo que se pretende lograr con la Actividad de Formación en el proceso de transferencia de conocimientos y/o experiencia. Debe expresarse con una forma verbal activa (por ejemplo: aumentar, apoyar, capacitar, habilitar u otras análogas).

Realizar curso internacional sobre Fisiología Nutricional de Peces.

Con este curso internacional en el tema de alimentación y nutrición de peces se logrará la capacitación de un grupo de personas que desarrollan investigación en el tema de alimentación y nutrición de peces y además a profesionales que se desempeñan en empresas elaboradoras de alimentos, profesionales que trabajan en el área de productos agrícolas para la salmonicultura y otros profesionales que se desempeñan en empresas afines a la acuicultura.

El curso será dictado preferentemente por un consultor experto en el tema de nutrición animal a alumnos del doctorado en acuicultura y abierto a profesionales del área.

5.2. OBJETIVOS ESPECÍFICOS

1. Desarrollar de competencias técnicas relacionadas con la nutrición y alimentación de salmones.
2. Integrar estructuras y funciones de los nutrientes alimenticios en relación a la fisiología de los peces.
3. Diseñar dietas para peces mediante el uso de software computacional de uso masivo.

MODALIDAD Y METODOLOGIA

En esta sección se debe entregar información respecto de la modalidad por la cual la Entidad postulante tiene previsto impartir la actividad de formación o capacitación, detallando si se utilizará modalidad presencial y/o a distancia. Si es presencial, dónde se impartirán los cursos; si es a distancia cuál o cuáles serán los puntos de entrega de información y de referencia para los estudiantes y el diseño del sistema de tutoría, entre otros aspectos. Adicionalmente, se deberá describir el carácter y estructura modular del programa. En este punto, en síntesis, se debe entregar toda la información necesaria para evaluar la pertinencia de la modalidad elegida.

6.1. MODALIDAD

Presencial, a desarrollar en la Escuela de Ciencias del Mar, de la Pontificia Universidad Católica de Valparaíso.

6.2. METODOLOGÍA

Se debe entregar información respecto de la metodología de enseñanza que se aplicará a lo largo de la actividad de formación o capacitación, sistema de evaluación, días y horarios de clases, indicadores a aplicar en el monitoreo y seguimiento de los participantes durante y después del curso, y todos los aspectos relevantes para una adecuada formación de los posibles participantes.

Clases expositivas (Presentaciones en Power Point) y prácticas (uso de software de uso masivo para el diseño de dietas)
El curso se hará en 5 días lectivos, en horarios de 09:00 a 13:00 Hrs y 14:30 a 18:00 Hrs.

falta especificar días y horario + entrega documentos del curso

PARTICIPANTES (DESTINATARIOS) EN LA ACTIVIDAD

En esta sección se deberá hacer explícito el perfil de los participantes a los cuales está dirigida la actividad de formación o capacitación. Para facilitar esta definición se puede utilizar la guía de apoyo al diseño de iniciativas de formación con enfoque de competencias laborales, la cual se entrega adjunta a este documento.

7.1. PERFIL DE LOS PARTICIPANTES (destinatarios de la actividad) Profesionales que laboran en empresas del sector acuícola, poseedores de conocimientos relacionados al cultivo de peces. Los participantes son poseedores de títulos profesionales en el área de biología, acuicultura, medicina veterinaria, nutrición, alimentación.
Estudiantes del único programa de doctorado en acuicultura existente en el país, que se dictan conjunto las Universidades de Chile, Católica del Norte y Pontificia Católica de Valparaíso.

7.2. REQUISITOS DE POSTULACIÓN (de los participantes)

Indicar si el participante debe cumplir con algún requisito para postular en función de las características específicas de la actividad de formación o capacitación propuesto.

Poseer conocimientos relacionados al cultivo de peces. Poseer títulos profesionales en el área de biología, acuicultura, medicina veterinaria, nutrición y alimentación.

7.3. CRITERIOS DE ELEGIBILIDAD O SELECCIÓN (de los participantes)
Indicar los criterios de elegibilidad y selección de los participantes.
Acreditar estar desempeñándose en el área de la acuicultura o afines. - como estudiante de doctorado o profesional.

7.4. DOCUMENTACIÓN REQUERIDA PARA POSTULAR
Especificar la documentación requerida para postular.

Certificado de la Institución en la cual se desempeña.

7.5. CUPO DE ASISTENCIA (máximo y mínimo)

Indicar el número máximo de participantes por curso y el número mínimo con el cual se recomienda y es posible impartirlo.

Maximo: 30
Mínimo: 10

8. ESTRUCTURA DE LA INICIATIVA DE FORMACIÓN

En esta sección se solicita que los postulantes definan las líneas temáticas que tienen previsto desarrollar, y a partir de las cuales se configuran y estructuran los contenidos.

Se debe entregar también la estructura en que se ordenan los módulos, indicando la organización temporal y secuencial que propone la Entidad postulante.

Además se debe indicar la relación entre los distintos módulos y/o talleres que configuran la malla curricular de la actividad de formación o capacitación.

Para cada curso y/o taller se debe indicar el número de horas que comprende e indicar si se trata de módulos obligatorios, o bien complementarios o electivos, dependiendo de las características y naturaleza de la actividad de formación o capacitación propuesta.

Para preparar esta sección se recomienda revisar previamente la **"Guía de apoyo para el diseño de iniciativas de formación con enfoque de competencias laborales".**

8.1. ESTRUCTURA DE LA INICIATIVA DE FORMACIÓN

Introducción (3.5 Hrs)

1. Definición de Ciencia Nutricional, Fisiología Animal y Bioquímica.
2. Clasificación de nutricionistas y Nutrientes.

Metabolismo Energético (1.5 Hrs)

1. Definición de unidades básicas.
2. Energía y nomenclatura.
3. Ventajas del metabolismo energético en peces.

Metabolismo de Nutrientes (19 Hrs)

1. ~~Proteínas y aminoácidos~~.
2. Toxicidad del amonio.
3. ~~Lípidos y ácidos grasos~~.
4. ~~Carbohidratos~~.
5. ~~Vitaminas~~.
6. ~~Minerales~~.
7. Alimentos y sustancias alimenticias.
8. ~~Formulación de alimentos y practica~~.

Acuicultura y Nuevas Áreas de Investigación (9.5 Hrs)

1. Futuro de la Acuicultura (Directrices).
2. Desarrollo de nuevas especies.
3. Desarrollo de dietas no contaminantes.
4. Investigación de nuevas técnicas.

8.2. MALLA CURRICULAR

No corresponde.

DESCRIPCIÓN DE CADA MÓDULO

La actividad de formación o capacitación debe presentarse estructurada en función de módulos y/o talleres, los cuales corresponden a la unidad mínima para el desarrollo de temas u objetivos más específicos. Para preparar esta sección se recomienda revisar previamente la **"Guía de apoyo para el diseño de iniciativas de formación con enfoque de *competencias laborales*"**.

Se debe realizar una descripción detallada de cada módulo o taller, a modo de ficha, en la cual se debe entregar información sobre los siguientes aspectos: objetivo(s), contenidos temáticos, docente responsable, equipo docente (si se trata de varios docentes), metodología, sistema de evaluación, material a entregar, aprendizajes esperados y horas de duración.

Cada Ficha debe ser repetida según el número de módulos o talleres contemplados en la actividad de formación o capacitación (se debe identificar con un número correlativo la ficha correspondiente a cada módulo).

En el **Anexo 4** se deben incluir antecedentes adicionales sobre los contenidos de la actividad a realizar que los proponentes consideren importantes para la adecuada evaluación de la propuesta.

NÚMERO DE HORAS:	3:5	**Ficha Nº:**	1
NOMBRE DEL MÓDULO:	Introducción		
RESPONSABLE:	S. Bai		
EQUIPO DOCENTE:	S. Bai		
OBJETIVO DEL CURSO: (Debe hacer referencia a lo que busca lograr específicamente el módulo dentro de la actividad global)	Introducir a los alumnos en el tópico general del curso.		
CONTENIDOS TEMÁTICOS: (Deben ser presentados en detalle)	Definición de Ciencia Nutricional, Fisiología Animal y Bioquímica. Clasificación de nutricionistas y Nutrientes.		
MÉTODO DE ENSEÑANZA:	Clases expositivas y talleres de aplicación y discusión.		
SISTEMA DE EVALUACIÓN:	Asistencia		

MATERIAL A ENTREGAR:	Presentacion del expositor (Apuntes) Papers del expositor en el tema tratado.
APRENDIZAJES ESPERADOS: (Deben ser coherentes con los objetivos y contendidos propuestos)	Entender y manejar conceptos básicos a utilizar en el desarrollo del curso.
NÚMERO DE HORAS:	1.5 **Ficha Nº:** 2
NOMBRE DEL MÓDULO:	Metabolismo Energético
RESPONSABLE:	S. Bai
EQUIPO DOCENTE:	S. Bai
OBJETIVO DEL CURSO: (Debe hacer referencia a lo que busca lograr específicamente el módulo dentro de la actividad global)	Conocer el funcionamiento del metabolismo energético de los peces.
CONTENIDOS TEMÁTICOS: (Deben ser presentados en detalle)	Definición de unidades básicas. Energía y nomenclatura. Ventajas del metabolismo energético en peces

METODO DE ENSEÑANZA:	Clases expositivas y talleres de aplicación y discusión.
SISTEMA DE EVALUACIÓN:	Asistencia
MATERIAL A ENTREGAR:	Apuntes del Profesor
APRENDIZAJES ESPERADOS: (Deben ser coherentes con los objetivos y contendidos propuestos)	Entender los mecanismos del metabolismo energético en los peces.
NÚMERO DE HORAS:	19 **Ficha Nº:** 3
NOMBRE DEL MÓDULO:	Metabolismo de Nutrientes
RESPONSABLE:	S. Bai
EQUIPO DOCENTE:	S. Bai
OBJETIVO DEL CURSO: (Debe hacer referencia a lo que busca lograr especificamente el módulo dentro de la actividad global)	Conocer clasificación y función de diferentes nutrientes en el metabolismo de los peces.
CONTENIDOS TEMÁTICOS: (Deben ser presentados en detalle)	Proteínas y aminoácidos. Toxicidad del amonio. Lípidos y ácidos grasos. Carbohidratos. Vitaminas. Minerales. Alimentos y sustancias alimenticias. Formulación de alimentos y práctica.

METODO DE ENSEÑANZA:	Clases expositivas y talleres de aplicación y discusión. Prácticas en el diseño de dietas para peces.
SISTEMA DE EVALUACIÓN:	Asistencia
MATERIAL A ENTREGAR:	Apuntes del Profesor. Cd con programa de elaboración de dietas (desarrollado por el equipo de LABCPAC)
APRENDIZAJES ESPERADOS: (Deben ser coherentes con los objetivos y contendidos propuestos)	Poder elaborar dietas para peces adecuadas para la nutrición y salud de éstos.

NÚMERO DE HORAS: 9.5 **Ficha Nº:** 4

NOMBRE DEL MÓDULO: Acuicultura y Nuevas Áreas de Investigación

RESPONSABLE: S. Bai

EQUIPO DOCENTE: S. Bai

OBJETIVO DEL CURSO: (Debe hacer referencia a lo que busca lograr específicamente el módulo dentro de la actividad global)	Conocer nuevas tendencias en nutrición de peces

CONTENIDOS TEMÁTICOS: (Deben ser presentados en detalle)	Futuro de la Acuicultura (Directrices). Desarrollo de nuevas especies. Desarrollo de dietas no contaminantes. Investigación de nuevas técnicas.
METODO DE ENSEÑANZA:	Clases expositivas y talleres de aplicación y discusión.
SISTEMA DE EVALUACIÓN:	Asistencia
MATERIAL A ENTREGAR:	Apuntes del profesor.
APRENDIZAJES ESPERADOS: (Deben ser coherentes con los objetivos y contendidos propuestos)	Actualizar conocimientos en cuanto a las tendencias y desafios en la temática del curso.

10. APROBACIÓN DEL PROGRAMA Y OBTENCIÓN DEL CERTIFICADO

Indicar en detalle cuales serán los requisitos para la aprobación de la actividad de formación o capacitación por parte de los participantes y para la obtención del certificado correspondiente. Además se debe indicar cuál o cuáles serán las instituciones que otorguen el certificado

10.1. REQUISITOS PARA LA APROBACIÓN DEL PROGRAMA DE FORMACIÓN

85% ASISTENCIA + aprobar de un 60% de la prueba (única)

10.2. ENTIDAD(ES) QUE ENTREGARÁ(N) EL CERTIFICADO

PONTIFICIA UNIVERSIDAD CATOLICA DE VALPARAISO
ESCUELA DE CIENCIAS DEL MAR

11. ASPECTOS ECONÓMICOS DEL PROGRAMA DE FORMACIÓN

En esta sección se debe entregar información sobre el costo de matrícula o inscripción, los beneficios que tiene su pago, las facilidades de pago posibles, el destino que se dará a estos recursos, entre otros aspectos.

11.1. CARACTERÍSTICAS DE LA MATRÍCULA O INSCRIPCIÓN (monto, modalidad de pago, beneficios y materiales que incluye)

$200.000, pago con cheque o contado, incluye materiales del curso, coffe-breaks y almuerzos.

11.2. SISTEMA DE BECAS Y AYUDAS

Entregar información sobre el sistema de ayudas y becas (liberación del pago de la matrícula en forma total o parcial) que se propone, señalando el número de becas, objetivos de las becas y ayudas, tipo o naturaleza, entre otros.

Para todos los asistentes al curso se debe contemplar la posibilidad de asignar ayudas tanto en materia de traslados como en alojamiento y alimentación. Así también se debe considerar dentro de la gestión de la actividad de formación o capacitación la administración de los recursos destinados a dichos fines y de su entrega a los participantes, es decir, la Entidad Responsable deberá organizar estos aspectos de modo de velar por que los participantes reciban los servicios de alimentación, traslado y alojamiento necesarios.

10 becas completas a alumnos de doctorado en acuicultura e integrantes del equipo del Laboratorio de Cultivo de Peces y Alimentación Para la Acuicultura (PUCV) que participan en el Proyecto FIA-PI-C-2004-1-D-089 "Aplicación del tomillo *Thymus vulgaris* en el tratamiento de enfermedades de la salmonicultura."

12. RESULTADOS E IMPACTOS ESPERADOS

Indicar cómo se espera que los participantes en la actividad de formación o capacitación aplicarán posteriormente los conocimientos y capacidades adquiridas, explicando cuál será la contribución concreta de la actividad realizada en su quehacer futuro, así como sus implicancias y proyecciones en el ámbito local, regional y/o nacional.

Toda la información que se entregará (metodologías y técnicas) serán aplicables en aquellas empresas dedicadas a la producción de alimentos para la industria acuícola, tanto en la etapa de formulación y elaboración de las dietas, como también en la etapa de selección de los ingredientes a utilizar sobre todo respecto a aquellos de origen vegetal.

Además, los contenidos del curso podrán ser aplicados en aquellas empresas agrícolas, productoras de insumos para la industria de alimentos para la acuicultura, con lo cual se impulsará la producción de productos agrícolas con las características requeridas para para un mejor aprovechamiento de los peces de cada uno de los nutrientes involucrados.

La contratación del Dr. Bai para la dictación del curso de Fisiología Nutricional, permitirá que los participantes puedan tener una visión integrada de todos los componentes necesarios para lograr el mejor aprovechamiento de los recursos disponibles en las dietas por parte de los peces. Lo que significa que los participantes podrán seleccionar los mejores ingredientes y utilizar estos de la mejor forma para lograr un buen desarrollo de los peces con un bajo impacto de los desechos producidos en el ambiente en que éstos son cultivados.
Los participantes del curso tendrán la oportunidad de intercambiar y discutir con el Dr. Bai sus propios proyectos innovadores en curso.

13. EQUIPO DOCENTE (Adjuntar curriculum vitae de cada integrante del equipo docente en Anexo N°5)

Incluir la nómina del equipo docente, identificando para cada uno de sus integrantes nombre completo, RUT, entidad en la cual trabaja, teléfono, dirección postal, actividad principal y firma de cada uno de ellos.

Además en el **Anexo 5** se deberá completar la ficha para cada uno de los docentes que participan en la propuesta.

Este cuadro se entrega en el disquet en programa Microsoft Excel, de modo que deberá ser construido en dicho programa, impreso y luego compaginado con el resto del formulario para la entrega de las copias impresas señaladas en las presentes Bases. Es preciso tener presente que el disquet que debe ser entregado junto con la propuesta impresa debe contener ambos archivos (Microsoft Word y Microsoft Excel).

NOMBRE	NIVEL DE FORMACIÓN	REGIÓN (ciudad y país si corresponde)	LUGAR DE TRABAJO	ACTIVIDAD PRINCIPAL	ROL A CUMPLIR (organizador o expositor	FIRMA
1 María Isabel Toledo	Candidato Ph.D	V REGION, VALPARAISO	PUCV	DOCENTE	ORGANIZADOR	
2 Gabriel Yany	Ph.D.	V REGION, VALPARAISO	PUCV	DOCENTE	ORGANIZADOR	
3 Sungchul Bai	Ph.D	PUSAN, KOREA	Pukyon University	DOCENTE/INVESTIGADOR	EXPOSITOR	

Clases expositivas (Presentaciones en Power Point) y prácticas (uso de software de uso masivo para el diseño de dietas)
El curso se hará en 5 días lectivos, en horarios de 09:00 a 13:00 Hrs y 14:30 a 18:00 Hrs.

PARTICIPANTES (DESTINATARIOS) EN LA ACTIVIDAD

En esta sección se deberá hacer explícito el perfil de los participantes a los cuales está dirigida la actividad de formación o capacitación. Para facilitar esta definición se puede utilizar la guía de apoyo al diseño de iniciativas de formación con enfoque de competencias laborales, la cual se entrega adjunta a este documento.

7.1. PERFIL DE LOS PARTICIPANTES (destinatarios de la actividad) Profesionales que laboran en empresas del sector acuícola, poseedores de conocimientos relacionados al cultivo de peces. Los participantes son poseedores de títulos profesionales en el área de biología, acuicultura, medicina veterinaria, nutrición, alimentación.
Estudiantes del único programa de doctorado en acuicultura existente en el país, que se dictan conjunto las Universidades de Chile, Católica del Norte y Pontificia Católica de Valparaíso.

7.2. REQUISITOS DE POSTULACIÓN (de los participantes)

Indicar si el participante debe cumplir con algún requisito para postular en función de las características específicas de la actividad de formación o capacitación propuesto.

Poseer conocimientos relacionados al cultivo de peces. Poseer títulos profesionales en el área de biología, acuicultura, medicina veterinaria, nutrición y alimentación.

7.3. CRITERIOS DE ELEGIBILIDAD O SELECCIÓN (de los participantes)
Indicar los criterios de elegibilidad y selección de los participantes.
Acreditar estar desempeñándose en el área de la acuicultura o afines.

7.4. DOCUMENTACIÓN REQUERIDA PARA POSTULAR
Especificar la documentación requerida para postular.

Cerificado de la Institución en la cual se desempeña.

7.5. CUPO DE ASISTENCIA (máximo y mínimo)

Indicar el número máximo de participantes por curso y el número mínimo con el cual se recomienda y es posible impartirlo.

Maximo: 30
Minimo: 10

8. ESTRUCTURA DE LA INICIATIVA DE FORMACIÓN

En esta sección se solicita que los postulantes definan las líneas temáticas que tienen previsto desarrollar, y a partir de las cuales se configuran y estructuran los contenidos.

Se debe entregar también la estructura en que se ordenan los módulos, indicando la organización temporal y secuencial que propone la Entidad postulante.

Además se debe indicar la relación entre los distintos módulos y/o talleres que configuran la malla curricular de la actividad de formación o capacitación.

Para cada curso y/o taller se debe indicar el número de horas que comprende e indicar si se trata de módulos obligatorios, o bien complementarios o electivos, dependiendo de las características y naturaleza de la actividad de formación o capacitación propuesta.

Para preparar esta sección se recomienda revisar previamente la **"Guía de apoyo para el diseño de iniciativas de formación con enfoque de competencias laborales".**

8.1. ESTRUCTURA DE LA INICIATIVA DE FORMACIÓN

Introducción (3.5 Hrs)

1. Definición de Ciencia Nutricional, Fisiología Animal y Bioquímica.
2. Clasificación de nutricionistas y Nutrientes.

Metabolismo Energético (1.5 Hrs)

1. Definición de unidades básicas.
2. Energía y nomenclatura.
3. Ventajas del metabolismo energético en peces.

Metabolismo de Nutrientes (19 Hrs)

1. Proteínas y aminoácidos.
2. Toxicidad del amonio.
3. Lípidos y ácidos grasos.
4. Carbohidratos.
5. Vitaminas.
6. Minerales.
7. Alimentos y sustancias alimenticias.
8. Formulación de alimentos y practica.

Acuicultura y Nuevas Áreas de Investigación (9.5 Hrs)

1. Futuro de la Acuicultura (Directrices).
2. Desarrollo de nuevas especies.
3. Desarrollo de dietas no contaminantes.
4. Investigación de nuevas técnicas.

8.2. MALLA CURRICULAR

No corresponde.

13. EQUIPO DOCENTE (Adjuntar curriculum vitae de cada integrante del equipo docente en Anexo Nº5)

Incluir la nómina del equipo docente, identificando para cada uno de sus integrantes nombre completo, RUT, entidad en la cual trabaja, teléfono, dirección postal, actividad principal y firma de cada uno de ellos.

Además en el **Anexo 5** se deberá completar la ficha para cada uno de los docentes que participan en la propuesta.

Este cuadro se entrega en el disquet en programa Microsoft Excel, de modo que deberá ser construido en dicho programa, impreso y luego compaginado con el resto del formulario para la entrega de las copias impresas señaladas en las presentes Bases. Es preciso tener presente que el disquet que debe ser entregado junto con la propuesta impresa debe contener ambos archivos (Microsoft Word y Microsoft Excel).

NOMBRE	NIVEL DE FORMACIÓN	REGIÓN (ciudad y país si corresponde)	LUGAR DE TRABAJO	ACTIVIDAD PRINCIPAL	ROL A CUMPLIR (organizador o expositor	FIRMA
1 María Isabel Toledo	Candidato Ph.D	V REGION, VALPARAISO	PUCV	DOCENTE	ORGANIZADOR	
2 Gabriel Yany	Ph.D.	V REGION, VALPARAISO	PUCV	DOCENTE	ORGANIZADOR	
3 Sungchul Bai	Ph.D	PUSAN, KOREA	Pukyon University	DOCENTE/INVESTIGADOR	EXPOSITOR	

14. PROGRAMA DE ACTIVIDADES DE LA PROPUESTA

Describir todas las actividades que se realizarán para llevar a cabo la actividad de formación. Se deberán incluir tanto las actividades propias de la organización (por ejemplo, reuniones de organización, envío de invitaciones, publicación de aviso, proceso de postulación y selección de participantes, etc.) como de la ejecución de la actividad de formación o capacitación (por ejemplo, clases teóricas y prácticas, exposiciones, reuniones de trabajo, salidas a terreno, elaboración de informes, etc.). Las actividades deberán presentarse ordenadas secuencialmente, indicando la fecha probable en que se realizará cada una de ellas.

Este cuadro se entrega en el disquet en programa Microsoft Excel, de modo que deberá ser construido en dicho programa, impreso y luego compaginado con el resto del formulario para la entrega de las copias impresas señaladas en las presentes Bases. Es preciso tener presente que el disquet que debe ser entregado junto con la propuesta impresa debe contener ambos archivos (Microsoft Word y Microsoft Excel).

15-16/06/2006	PUCV	Organización	Introducir observaciones de FIA a Propuesta		Ajuste de la propuesta a solicitud de FIA
17/06/2006	PUCV/Imprenta	DIFUSIÓN	Elaborar material de difusión		Imprimir afiches y trípticos
19/06/2006	PUCV	DIFUSIÓN	Dar a conocer realización del curso		Envío del material de difusión a empresas, universidades y prensa
20-30/06/2006	PUCV	POSTULACIÓN	Inscribir a interesados en participar en curso		Inscripción de alumnos
20-30/06/2006	PUCV	Organización	Preparar material		Preparación de apuntes
17/07/2006	PUCV	CLASES	Introducir a los alumnos en el tema	30	1.Definir unidades básicas 2. Energía y nomenclatura
18/07/2006	PUCV	CLASES	Instruir respecto a principales nutrientes en nutrición	30	1. Proteínas y aminoácidos. 2. Lípidos y ácidos grasos. 3. Carbohidratos
19/07/2006	PUCV	CLASE	Instruir respecto a principales nutrientes en nutrición	30	1.Vitaminas. 2.Minerales. 3. Formulación de dietas.
20/07/2006	PUCV	TALLER	Poner en práctica lo aprendido	30	1. Formulación de alimentos (práctica)
17/18/19 y 20 / 07/2006	PUCV	Reunión investigadores Proyecto FIA con Dr. Bai	Revisión proyecto FIA FIA-PI-C-2004-1-D-089, análisis y discusión de resultados, proposición estrategias próximas actividades el proyecto	6	Antecedentes y resultados del proyecto FIA-PI-C-2004-1-D-089
21/07/2006	PUCV	CLASE	Conocer nuevas tendencias en nutrición acuícola	30	Dirección futura de la acuicultura. Desarrollo de nuevas dietas. Desarrollo de dietas de baja

					contaminación. Nuevas técnicas para investigación.
22/07/2006	PUCV	EVALUACION	Evaluación del curso y cierre.	Alumnos asistentes al curso.	Resultados de la Evaluación

16. ANEXOS

ANEXO 1: CURRICULUM VITAE DEL COORDINADOR DE LA PROPUESTA

Se deberá incluir en esta sección el *curriculum vitae* del coordinador de la propuesta y fotocopia de los certificados de títulos. Especificar función y responsabilidad en la ejecución de la propuesta, además se debe completar la ficha que sigue a continuación.

FICHA DE ANTECEDENTES PERSONALES RESUMIDA	
ANTECEDENTES PERSONALES (Obligatorio para todos los participantes)	
Nombre completo	Gabriel Yany Gonzalez
RUT	
Fecha de Nacimiento	3 de marzo de 1947
Nacionalidad	Chilena
Dirección particular (indicar comuna y región)	
Fono particular	
Celular	
E-mail	gyany@ucv.cl
Banco y número de cuenta corriente personal	
Género (Masculino o femenino)	Masculino
Indicar si pertenece a alguna etnia (mapuche, aymará, rapa nui, atacameña, quechua, collas, alacalufe, yagán, huilliche, pehuenche)	
Nombre y teléfono de la persona a quien avisar en caso de emergencia	

ACTIVIDAD PROFESIONAL Y/O COMERCIAL (ACTUAL)	
Nombre de la Institución o Empresa a la que pertenece	PUCV
Rut de la Institución o Empresa	
Nombre y Rut del Representante Legal de la empresa	Alfonso Muga N.
Cargo	Rector
Profesión	
Dirección comercial (Indicar comuna y región)	Brasil 2950, Valparaíso, V
Fono y Fax comercial	
E-mail	rector@ucv.cl
Clasificación de público o privado	
Banco y número de cuenta corriente de la institución	
ACTIVIDAD COMO AGRICULTOR (ACTUAL) (Completar sólo si se dedica a esta actividad)	
Tipo de Agricultor (pequeño, mediano o grande)	
Nombre de la propiedad en la cual trabaja	
Cargo (dueño, administrador, etc.)	
Superficie Total y Superficie Regada	
Ubicación (detallada)	
Rubros a los que se dedica (incluir desde cuando se trabaja en cada rubro) y niveles de producción en el rubro de interés	
Resumen de sus actividades	
Organizaciones (campesinas, gremiales o empresariales) a las que pertenece y cargo, si lo ocupa	

CURRICULUM VITAE

GABRIEL ELIAS YANY GONZALEZ

Profesor de Biología y Licenciado en Ciencias con mención en Biología por la Universidad Católica de Valparaíso y Doctor en Ciencias y Técnicas en Producción Animal, Opción Ictiología Aplicada por el Instituto Nacional Politécnico de Toulouse-Francia.

Profesor Titular de la Pontificia Universidad Católica de Valparaíso, realiza docencia de pregrado en la Escuela de Ciencias del Mar para las carreras de Ingeniería Pesquera y de Ingeniería en Acuicultura y de Postgrado para el Doctorado en Acuicultura y el Magíster en Ecología y Sistemática del Instituto de Biología. En esta función ha dirigido tesis de pregrado y formado parte de Comisiones de Tesis de pre y postgrado.

Su investigación la ha realizado fundamentalmente en el área de Fisiología de la alimentación, reproducción y osmoregulación de peces y en el cultivo de salmónidos. Ha participado en proyectos Fondecyt, Fondef, destacándose entre estos:

- Fondef D93, "Manejos reproductivos aplicados a la producción de salmónidos"
- Fondef D96I1038, "Elaboración y Comercialización de una vacuna di-valente para controlar los agentes piscirricketsia salmonis y virus de la necrosis pancreática infecciosa (P.S-IPNv) en la Industria Salmonicultura".
- Fondef D97T1003, "Transferencia Tecnológica en procesos de cultivo de salmones".
- Fondef D99I1055, "Optimización inmunología y genética de péptidos endógenos antimicrobianos en ostiones (*argopecten purpuratus)* para aumentar su capacidad exportadora".
- Fondef D01I1046, "Generación de nuevas materias primas para la alimentación de especies acuícolas basadas en productos algales: I Peces".

Ha publicado una veintena de artículos en revistas científicas y 4 manuales de innovación para la industria piscícola. También ha participado en proyectos de investigación financiados por FIP y FIA y presentado trabajos a Congresos nacionales e internacionales.

Ha sido asesor de empresas de cultivo de salmones y miembro del Grupo de Estudio de proyectos Fondecyt en el área de Producción Animal.

En la Pontificia Universidad Católica de Valparaíso, además, se ha desempeñado en los cargos administrativo-académicos de Director de la Escuela de Ciencias del Mar, Decano de la Facultad de Recursos Naturales, Vicerrector de Administración y Finanzas, Secretario General.

Actualmente es Consejero del Fondo de Investigación Pesquera, Vicerrector de Investigación y Estudios Avanzados en la PUCV, Director de la Fundación San Ignacio del Huinay, Director de Quintil S.A. y Director del Centro de Formación Técnica de la PUCV.

Valparaíso, Noviembre de 2005

FICHA DE ANTECEDENTES PERSONALES RESUMIDA	
ANTECEDENTES PERSONALES (Obligatorio para todos los participantes o postulantes)	
Nombre completo	María Isabel Toledo Donoso
RUT	
Fecha de Nacimiento	31/01/1953
Nacionalidad	Chilena
Dirección particular (indicar comuna y región)	
Fono particular	
Celular	9-3494714
E-mail	itoledo@ucv.cl
Banco y número de cuenta corriente personal	
Género (Masculino o femenino)	Femenino
Indicar si pertenece a alguna etnia (mapuche, aymará, rapa nui, atacameño, quechua, collas, alacalufe, yagán, huilliche, pehuenche)	
Nombre y teléfono de la persona a quien avisar en caso de emergencia	
ACTIVIDAD PROFESIONAL Y/O COMERCIAL (ACTUAL) (Los agricultores deben llenar la sección siguiente)	
Nombre de la Institución o Empresa a la que pertenece	
Rut de la Institución o Empresa	
Nombre y Rut del Representante Legal de la empresa	
Cargo	
Profesión	
Dirección comercial (Indicar comuna y región)	
Fono y Fax comercial	
E-mail	

Clasificación de público o privado	
Banco y número de cuenta corriente de la institución	

ACTIVIDAD COMO AGRICULTOR (ACTUAL) **(Completar sólo si se dedica a esta actividad)**	
Tipo de Agricultor (pequeño, mediano o grande)	
Nombre de la propiedad en la cual trabaja	
Cargo (dueño, administrador, etc.)	
Superficie Total y Superficie Regada	
Ubicación (detallada)	
Rubros a los que se dedica (incluir desde cuando se trabaja en cada rubro) y niveles de producción en el rubro de interés	
Resumen de sus actividades	
Organizaciones (campesinas, gremiales o empresariales) a las que pertenece y cargo, si lo ocupa	

CV: Mª Isabel Toledo Donoso.

1. Datos personales

Nombre: María Isabel Toledo Donoso		
Institución: Pontificia Universidad Católica de Valparaíso		
Cargo actual: Profesor Jornada. Adjunto de la Universidad Católica de Valparaíso.		
Dirección: Avenida Altamirano 1480 of. 204	**Ciudad:** Valparaíso	
Teléfonos: (32) 274279; 274263	**Fax:** (32) 274263; 274206	**E-Mail:** itoledo@ucv.cl

2. Datos curriculares

Grados	Lugar	Fecha
Maestría	Universidad de Oregon. Oregon EE.UU.	1982
Doctorado en Acuicultura	Universidad de Chile, Santiago, Chile	EN CURSO

Indicar cualquier otra actividad de entrenamiento que haya contribuido a su experiencia profesional actual.

- Curso Estrategias empresariales Universidad Adolfo Ibáñez, Viña del Mar, , 2000.
- Diplomado en Currículo basado en competencias. U. de Santiago PUCV, 2004.

2. **Publicaciones de los últimos 5 años.** (En caso de citar un libro por favor indique si éste es un volumen completo o un capítulo). Repita esta ficha las veces que sea necesario.

Título:. Capitulo 2 titulado *Problem Formulation and Options Assessment (PFOA): science-driven deliberation in risk assessment of transgenic fish* **.**		
Autores: Kristen C. Nelson, Zubaida Basiao, Anne Cooper, Madan Dey, Miguel Lorenzo Hernandez, Sukun Kunawasen, Li Sifa, Domingo Fonticiella, Blake D. Ratner, **Maria-Isabel Toledo,** Wattana Leelapatra Kapuscinski,Sifa and Hayes		
Nombre de la revista/editorial: Environmental Assesment of Genetically Modified Organisms	**Páginas**	**Año** 2006

2. Título del volumen (en caso de libro o capítulo de libro) Volumen Nº 3 de la serie CABI, serie titulada: Environmental Risk Assesment of Genetically Modified Organisms: Building Scientific Capacity for Transgenic Fish in developing Countries		
Editorial (Incluya lugar de edición) CABI Publishing. En revision.	**Páginas**	**Año**

Título: *Lupino dulce: Un ingrediente para la alimentación de truchas y salmones*		
Autores: Toledo, M., G. Olivares, P. Soto y A. Manriquez. 2004.. Universidad Católica de Valparaíso. Págs. 11-25.		
1. Nombre de la revista/editorial: Lupino dulce: Leguminosa en la producción de alimento para salmónidos,Pontificia	**Páginas** 43	**Año** 2004
2. Título del volumen (en caso de libro o capítulo de libro)		
Editorial (Incluya lugar de edición) Pontificia Universidad Católica dwe Valparaíso ISBN -956-17-0352-1, Inscripción Nº 138710 Derechos reservados.	**Páginas**	**Año** 2004

Título: *Reemplazo de harina de pescado por Harina de Lupino (**Lupinus albus**) en alimentos extruidos para trucha Arcoiris*		
Autores: TOLEDO M.I. , G. OLIVARES y P. SOTO		
Nombre de la revista/editorial: 3er simposio Avances y Proyecciones de la Acuicultura en Chile, X Congreso Latinoamericano de Acuicultura (ALA). *Ed*: Universidad Católica del Norte, Coquimbo, Chile.	**Páginas** 40	**Año** 2002
2. Título del volumen (en caso de libro o capítulo de libro)		
Editorial (Incluya lugar de edición)	**Páginas**	**Año**

Título: *Algas: nutrición y calidad para la acuicultura nacional*		
Autores: María Isabel Toledo		
Nombre de la revista/editorial: Chile Acuícola.	**Páginas**	**Año** 2003

2. Título del volumen (en caso de libro o capítulo de libro)		
Editorial (Incluya lugar de edición)	**Páginas**	**Año**

Título: *Premix de algas en alimentos para peces*		
Autores: María Isabel Toledo		
Nombre de la revista/editorial: Chile Acuícola.	**Páginas**	**Año** 2002
2. Título del volumen (en caso de libro o capítulo de libro)		
Editorial (Incluya lugar de edición)	**Páginas**	**Año**

3. Presentaciones en congresos internacionales/nacionales durante los últimos 5 años.
(Repetir esta ficha las veces que sea necesario)

Título de la presentación: *Marco legal nacional de bioseguridad en Chile*	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: Transgenic Fish Biosafety Writing Worshop, 17 – 21 Octubre 2005. Penang, MALASYA Scientific & Technical Advisory Panel (STAP) to the Global Environment Facility (GEF). Experto invitado.	**Año** 2005

Título de la presentación: Problem Formulation and Options Assessment (PFOA): science-driven deliberation in risk assessment of transgenic fish.	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: Transgenic Fish Biosafety Writing Worshop, 17 – 21 Octubre 2005. Penang, MALASYA Scientific & Technical Advisory Panel (STAP) to the Global Environment Facility (GEF). Experto invitado.	**Año** 2005

Título de la presentación: "Generación de fuentes alternativas de materias primas para la alimentación de especies acuícolas, basadas en productos algales: I. Peces.	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: *Algas enriquecidas como ingrediente en la producción de alimentos para salmónidos Puerto Montt*	**Año** 2005

Título de la presentación: Enricehd seaweed for salmonid feeding.	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: *XVII Internatinal Seaweed Symposium Bergen Noruega*	**Año** 2004

Título de la presentación: *Diversificación del uso del Lupino (Lupinus albus) utilizándolo como fuente proteica alternativa en la alimentación de la salmonicultura*	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: *Sector agrícola y la biotecnología, situación actual y desafíos,Santiago*	**Año** 2004

Título de la presentación: *Generación de fuentes alternativas de materia primas para la alimentación de especies acuícolas basadas en productos algales: I Peces*	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: *Feria Creativa Fondef*	**Año** 2003

Título de la presentación: *Manejo Alimentario de la Salmonicultura*	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: INTESAL - Aqua Sur	**Año** 2003

Título de la presentación: *Nuevos insumos para la alimentación de salmones*	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: *Feria internacional del Salmon Fisal*	**Año** 2003

Título de la presentación: *Reemplazo de harina de pescado por harina de lupino en alimentos extruidos para truchas arco iris*	
Autores: María Isabel Toledo	
Nombre y lugar del congreso: X Congreso Latinoamericano de Acuicultura. Santiago, Chile.	**Año** 2002

5. Proyectos en los cuales el investigador ha trabajado los últimos 5 años.

(Repetir esta ficha las veces que sea necesario)

Nombre del Proyecto Utilización de atractantes naturales en alimentos artificiales para trucha arco iris.	**Años** 2005-2006
Categoría en la que participó Director de Proyecto	**Fuente de financiamiento** **PUCV-UNIVERSIDAD DE CONCEPCION**

Nombre del Proyecto FIA PI-C-2004-1-D-089 Aplicación del Tomillo (*Thymus vulgaris*) en el manejo de enfermedades de la salmonicultura.	**Años** 2004
Categoría en la que participó Director de Proyecto	**Fuente de financiamiento** **FUNDACION PARA LA INNOVACION AGRARIA**

Nombre del Proyecto DI 223796/2004 "Utilización de *Leptocarphus ruvilares*, planta medicinal, en el tratamiento de hematomas cancerígenos en hígados de truchas de cultivo".	**Años** 2004
Categoría en la que participó Director de Proyecto	**Fuente de financiamiento** **PUCV**

Nombre del Proyecto	**Años**
Proyecto FIA C-01-1-D060 . "Diversificar el uso del lupino, utilizándolo como fuente proteica alternativa en la alimentación de la salmonicultura	2001
Categoría en la que participó	**Fuente de financiamiento**
Director de Proyecto	**FUNDACION PARA LA INNOVACION AGRARIA**

Nombre del Proyecto	**Años**
Evaluación de la harina de lupino como fuente proteíca alternativa en formulación de alimentos para reproductores de trucha arco iris	2001
Categoría en la que participó	**Fuente de financiamiento**
Director de Proyecto	**PUCV**

Nombre del Proyecto	**Años**
FONDEF D01-I-1046 "Generación de fuentes alternativas de materias primas para la alimentación de especies acuícolas, basadas en productos algales: I. Peces.	2001
Categoría en la que participó	**Fuente de financiamiento**
Director de Proyecto	**FONDEF**

4. **Formación de estudiantes y de investigadores jóvenes.** Incluya tutoría y co-tutoría de tesis,
Entrenamiento a través de seminarios individuales o colectivos, cursos, etc.
(Repetir esta ficha las veces que sea necesario)

Nombre de la tesis/seminario/curso		
Utilización de harina de lupino -lupino albus- en formulaciones de alimento para juveniles de trucha arcoiris -oncorhynchus mykiss.		
Grado postulado	**Lugar/institución**	**Año**
Ingeniero Pesquero	PUCV	2004

Nombre de la tesis/seminario/curso		
Evaluación de la prefactibilidad técnica-económica de cultivo de carpa -cyprinus carpio- utilizando agua proveniente del sistema de refrigeración de una central termoeléctrica.		
Grado postulado	**Lugar/institución**	**Año**
Ingeniero Pesquero	PUCV	2001

Nombre de la tesis/seminario/curso		
Prefactibilidad técnico-económica para el cultivo del puye -galaxias maculatus- a escala comercial, en la IX región.		
Grado postulado	**Lugar/institución**	**Año**
Ingeniero Pesquero	PUCV	2002

Nombre de la tesis/seminario/curso		
Diseño y dimensionamiento de un sistema de remoción de desechos orgánicos para ser implementado en balsas-jaulas.		
Grado postulado	**Lugar/institución**	**Año**
Ingeniero Pesquero	PUCV	2001

7. Consultorías a particulares, empresas o instituciones (públicas y privadas)

(Describa en detalle).

- Se desempeña como Jefe de Proyecto en Los estudios de Pesca o Acuicultura que realiza Aquambiente Ltda.
- Se desempeña como perito judicial en materias acuícola pesqueras
- Diagnóstico para el Fortalecimiento de las Políticas Ambientales Referentes a la Introducción de Especies Hidrobiológicas Exóticas. Para Comisión Nacional del Medio Ambiente.
- Supervisión del Estudio de Potencial de Cultivo Hidrobiológico Estuarinos y selección de sitios aptos en la VII Región. Para Gobierno Regional de la VII Región.
- Informe Pericial- Tasación de Aparejos de Pesca. Determinación del valor de las especies incautadas a bordo del pesquero Pan Atlantis I. Ordenado por el 2º Juzgado Civil. Valparaíso.
- Estudio Ambiental para 5 empresas procesadoras de Puerto Natales (Pesquera Edén 1 y 2, Pesquera Froward, Comercial Mac Clean y Cía., Pesquera Cold S.A.).Con financiamiento Corfo.
- Estudio de antecedentes Biológico Técnico de Cultivo de Pejerreyes en Terrenos Adyacentes al lago Rapel. Prefactibilidad Técnica-Económica. Sercotec VI Región.
- Caracterización de las Aguas de la XI y XII regiones, para evaluar el potencial de Cultivos Marinos. Proyecto FTT Austral.
- Supervisión en Estudio de Mercado de Productos Pesqueros de Caletas Artesanales de la VI Región. Diseño de Plan Estratégico Encargado por Gobierno Regional de la VI Región.
- Determinación de Porción de Agua y Fondo para Cultivo en Nehuentue, IX Región.
- Diagnóstico y Difusión de Pisciculturas en Aguas Interiores de la VI Región. SERNAPesca V Región.
- Evaluación Impacto Ambiental en Ecosistema costero adyacente Planta Termoeléctrica-Tocopilla.
- Alternativas de aprovechamiento de capturas pesqueras en la industria de la acuicultura (Pesca Chile).
- Estudio de Impacto Ambiental Marítimo para Celulosa Arauco en Laraquete (VIII Región).

FICHA DE ANTECEDENTES PERSONALES RESUMIDA	
ANTECEDENTES PERSONALES **(Obligatorio para todos los participantes)**	
Nombre completo	Bai Sungchul Charles
RUT	
Fecha de Nacimiento	23 de Febrero de 1954
Nacionalidad	Koreana
Dirección particular (indicar comuna y región)	Feeds and Foods Nutrition Research Center Pukyong National University Busan 608-737, Korea
Fono particular	(82-51) 620-6137/6874
Celular	
E-mail	scbai@pknu.ac.kr
Banco y número de cuenta corriente personal	
Género (Masculino o femenino)	Masculino
Indicar si pertenece a alguna etnia (mapuche, aymará, rapa nui, atacameña, quechua, collas, alacalufe, yagán, huilliche, pehuenche)	
Nombre y teléfono de la persona a quien avisar en caso de emergencia	

ACTIVIDAD PROFESIONAL Y/O COMERCIAL (ACTUAL)	
Nombre de la Institución o Empresa a la que pertenece	Feeds and Foods Nutrition Research Center Pukyong National University, Pusan
Rut de la Institución o Empresa	
Nombre y Rut del Representante Legal de la empresa	
Cargo	Investigador/Docente
Profesión	Dr. en Nutricion Animal
Dirección comercial (Indicar comuna y región)	Feeds and Foods Nutrition Research Center Pukyong National University Busan 608-737, Korea
Fono y Fax comercial	
E-mail	
Clasificación de público o privado	
Banco y número de cuenta corriente de la institución	
ACTIVIDAD COMO AGRICULTOR (ACTUAL) **(Completar sólo si se dedica a esta actividad)**	
Tipo de Agricultor (pequeño, mediano o grande)	
Nombre de la propiedad en la cual trabaja	
Cargo (dueño, administrador, etc.)	
Superficie Total y Superficie Regada	
Ubicación (detallada)	
Rubros a los que se dedica (incluir desde cuando se trabaja en cada rubro) y niveles de producción en el rubro de interés	
Resumen de sus actividades	
Organizaciones (campesinas, gremiales o empresariales) a las que pertenece y cargo, si lo ocupa	

ANEXO 2: ANTECEDENTES DE LA ENTIDAD RESPONSABLE

2. INGRESOS OPERACIONALES (miles de $)

Los principales ingresos que registra la Universidad cada año provienen de los aranceles de matrículas de sus estudiantes de Pre Grado y Post Grado, investigaciones académicas, aportes fiscales directo e indirecto, fondos concursables del sector público y donaciones del sector privado

	2005	2004
Donaciones Privadas		
Recursos con Restricción al Cierre del Ejercicio		
Donaciones sin Restricción al Cierre del Ejercicio		
Subvenciones y Aportes		
Aporte Fiscal Directo		
Aporte Fiscal Indirecto		
Aporte Fiscal para Becas		
Otras Transferencias Fiscales		
Fondos Concursables		
Recursos con Restricción A Ant.		
Recursos con Restricción al Cierre del Ejercicio		
Subvenciones / Aportes sin Restricción al Cierre del Ejercicio		
Servicios Académicos		
Matrículas		
Derechos Universitarios		
Investigaciones Académicas		
Servicios No Académicos		
Servicios Generales		
Arriendos en General		
Venta de Bienes y Productos		

3. GASTOS OPERACIONALES (Miles de $)

Personal

Sueldos
Honorarios y Becas
Colaciones y Viáticos
Provisión Vacaciones Devengadas
Indemnizaciones y otros beneficios
Nota: En el año 2005 quedan provisionados $ 1.201.419 M., por desvinculación profesores 2005/2006 valor final acordado a pagar en Marzo 2006.- El 2004 la prov.fue de $ 450.235M

Bienes de Consumo

Bienes de Consumo Corriente

Bienes no Inventariables
Combustibles y Lubricantes

Servicios No Personales

Servicios Básicos
Servicios de Transporte y Comunicación
Arriendos Pagados
Seguros
Manutención
Otros Servicios

Transferencias

Becas y Beneficios Alumnos
Fondo Bienestar del Personal
Consejo de Rectores
Fondo Solidario Crédito Universitario
Otros Organismos

4. INGRESOS NO OPERACIONALES (Miles de $

Renta de Inversiones

Intereses de Inversiones
Dividendos
Intereses por Préstamos al Personal

Tasas Recargo Matrículas y otros

Tasas de Recargo Matrículas
Ingresos Años Anteriores
Ingresos No Clasificados

5. GASTOS NO OPERACIONALES (Miles de $)

Gastos Legales y Financieros

Intereses y Reajustes
Comisiones y Gastos Bancarios
Permisos de Circulación, Gastos
Notariales, Judic., Impuestos y Otros
Crédito Fiscal IVA Rechazado
Descuento por Pronto Pago Matrículas
Diferencias de Cambio

6. SINIESTROS Y CASTIGOS DEL PERIODO (Miles de $)

Siniestros y Castigos del Período

Castigo Activo Fijo y Bodega Reactivos
Provisión Incobrabilidad Alumnos, otros
Deudores y Condonación Deuda
Matrículas
Provisión Incobrabilidad Préstamos al
Personal

7. RESUMEN DE LOS PRINCIPALES CRITERIOS CONTABLES APLICADOS

A) BASES DE PREPARACION DE LOS ESTADOS FINANCIEROS

Los estados financieros han sido preparados de acuerdo con principios de contabilidad generalmente aceptados y consistentes con los aplicados en años anteriores. Conforme lo establecido por el Boletín Técnico # 63 del Colegio de Contadores de Chile A.G. para las organizaciones sin fines de lucro, se han aplicado los puntos relativos a reconocer las restricciones temporales y permanentes que puedan afectar a los activos Circulantes y Fijos. De igual forma se aplicó lo establecido en el Boletín Técnico # 47 que dice relación con el reconocimiento del costo de las vacaciones devengadas por el personal al cierre del

ejercicio, reconociéndose la variación de las mismas con cargo a los resultados del ejercicio

B) CORRECCION MONETARIA

Los estados financieros han sido ajustados mediante la aplicación de las normas legales de corrección monetaria vigentes, de acuerdo a principios de aceptación general. Para el presente ejercicio, se han corregido monetariamente las cuentas de Ingresos y Gastos. El saldo de la aplicación de dicha corrección se origina de la siguiente forma:

Activo Circulante

Pasivo Circulante
Activo Fijo
Fondo Depreciaciones
Capital Propio
Otros Activos
Ingresos
Gastos

Cargo Neto a Resultados

El Balance y el Estado de Actividades del periodo anterior han sido ajustados en un 3,6%, correspondiente a la variación del IPC en el periodo relevante, para efectos de permitir una adecuada comparación con los estados financieros del presente ejercicio.

C) DEPOSITOS A PLAZO E INVERSIONES

Los depósitos a plazo e inversiones se presentan a sus valores de inversión más los reajustes e intereses devengados al 31 de Diciembre.

D) DEUDORES MATRICULAS

Por primera vez, se ha efectuado el proceso de matrícula para alumnos de primer año del año académico siguiente, durante el mes de Diciembre del año en curso, lo anterior ha significado reconocer las deudas de estos alumnos con la Universidad descontando al arancel de inscripción respectivo, como igualmente reconocer el ingreso anticipado de dichas matrículas, dado lo anterior la composición de las deudas alumnos al 31 de Diciembre es la siguiente

-Deudas del año académico 2005	Activo Circulante
-Deudas del año académico 2006	Otros Activos
Total deudas alumnos	

E) ACTIVO FIJO

El Activo Fijo se presenta a su costo de adquisición más las revalorizaciones legales acumuladas al 31 de Diciembre del año de cierre. La depreciación de dichos bienes, se ha calculado de acuerdo al sistema lineal, considerando los años de vida útil remanente de los respectivos bienes, para el año 2005 ésta fue de $1.739.482.694 y para el año 2004 fue de $1.685 594.015.

8. COMPROMISOS CON BANCOS

La Universidad ha contratado créditos con bancos privados nacionales tanto para el corto plazo como para el largo plazo. Estos fondos han sido utilizados para cancelar indemnizaciones por desvinculación del personal académico desde el año 2001 a la fecha y una parte para cubrir necesidades normales de operación. Los compromisos pendientes al 31 de Diciembre del 2005 son los siguientes:

Banco del Estado	
Banco Scotiabank CR 1	
Banco Chile (deuda en $)	
Banco Scotiabank CR.2	
Banco Scotiabank CR.3 Curauma	
Banco Chile (Desvinculación 2005)	
Intereses Devengados al Cierre del Ejercicio	
Totales	

9.-INGRESOS ANTICIPADOS

Durante el mes de Diciembre de este ejercicio, por primera vez tuvo lugar al proceso de matrículas de alumnos de primer año del año siguiente, por este motivo se reconoció un ingreso anticipado por concepto de matrículas año académico 2006 por $ 5.358.347.000.-

10. PROVISIONES Y CASTIGOS

Durante el año 2005 la Universidad procedió al igual que años anteriores, ha efectuar una provisión de castigo de deudas incobrables de alumnos del año 2004 y anteriores como también provisiones contables sobre deudores varios de dudosa recuperación lo cual, unido al castigo de algunas existencias significó un cargo a resultados final de $ 456.414 405 -

10. IMPUESTO RENTA

La Universidad no ha constituido provisión para impuesto a la renta de primera categoría, dado que según el Decreto Ley 1604 Art.14 publicado en el Diario Oficial del 03/12/76 y en conformidad con el Decreto Ley 824 Art.40 N°2, las Universidades mantienen su exención por los ingresos que le son propios a su actividad, quedando sujetas solamente al pago de los impuestos sobre las utilidades generadas por las actividades del art.20 N° 3 y N° 4 que puedan estas realizar.

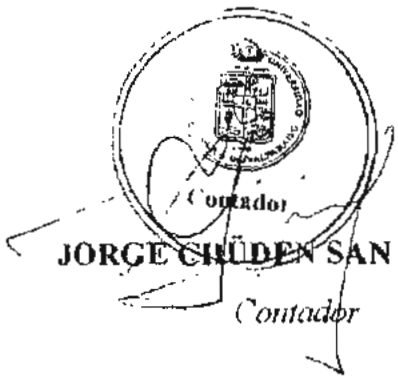
11. GARANTIAS Y AVALES

La Universidad por acuerdo del Honorable Consejo Superior, se ha constituído como aval de los créditos bancarios que han obtenido las empresas relacionadas Corporación de Televisión de la Universidad Católica de Valparaíso y CFT UCEVALPO , además se han otorgado garantías con boletas extendidas por bancos, a favor de terceros para caucionar recursos provenientes de Proyectos.

Al cierre del ejercicio estos tipos de créditos y las garantías de proyectos son los siguientes.

12. HECHOS POSTERIORES

Hasta la fecha de la emisión de los presentes Estados Financieros no han ocurridos hechos relevantes que pudieran tener un efecto significativo en las cifras presentadas al 31 de diciembre de 2005



JORGE CRUDEN SAN MARTIN

Contador

ALEX PAZ BECERRA

Director de Finanzas

ANEXO 3: ANTECEDENTES DE LA(S) ENTIDAD(ES) ASOCIADAS

ANEXO 4: CONTENIDOS DE LA ACTIVIDAD

Short Course
in
Physiology of Fish Nutrition

July 17 to 21, 2006.

Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso,
Av. Altamirano 1480, Valparaíso.

Professor: Dr. Charles Bai, Ph.D., Nutrition/Physiological Chemistry. (See attached CV)

Units and Topics of the course.

Unit I.-

Introduction: 1. Define Nutritional Sciences, Biochemistry and Animal Physiology
2. Classification of Nutritionists and Nutrients

1-1. Definition of Fish Nutrition and Biochemistry:
1-2. Definition of Physiology:
1-3. Important subjects of physiology:

2-1. Nutritionists:
2-2. Nutrients:
2-2-1. Major nutrients groups: Protein, Lipids, Carbohydrates, Vitamins, Minerals and Water

Unit II.-

Energy metabolism: 1. Define the basic units
2. Energy partitioning and nomenclatures
3. Advantages of E metabolism in Fish

1-1. Calorie
1-2. Joule
2-1. Energy partitioning
2-2. Nomenclatures
3. Advantages of Energy metabolism in Fish

Nutrients metabolism: 1. Protein & amino acids
2. Ammonia toxicity

1-1. Protein's structures, function and roles
1-2. Evaluation of protein quality
1-3. Nitrogen excretion (urea, uric acids, ammonia)
1-4. Amino acids' structure function
1-5. Definition of Essential Amino Acids (EAA) and NEAA
1-6. Definition of Biochemical Amino Acids and the Definition of Life Status
2-1. Ammonia Toxicity via TCA
2-2. via Neuro-transmitter

2-3. via unbalance of Cation in and out side of Cell (Na-K ATPase)

Unit III.-

Nutrients metabolism: 1. Lipids and fatty acids
2. Carbohydrates
3. Vitamins
4. Minerals

1-1. Utilization, Classification and Function of Lipids
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PARA LA ACUICULTURA

Mayor Información:

itoledo@ucv.cl

Fonos: (56) (32) 274263, 274279,
Fax: (56) (32) 274263, 274206

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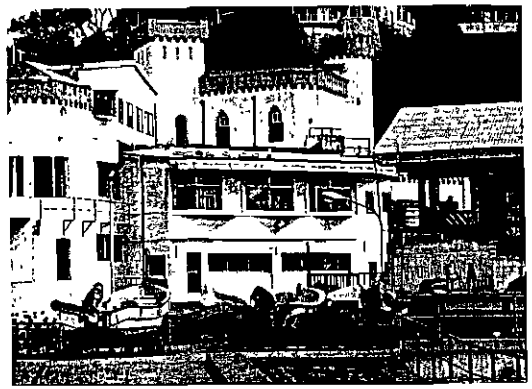

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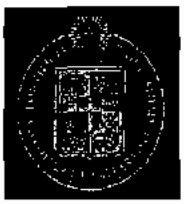
Pukyong National University, Pusan, Korea
y
Laboratorio de Cultivo de Peces y Alimentación para la Acuicultura (*LABCPAC*), Escuela de Ciencias del Mar PUCV

e-mail: itoledo@ucv.cl Fonos: (56) (32) 274263 - 274241- 274279
Fax: (56) (32) 274263 - 274206
CODIGO SENCE 1237761415



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Post Prandial Plasma Free Arginine Concentrations Increase in Rainbow Trout Fed Arginine-deficient Diets

Gunjun Park, Sungchul C. Bai*, Im-ho Ok, Kyungmin Han, Silas S. O. Hung[1]
Quinton R. Rogers[2] and Taesun Min[3]
Department of Aquaculture, Pukyong National University, Busan 608-737, Korea

ABSTRACT : Three experiments were conducted to determine the effects of dietary arginine concentrations on plasma free amino acid (PAA) concentrations in rainbow trout, *Oncorhynchus mykiss* (Walbaum) The first experiment was conducted to determine appropriate post-prandial and food deprivation sampling times in dorsal aorta cannulated rainbow trout averaging 519±9.5 g (mean±SD) at 16°C. Blood samples were taken at 0, 2, 3, 4, 5, 6 and 24 h after feeding (0 and 24 h blood samples were taken from the same group of fish). PAA concentrations increased by 2 h post-feeding and the concentration of all essential amino acids except histidine peaked at 5 h and returned to 0 time values by 24 h In the second experiment dorsal aorta cannulated rainbow trout averaging 528±11.3 g (mean±SD) were divided into 6 groups of 4 fish to study the effect of dietary arginine levels on PAA. After 24 h food deprivation, each group of fish was fed one of six L-amino acid diets containing graded levels of arginine (0.48, 1.08, 1.38, 1.68, 1.98 or 2.58%) by intubation. Blood samples were taken at 0, 5 and 24 h after feeding. Post-prandial (5 h after feeding) plasma-free arginine concentrations (PParg) showed a breakpoint at 1.03% arginine in the diet and post-absorptive (24 h after feeding) plasma free-arginine concentrations (PAarg) showed a breakpoint at 1.38% arginine. PAarg increased linearly from fish fed diets containing arginine between 0.48% and 1.38%, and the concentrations remained constant from fish fed diets containing arginine at or above 1.38%, but were all below PParg at all time points. Results of the third experiment confirm the results that PParg concentrations from fish fed arginine deficient diets were higher than PAarg (0 or 24 h values). Thus, in contrast to mammals and birds, the PParg when arginine is present in the diet as the most limiting amino acid such that it severely limits growth, increases in plasma rather than decreases. *(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 396-402)*

Key Words : Arginine, Rainbow Trout, Dorsal Aorta Cannulation, Plasma Free Amino Acids

INTRODUCTION

Evaluation of plasma free amino acids concentrations in mammals and birds has led to discoveries involving the genetic defects of amino acid metabolism, the secondary perturbations of amino acid metabolism as a result of primary renal or liver disease and the effects of amino acid deficiencies, imbalances and toxicities on amino acid metabolism (Zicker and Rogers, 1990) Factors influencing plasma free amino acids (PAA) concentrations in growing animals have been studied extensively. Although assay procedures used in PAA studies have varied considerably, the results obtained have been quite consistent in demonstrating that dietary amino acid deficiencies result in reduced plasma concentration of that amino acid, post-prandially (Hill and Olsen, 1963), whereas dietary amino acid excesses have resulted in increase of that amino acid in the plasma (Richardson et al., 1953).

Studies in the chick (Richardson et al., 1953, Hill and Olsen, 1963), rat (Swendseid et al., 1963; Young and Zamora, 1968), pig (Puchal et al., 1962), human (Longenecker and Hause, 1961; Snyderman et al., 1964) and fish (Thebault, 1985) have clearly established that a reduced concentration of an essential amino acid (EAA) in plasma reflects a deficient level of that amino acid in the diet. Others (Munro, 1970; Young and Scrimshaw, 1970; Young et al., 1971) showed that the pattern of amino acids and the level of a specific EAA in plasma correlate with the ability of the dietary protein to support growth The relationships between the concentration of PAA and dietary amino acid intake have been the subject of reviews (Leathem, 1968; McLaughlan and Morrison, 1968; Munro, 1970; Young and Scrimshaw, 1970; Zicker and Rogers, 1990).

Although the effects of dietary protein sources and amino acid mixtures on plasma free essential amino acid concentrations in sea bass (Thebault, 1985) and in rainbow trout (Schuhmacher et al., 1997; Vermeirssen et al., 1997) have been reported, the complete dose-response relationships for arginine have not been investigated Therefore, the objectives of the present study were to

* Corresponding Author. Sungchul C. Bai Tel: +82-51-620-6137, Fax: +82-51-628-6873, E-mail: scbai@mail.pknu.ac.kr
[1] Department of Animal Science, College of Agriculture and Environmental Sciences, University of California, Davis, CA 95616 USA.
[2] Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616 USA.
[3] Korea Science and Engineering Foundation, Gajeong-dong, Yuseong-Gu, Daejeon 305-350, Korea.
Received May 5, 2004; Accepted November 26, 2004

Table 1. Composition of the basal diet (% of dry matter)[1]

Ingredients	%
EAA[2]	17.27
NEAA[3]	12 36
Casein[4]	5 00
Gelatin[4]	2.00
Dextrin[4]	27.97
Dextrose[4]	5.00
α-cellulose[4]	8.20
Fish oil[5]	10.00
Carboxymethyl cellulose[4]	1.00
$Ca(H_2PO_4)_2H_2O$	3.00
Choline bitartrate[4]	1.20
Vitamin mixture[6]	3.00
Mineral mixture[7]	4 00

[1] Diets were neutralized with NaOH to give a final pH 6.6.
[2] EAA: Essential amino acids, Ajinomoto, Tokyo, Japan.
[3] NEAA: Non-Essential amino acids, Ajinomoto, Tokyo, Japan.
[4] United States Biochemical (USB), Cleveland, Ohio.
[5] Ewha Oil Company, Pusan, Korea.
[6] Vitamin mixture (mg/kg feed unless indicated otherwise): vit. A, 3,000 IU; vit. D_3, 2,400 IU; vit. E, 120 IU; menadione sodium bisulfate, 6, vit. B_1-HCl, 15; vit B_2, 30; vit B_6-HCl, 15; vit B_{12}, 0.06; vit C, 300; calcium pantothenate, 150; nicotinamide, 150; inositol, 150; d-biotin, 1.5; choline chloride, 3,000, pancreatin, 12.5. Vitamin mixture prepare by our laboratory and the individual vitamins purchased from USB, Cleveland, Ohio, USA.
[7] Mineral mixture (mg/kg feed): $MnSO_4$, 320, $ZnSO_4$, 270, $FeSO_4$, 750; $CuSO_4$, 60, $CoSO_4$, 7; $MgSO_4$, 17.3; K_2SO_4, 212; NaCl, 519; K_2HPO_4, 136; $NaSeO_3$, 0 01; KI, 0.15. Mineral mixture was prepared by our laboratory and the individual minerals purchased from Junsei Chemical, Tokyo, Japan.

Table 2. Amino acid composition of the basal diet (%of dry matter)

Amino acids	From casein +gelatin	From crystalline amino acids	Total[1]
EAA			
Arginine	0.353	1.924[2]	2.277
Histidine	0.194	0.725	0.919
Isoleucine	0.252	1.674	1.926
Leucine	0 493	2.702	3.195
Lysine	0.502	1.904	2.406
Methionine	0.152	1.030	1.182
Cystine	0.019	0.172	0.191
Phenylalanine	0.271	1.742	2.013
Tyrosine	0.270	1.335	1.605
Threonine	0.221	1.601	1.822
Tryptophan	0.065	0.462	0.527
Valine	0.350	1.999	2.349
NEAA			
Alanine	0.345	1.741	2.086
Aspartic acid	0.483	3 280	3.763
Glycine	0.538	0.758	1.296
Glutamic acid	1.298	3.616	4.914
Proline	0.790	0.568	1.358
Serine	0.374	2.398	2.772

[1] The amino acid profile simulated that of 35% whole chicken egg protein (Robinson et al., 1981).
[2] Six experimental diets were formulated to have graded levels of arginine (0.48, 1.08, 1.38, 1.68, 1.98 or 2.58%); equal amounts of aspartic acid and glutamic acid by weight were substituted by arginine in the basal diet.

determine the effects of the different dietary arginine levels on PAA concentrations and to estimate the dietary arginine requirement by using surgically modified young growing rainbow trout (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Animals and husbandry

Rainbow trout averaging 519±9.5 g (Experiment I), 528±11.3 g (Experiment II) and 521±13.1 g (Experiment III) were obtained from Ewhajung Trout Farm in Sang Joo, Korea. For all experiments, net cages (1.3 m×1.3 m×1.3 m) were placed in a flow-through raceway with a water flow of 60 L/min Supplemental aeration was also provided to maintain the dissolved oxygen near 7.2±0.4 mg/L. Water temperature was maintained at 16±0.2°C.

Dorsal aorta cannulation and intubation

The trout were anesthetized with 200 mg/L 3-aminobenzoic acid ethyl ester methansulfonate (MS 222, Sigma Chemical Company, St. Louis, MO) for 3 to 5 minutes, placed on a V-shape table and gills were continuously irrigated with 16°C water containing 100 mg/L of MS 222 during the operation. A 50 cm-long cannula (Clay Adams PE 50 tubing, Parsippany, NJ) with a bubble about 5-6 cm from one end was washed with heparinized Cortland saline solution (Houston, 1990) and a 13-gauge needle was used to pierce a hole on the right nostrum (ventral side up) for the cannula to exit. A 19-gauge needle was used to bore a small hole in the roof of the mouth at the mid-line behind the third gill arc at a 30° angle and a piano wire was inserted into the PE 50 tubing as a guide. The proper insertion was verified by the observation of a slow blood flow after the wire was withdrawn from the cannula. A 3 ml syringe with a 23-gauge needle was used to remove air and blood clot and the cannula was flushed with the heparin solution. The cannula was sutured behind the bubble on the roof of the mouth, led out from the right nostrum, plugged with a color head pin, and sutured at the dorsal fin (I-H Ok et al., 2001; Bai et al., 2003).

Experimental design and diets

Experiment I was conducted to determine the appropriate post-prandial and post-absorptive time for blood sampling in dorsal aorta cannulated rainbow trout (I-H Ok et al., 2001; Bai et al., 2003). After dorsal aorta cannulation, the trout were divided into 6 groups (4 fish per group) in each net cage and were fed a commercial rainbow

trout diet (Woosung Feed Co Ltd., Taejon-Si, Korea) for 3 days until the fish recovered from the operation of dorsal aorta cannulation. After 24 h food deprivation, these fish were intubated with the L-amino acid based diet at 1% body weight (dry-matter), anesthetized with MS222 and blood was sampled at 0, 2, 3, 4, 5, 6 and 24 h thereafter (0 and 24 h blood samples were taken from the same group of fish). The basal diet was formulated by the modification of Kim (1997) and contained a 29.6% crystalline amino acid mixture, 5% casein and 2% gelatin. Ingredients and amino acid composition of the basal diet are shown in Table 1 and 2, respectively. The ingredient mixtures without oil were stored at -80°C until used and basal diet was prepared by adding fish oil (10% of diet) and water (0.4 part of distilled water/diet, w/w) before intubation.

Experiment II was conducted to determine the effects of different dietary arginine levels on post-prandial (5 h after feeding, PParg) and post absorptive (24 h after feeding, PAarg) plasma free arginine concentrations in rainbow trout. Rainbow trout were divided into 6 groups of 4 fish each in net cage and fed a commercial diet (Woosung Feed Co. Ltd., Taejon-Si, Korea) for 3 days until the fish recovered from the operation of dorsal aorta cannulation. After 24 h food deprivation, these fish were intubated with 1% body weight (dry-matter) of the experimental diets. Six diets were formulated to contain 0 48, 1.08, 1.38, 1.68, 1.98 or 2.58% of arginine. Equal amounts of aspartic acid and glutamic acid by weight were substituted for the proper amounts of arginine in the diets. Each group of fish was anesthetized and blood was sampled from each fish within a group at 0, 5 and 24 h after intubating the experimental diets (0.4 parts of distilled water diet, w/w) by using a 3 ml syringe.

Experiment III was conducted to confirm the results from Experiment II in which PParg were higher than PAarg from fish fed the arginine deficient diet (0.48%). Four rainbow trout were fed a basal diet for 3 days until the fish recovered from the operation of dorsal aorta cannulation. After 24 h food deprivation, these fish were intubated at 1% body weight (dry-matter) of the 0.48 or 2.58% arginine diet Two consecutive dietary periods were used: the first period, 3 days of the 2.58% arginine diet; the second period, 3 days of the 0.48% arginine diet. In the first period, 0 h post feeding blood samples were taken at the beginning of day 1 and 24 h later (24 h after feeding). During the second period, 0 h and postprandial (5 h after feeding) blood samples were taken for 3 days (day 1, 2 and 3). The post-absorptive (0 h) blood samples were taken on day 4 (24 h after feeding fish on day 3).

Sample collection and analysis

Fish were anesthetized with 200 mg/l MS222 and 300 μl blood were sampled from each fish. Plasma samples were prepared by centrifugation at 3,000×g for 10 min. For deproteinization, the plasma samples were mixed with 10%

Table 3. Plasma free amino acid concentrations (nmol/ml) after feeding the basal diet (Experiment I)[1]

Amino acids	Time (h) after feeding							Pooled SEM
	0	2	3	4	5	6	24	
EAA								
Arginine	79^{c}	131^{b}	119^{b}	128^{b}	228^{a}	78^{c}	131^{b}	9
Histidine	114^{d}	244^{a}	186^{b}	143^{c}	185^{b}	96^{d}	113^{d}	10
Isoleucine	84^{e}	200^{d}	260^{c}	346^{b}	488^{a}	367^{b}	110^{e}	26
Leucine	146^{f}	307^{e}	392^{d}	516^{c}	784^{a}	581^{b}	166^{f}	41
Lysine	108^{e}	270^{d}	354^{c}	422^{b}	515^{a}	237^{d}	248^{d}	24
Methionine	49^{d}	149^{c}	155^{c}	222^{b}	321^{a}	235^{b}	62^{d}	17
Phenylalanine	93^{e}	178^{d}	225^{c}	426^{b}	714^{a}	239^{c}	98^{d}	40
Threonine	123^{e}	432^{c}	532^{b}	450^{c}	793^{a}	518^{b}	165^{d}	41
Tryptophan	10^{d}	23^{ab}	26^{a}	25^{a}	20^{bc}	18^{c}	10^{d}	1
Valine	222^{c}	530^{b}	594^{b}	560^{b}	783^{a}	579^{b}	238^{c}	37
Total	1,028^{e}	2,464^{d}	2,843^{c}	3,238^{b}	4,831^{a}	2,948^{c}	1,341^{e}	181
NEAA								
Alanine	603de	1086^{b}	1248^{a}	715^{cd}	845^{c}	671^{d}	465^{e}	52
Aspartic acid	474^{e}	627^{d}	694^{c}	795^{b}	976^{a}	892^{ab}	512^{e}	36
Asparagine	103^{c}	165^{b}	185^{a}	153^{b}	156^{b}	98^{c}	115^{c}	8
Citrulline	38^{bc}	45^{b}	40^{b}	49^{a}	37^{c}	31^{d}	18^{e}	2
Glycine	329^{b}	446^{a}	241^{c}	143^{d}	140^{d}	242^{c}	396^{ab}	23
Glutamic acid	272^{d}	463^{c}	614^{b}	454^{c}	1,092^{a}	614^{b}	261^{d}	52
Ornithine	111^{b}	105^{b}	126^{a}	131^{a}	118^{ab}	70^{c}	48^{d}	8
Serine	112^{c}	253^{b}	255^{b}	282^{b}	377^{a}	415^{a}	134^{c}	21
Tyrosine	39^{e}	88^{d}	136^{c}	195^{b}	300^{a}	175^{b}	57^{e}	16
1-Methylhistidine	33^{e}	76^{c}	88^{c}	111^{b}	127^{a}	87^{c}	53^{d}	6
3-Methylhistidine	31^{d}	54^{c}	72^{c}	119^{a}	115^{a}	101^{b}	39^{d}	5

[1] Values are means of four fish where the means in each row with different superscripts are significantly different ($p<0.05$).

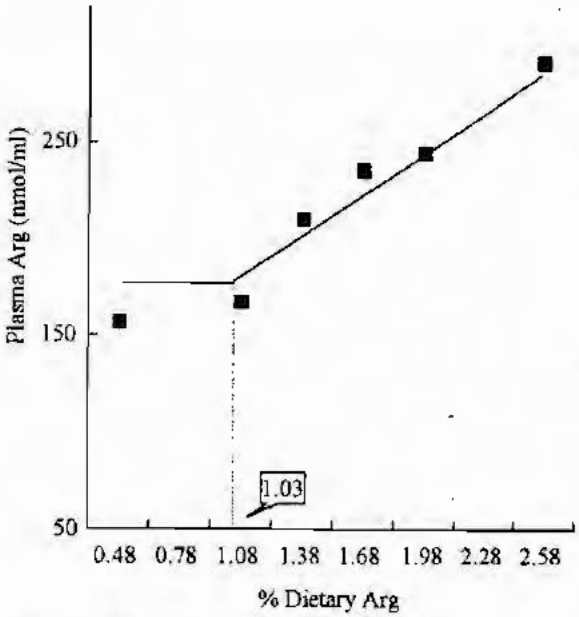

Figure 1. Plasma free arginine concentrations (nmol/ml) at 5 h (post-prandial arginine) after feeding in fish fed graded levels of dietary arginine (Experiment II). Y=175.1-33.2 (R-X), R=1.03± 0.299 (SE).

Table 4. Post-prandial and post-absorptive plasma free arginine concentrations (nmol/ml) of rainbow trout fed graded levels of dietary arginine (Experiment II) [1]

Level of arginine (%)						Pooled
0.48	1.08	1.38	1.68	1.98	2.58	SEM[2]
Post-prandial values						
157^{c}	168^{c}	211^{b}	236^{b}	245^{b}	292^{a}	1.98
Post-absorptive values						
66^{d}	93^{c}	117^{ab}	113^{b}	121^{ab}	132^{a}	0.82

[1] Values are means (n=5) and means with different superscripts are significantly different ($p<0.05$).

[2] Pooled standard error of mean: $SD/\sqrt{n}$.

5-sulfosalicylic acid in the ratio of four to one, cooled on ice for 30 min and centrifuged. The protein-free supernatant was diluted in pH 2.2 lithium citrate sample dilution buffer in the ratio of one to one and the samples were stored at -80°C until analysis. The plasma free amino acids were separated and quantified using a S433 amino acid analyzer (Sykam, Germany) using the ninhydrin method.

Statistical analysis

Data were subjected to analysis of variance test by using Statistix 3.1 (Analytical Software, St. Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $p<0.05$. The breakpoints for both PParg and PAarg were estimated by using the broken line model of Robbins et al. (1979).

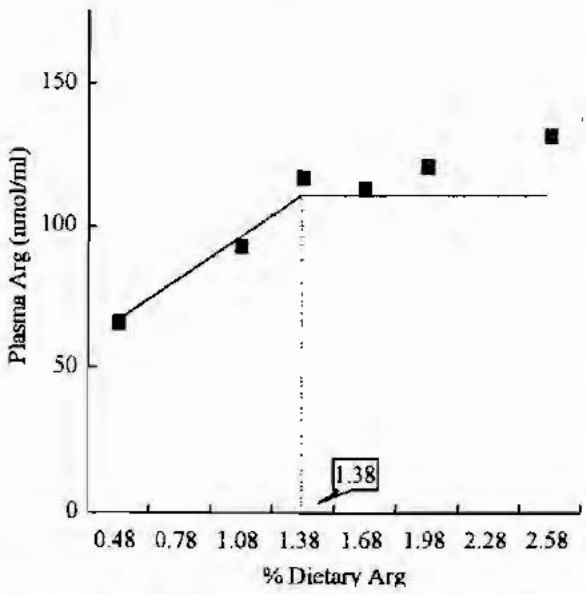

Figure 2. Plasma free arginine concentrations (nmol/ml) at 24 h (post-absorptive arginine) after feeding in fish fed graded levels of dietary arginine (Experiment II). Y=175.1-33.2 (R-X) R=1.03± 0.299 (SE).

RESULTS

Experiment I

Plasma free amino acid concentrations from fish force-fed the basal diet are shown in Table 3. Plasma free essential amino acid concentrations, with exception of histidine, lysine and tryptophan, began to increase at 2 h, peaked at 5 h and returned to near basal level at 24 h. Plasma free histidine concentration peaked at 2 h and returned to the basal level at 6 h. Plasma free lysine concentration peaked at 5 h and did not return to the basal level by 24 h. Plasma free tryptophan concentrations peaked at 2 h, remained constant between 2 h and 4 h and returned to the basal level at 24 h. Total plasma free essential amino acid concentrations peaked at 5 h and returned to near basal level at 24 h. Plasma dispensable amino acid concentrations peaked between 2 h and 6 h and returned to near basal levels at 24 h.

Experiment II

Post-prandial plasma free arginine concentrations (PParg) and post-absorptive plasma free arginine concentrations (PAarg) of fish fed graded levels of arginine are shown in Figure 1 and 2. PParg concentrations declined with decreasing dietary arginine. PParg from fish fed 0.48 and 1.08% arginine diets were not significantly different ($p>0.05$); however, PParg increased with dietary arginine levels among fish fed diets containing from 1.08 to 2.58%. PAarg significantly increased with dietary arginine levels

Table 5. Plasma free arginine concentrations (nmol/ml) from fish fed diets containing 0 48 and 2.58% dietary arginine (Experiment III)[1]

Dietary arginine (%)	Time (h) after feeding				
	0 h[2]	5 h (d1)	5 h (d 2)	5 h (d 3)	24 h (d 4)
0.48		153±25[a]	158±21[a]	168±34[a]	87±18[b]
2.58	143±27[a]				135±33[a]

[1] Values are means±SD from four fish and means with different superscripts are significantly different ($p<0.05$).

[2] 5 h postprandial blood samples were taken daily for 3 days (d1, 2 and 3) and 24 h post-absorptive blood samples were taken on day 4 (at 24 h after feeding fish on d 3). 0 h post-prandial blood samples were taken at the beginning of day 1 and feeding fish once a day, 24 h post-absorptive feeding blood samples were taken on day 4 (at 24 h after feeding fish on day 3).

from 0.48% to 1.38%, while it increased at a lower rate beyond 1.38% The breakpoints, using the broken-line model, were 1.03 and 1.38% for PParg and PAarg, respectively.

Experiment III

There were no significant differences between PParg from fish fed 0.48% arginine diet and PAarg from fish fed 2.58% arginine diet. However, the fourth day PAarg from fish fed 0.48% arginine diet was significantly lower than PParg from fish fed the same diet and the PAarg from fish fed 2.58% arginine diet ($p<0.05$).

DISCUSSION

Experiment I demonstrated that most amino acid concentrations peaked at 5 h and returned to near basal level at 24 h after feeding. From the 6 h plasma amino acid concentrations it would appear that post-absorptive concentrations might have been reached long before 24 h In mammals, including rats (Swendseid et al., 1963, Young and Zamora, 1968), pigs (Puchal et al., 1962), dogs (Longenecker and Hause, 1959) and humans (Young and Scrimshaw, 1970) severe essential amino acid deficiency causes a decrease in the limiting amino acid during the absorptive phase, with a return toward normal 12-24 h after meal. Murai et al. (1987) and Schuhmacher et al. (1997) reported similar results that the plasma concentrations of arginine, leucine, isoleucine, valine, phenylalanine and threonine from fish force-fed crystalline amino acids at 1% body weight (dry-matter) peaked at 6-9 h and returned to baseline by 24-32 h post feeding in rainbow trout

Post-absorptive (24 h after feeding) plasma free arginine concentrations (PAarg) of the trout increased as dietary protein increased, with somewhat of a plateau occurring at about 1.38% of dietary arginine. Perhaps this is the concentration of dietary arginine that results in "regulation" (either reutilization for protein synthesis or oxidation of excess of that mobilized) of body arginine during the post-absorptive phase.

Table 6. 0 h and 24 h post feeding plasma free arginine concentrations (nmol/ml) from fish fed the various levels of dietary arginine (Experiment I, II and III)[1]

	0 h post feeding value	24 h post feeding value
Exp I	79±11 (1.12%)[2]	131±13 (2.28%)[3]
Exp. II		66±5 (0 48%)
		132±15 (2.58%)
Exp. III	143±27 (2.28%)[4]	87±18 (0.48%)
		135±33 (2 58%)

[1] Values are means±SD from four fish where the means in each row with different superscripts are significantly different ($p<0.05$).

[2] 0 h post feeding value is 24 h post feeding value from fish fed commercial diet containing 1.21% arginine.

[3] 24 h post feeding value from fish fed experimental diet (dietary arginine levels).

[4] 0 h post feeding value is 24 h post feeding value from fish fed basal diet containing 2 28% arginine.

In Experiment II the effects of alterations of dietary arginine intake on post-prandial (5 h after feeding) plasma free arginine concentrations (PParg) were dependent upon the relative adequacy of the dietary arginine supply. PParg from fish fed 0 48 and 1.08% arginine diets were not significantly different; however, PParg increased with increasing dietary arginine from 1.08 to 2.58%. In the chicks, Zimmerman and Scott (1967) found that the dose-response curves for lysine, valine and arginine in the plasma remained almost flat initially and then increased at the point when the intake of each amino acid just exceeded the level required for maximum growth. In rats, McLaughlan and Illman (1967) found that the dietary level of each essential amino acid that supported the concentration of the amino acids after overnight food deprivation, was the same as that published for the requirements for each amino acid.

If the breakpoint was taken as the requirement of arginine for the trout in the present experiment, the requirement would be 1.03% of diet on the basis of PParg, considerably lower than that shown for the dose-response curve using maximum growth (Ogino, 1980; Walton et al., 1986). PAarg increased with dietary arginine from 0.48% to 1.38%, then showed a breakpoint with a slight continued slope If this breakpoint were used as the arginine requirement the arginine requirement of trout would be 1.38% dietary arginine. The latter breakpoint is close to the requirement as determined by Ogino (1980) who reported that the arginine requirement of rainbow trout was 1.4% of diet. Kim et al. (1992) estimated the arginine requirement of rainbow trout as 1.41% of diet based on the growth data when L-amino acid mixture, casein and gelatin were used as protein source. Other reported estimates of the arginine requirement of trout ranged between 1.2-1.8% of the diet (Kaushik, 1979; Walton et al., 1986). Since the breakpoint of post-prandial plasma essential amino acids has not been consistently found at the requirement for all essential amino

acids in other species, more work needs to be done before the breakpoint of post-prandial plasma essential amino acids should be taken as the requirement in trout or other fish.

Experiment II showed that PParg (157±22 nmol/ml) was higher than PAarg (66±22 nmol/ml) for fish fed 0.48% arginine diet (less than half of the estimated requirement). This response indicates a basic difference between rainbow trout and mammals and birds in the metabolic response to a dietary deficiency of arginine. Perhaps arginine is catabolized more slowly and thus is available for protein synthesis and gluconeogenesis over a longer period of time after a given meal in rainbow trout. This is not true for all amino acids in fish since for methionine in sea bass (Thebault, 1985) and lysine in rainbow trout (Schuhmacher et al., 1997) dietary deficiencies of these amino acids cause a decrease in their concentrations in post-prandial plasma.

Experiment III confirmed the results from Experiment II that PParg from fish fed the arginine deficient diet were higher than PAarg from fish fed either the arginine deficient diet (0.48%) or the arginine adequate diets (1 68-2.58%). This experiment shows that the response of PAarg pattern in trout is not similar to those of mammals and birds. PAarg from fish fed the arginine deficient diet was lower than that from fish fed the arginine adequate diet (Table 5). This might indicate that PAarg concentrations are dependent upon the previous arginine intake.

In conclusion, these results show that post-prandial plasma arginine concentrations are responsive to dietary arginine level. Feeding a diet severely deficient in arginine to trout results in a postprandial rise, not fall in plasma arginine concentration, in contrast to decreases found in birds and mammals. Breakpoint analysis using PParg resulted in a breakpoint at 1 03% dietary arginine, whereas using PAarg it resulted in a breakpoint at 1.38% dietary arginine. However, validity of using these breakpoints to estimate arginine requirement needs further study.

ACKNOWLEDGES

Supported in part by the funds of the Feeds and Foods Nutrition Research Center, Korea Sea Grant Program and Brain Korea 21 Program at Pukyong National University, Pusan, Republic of Korea.

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J. Anim. Physiol. a. Anim. Nutr. 86 (2002), 201–213 *Eingang des Ms.: 28. 12. 2001*

ISSN 0931–2439

[1]*School of Natural Resources, The Ohio State University, Columbus, USA,* [2]*Department of Aquaculture, Pukyong National University, Pusan, Korea,* [3]*Department of Veterinary Biosciences, The Ohio State University, Columbus, USA*

A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*)

By K.-J. Lee[1], K. Dabrowski[1], J. H. Blom[1], S. C. Bai[2] and P. C. Stromberg[3]

Summary

Diets incorporating three different sources of extracted cottonseed meal (CM), soybean meal and an animal protein mixture were evaluated for juvenile rainbow trout. Fish averaging 0.96 g were divided into groups of 30; 3 groups per treatment, and each group was fed one of four diets for a 16-week period. Fish meal (FM) was replaced on a 25% protein basis by each of three different sources of CM from California (CA), Tennessee (TN), and Arkansas (AR), U.S.A. In the three CM-containing diets another 25% soybean meal protein and 50% animal protein mixture were also incorporated to completely replace FM protein. The results of growth rate and feed utilization showed that FM could be entirely replaced by a mixture of plant proteins (CM and soybean meal) and animal by-product proteins. Hematocrit levels were significantly lower in the group fed CM-containing diets than in the control. The findings suggest that CM can be used as a good protein source by the incorporation of at least 15% in diets (25% of fish meal protein replacement), and that the nutritive values of CM in juvenile trout can be different due to their different origin. Significantly higher concentrations of total gossypol were found in faeces of CM-TN (5.8 ± 0.4 μmol/g) and CM-AR (5.6 ± 0.6) groups than in that of CM-CA (3.7 ± 0.4) group. It was documented that gossypol enantiomers, present in an equal proportion in diets, selectively accumulated in liver and bile, whereas equal proportions of (+)- and (−)-enantiomers were found in whole-body and faeces. Depending on CM source, fish can absorb approximately 35–50% of dietary gossypol, and the majority of the absorbed gossypol seemed to be excreted.

Introduction

Feedstuffs of animal origin are generally considered to be of higher quality than those of plant origin, primarily because of their higher protein content and superior complement of indispensable amino acids (Robinson and Li 1998). In recent years there have been efforts to increase the amount of ingredients of plant origin and studies have reported some success in replacement of fish meal (FM) in diets for large rainbow trout, *Oncorhynchus mykiss*, using soybean meals and protein concentrates (Kaushik et al. 1995; Moyano et al. 1992), soybean and corn gluten meal mixtures (Gomes et al. 1995), and a combination of several alternative protein sources (Yamamoto et al. 1995). Soybean meal was the most frequently studied dietary ingredient as a FM replacement in diets for many fish. Animal by-products, such as poultry by-product meal, meat and bone meal, feather meal, and blood meal, have

also been incorporated in practical fish feeds (Murai 1992), and have individually been used as an animal protein source for FM replacement (Davies et al., 1990; Fowler 1991; Higgs et al., 1979; Luzier et al., 1995). However, when compared to FM-based control diets, diets free of fish meal resulted in general inferior growth in salmonids, (Mambrini et al., 1999). By-products of cottonseed are used in diets for both terrestrial animals (Colin-Negrete et al. 1996) and fish (Hendricks et al., 1980) because of its high protein content. Cottonseed meal (CM) has been examined in diets of fish such as channel catfish, *Ictalurus punctatus* (Dorsa et al., 1982; Robinson and Brent 1989; Robinson and Li 1994; Robinson and Tiersch 1995), rainbow trout (Hendricks et al., 1980; Herman 1970; Roehm et al., 1967), and tilapia, *Oreochromis niloticus* (El-Sayed 1990; Robinson et al., 1984). Despite its high nutritional value, cottonseed contains gossypol, a polyphenolic compound, which is toxic to fish (Herman 1970; Rinchard et al., 2000) and terrestrial animals (Colin-Negrete et al., 1996; Makinde et al., 1997). In most studies, gossypol concentrations in fish tissue were either not analysed or were analysed by a colorimetric method with anisidine (Chamkasem 1988; Fisher et al., 1987), which can overestimate gossypol. Data on utilization of CM in fish diets in combination with animal by-product and the resulting gossypol accumulation and/or excretion are not available. There are wide variations in the ratio of gossypol enantiomers present in different species of cotton plants (*Gossypium* species) and even within different tissues in the same plant (Cass et al., 1991; Jaroszewski et al., 1992). However, only few authors reported the selective accumulation of enantiomers in tissues of animals ingesting gossypol (Kim et al., 1996), and only one report exists on catfish (Robinson and Tiersch 1995). We, therefore, evaluated three different sources of solvent extracted CM containing an equal proportion of gossypol isomers, as a FM substitute, incorporated in combination with soybean meal and an animal protein mixture in diets for juvenile rainbow trout. Diets were evaluated by fish growth rate, feed utilization, gossypol 'absorption' and concentrations in tissues, and the digestibility and concentrations of protein and minerals (phosphorus and iron) in fish body and faeces. For the first time concentrations of separate gossypol isomers in tissues and faeces of fish were analysed using specific high performance liquid chromatography (HPLC) methods employing simultaneously both UV and electrochemical detectors.

Materials and methods

Diets

Four experimental diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (47%) and gross energy (17.5 MJ/kg) (Table 1). The energy value of each diet was estimated on the basis of mammalian physiological fuel values, i.e. 16.7 KJ/g protein or carbohydrate and 37.7 KJ/g lipid (Lee and Putnam 1973). Dietary FM protein was substituted with 50 or 100% animal protein mixture (APM) and the diets marked as APM50 and APM100, respectively. The APM consisted of equal amounts of meat and bone meal (50% protein, 8.5% lipid), blood meal (92% protein, 0.3% lipid), poultry by-product meal (58% protein, 14% lipid) and feather meal (85% protein, 2.5% lipid). For CM-containing diets, FM was replaced on a 25% protein basis by one of three different sources of CM from California (CA), Tennessee (TN), and Arkansas (AR), USA. These diets are referred to as CM-CA, CM-TN, and CM-AR, respectively. In the CM-containing diets the remaining protein consisted of 25% soybean meal (SM) and 50% APM. The three different sources of CM were solvent extracted meals and the total gossypol concentrations were 1.07, 1.65, and 1.53%, for the CM from California, Tennessee, and Arkansas, respectively. The proportions of (+)- and (–)-isomers of the CM were 53 : 47, 54 : 46, and 53 : 47, respectively. The analysed concentrations of dietary total gossypol were 0.11, 0.16, and 0.16% for the CM-CA, CM-TN, and CM-AR, respectively. Experimental diets were

Table 1. **Composition of the experimental diets (% of dry matter)**

Experimental diets	Control	APM50	APM100	CM-CA	CM-TN	CM-AR
Ingredients[1]						
Fish meal, menhaden	20.00	10.00	–	–	–	–
Fish meal, herring	20.00	10.00	–	–	–	–
Animal Protein Mixture[2]	0.00	19.84	39.67	19.84	19.84	19.84
Cottonseed meal-CA[3]	–	–	–	15.66	–	–
Cottonseed meal-TN[3]	–	–	–	–	14.71	–
Cottonseed meal-AR[3]	–	–	–	–	–	15.97
Soybean meal	–	–	–	14.72	14.72	14.72
Krill meal (hydrolysate)	5.00	5.00	5.00	5.00	5.00	5.00
Wheat middling	28.00	27.00	26.00	11.80	13.00	11.40
Corn gluten meal	11.60	11.80	12.00	15.30	15.00	15.40
Yeast (brewer)	6.00	6.00	6.00	6.00	6.00	6.00
Vitamin mixture[4]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mixture[5]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin C[6]	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Menhaden fish oil	8.00	8.90	9.80	10.50	10.50	10.50
Cellulose	0.25	0.31	0.38	0.03	0.08	0.02
Proximate analyses						
Crude protein	46.8	47.2	48.3	48.2	48.3	47.4
Crude Lipid[7]	14.4	14.4	14.3	14.0	14.0	14.0
Gross energy (MJ/kg)[7]	17.5	17.5	17.5	17.5	17.5	17.5

[1] The ingredients were purchased from: fish meal (herring), Ampro Fisheries Co. Reedville, Virginia, USA; fish meal (menhaden), Baker Co., Stamford, Connecticut, USA; soybean meal, Archer Damets Midland Co., Fostoria, Ohio, USA; Krill meal, Specialty Marine Products Ltd, Vancouver, Canada; wheat middling, ADM Co., Loudonville, Ohio, USA; corngluten meal, Baker Trading Co., Dayton, Ohio, USA; Yeast (brewer), Alltech, Chicago, Illinois, USA; mineral mixture (Bernhart Tomarelli salt mixture), choline chloride, and cellulose, ICN Pharmaceuticals Inc., Costa Mesa, California, USA; fish oil (menhaden), Cereal By-products Co., Chicago, Illinois, USA

[2] Animal protein mixture was composed of equal amounts of blood meal (American Protein Co., Ames, IA), meat & bone meal (Inland Products Co., Clyde, OH), feather meal (American Protein Co., Ames, IA), and poultry by-product meal (Holmes By-products Co., Millorsburg, OH). Its crude protein and lipid levels were 68.25% and 6.5%, respectively

[3] CA, California source; TN, Tennessee source; AR, Arkansas source

[4] Roche Performance Premix composition per g of the vitamin mixture: vitamin A, 2646 IU; vitamin D3, 221 IU; vitamin E, 66.1 IU; vitamin B12, 13 µg; riboflavin, 13.2 mg; niacin, 61.7 mg; d-pantothenic acid, 22.1 mg; menadione, 1.32 mg; folic acid, 1.76 mg; pyridoxine, 4.42 mg; thiamin, 7.95 mg; d-biotin, 0.31 mg. Hoffman-La Roche, Inc., Nutley, NJ

[5] Five mg Se in the form of sodium selenite per kg Bernhart Tomarelli salt mixture (ICN Pharmaceuticals Inc., Costa Mesa, CA)

[6] Phospitan C (Mg-L-ascorbyl-2-Phosphate), Showa Denko K. K. Tokyo, Japan

[7] Calculated based on compositions of the ingredients used (NRC 1993)

cold-pelleted into 2.0 mm diameter size, freeze-dried to approximately 5% moisture, crushed into desirable particle size (0.4–2.0 mm), and stored at −20 °C until use.

Fish, facility and feeding trial

The feeding trial was performed at the Piketon Research and Extension Center aquaculture facility with juvenile rainbow trout (London, Ohio, registered strain) averaging 0.96 ± 0.07 g initial weight. Prior to the feeding trial fish were fed a commercial diet (Bioproducts, Inc., Warrington, OR) for two weeks to allow for adjustment to the experimental conditions. Fish were randomly distributed into groups of 30; 3 groups per treatment. Each

experimental diet was fed to triplicate groups of fish with the feeding rates ranging from 4% of fish weight at the beginning to 2% at the end of the feeding trial (NRC 1993). All procedures and handling of animals were conducted in compliance with the guidelines of the Institutional Laboratory Animal Care and Use Committee, The Ohio State University. The fish were fed three times per day, 7 days a week, for 16 weeks. The feeding trial was conducted in 50 l flow-through circular fibreglass tanks, supplied with well water at a flow rate of 1.8–2.0 l/min. Supplemental aeration was also provided to maintain dissolved oxygen levels near saturation. Water temperature increased gradually from 8 to 15 °C during the experiment and a diurnal light:dark cycle was regulated at 12 h:12 h. Total fish weight in each tank was determined every 4 weeks to check their growth and to adjust the feeding rate. Feeding was stopped 24 h prior to weighing.

Sample collection and analysis

Analyses of crude protein, moisture and ash were performed by standard procedures (AOAC 1995). At the end of the feeding trial all fish were weighed and counted to calculate percent body weight gain (PWG; body wt gain × 100/initial body wt), feed conversion (FC; body wt gain/dry feed consumed), protein efficiency ratio (PER; body wt gain/protein intake), specific growth rate (SGR; [ln final body wt – ln initial body wt] × 100/days), and survival. Hematocrit was determined by the microhematocrit method (Brown 1980) on three fish randomly selected per group (total 9 fish per treatment). For histological examination, livers from three randomly selected fish in each dietary replicate were used. Tissues were preserved in 10% neutral buffered formalin, dehydrated through a graded series of alcohol, and embedded in paraffin. Sections were cut at 3–4 μm, mounted on glass slides and stained routinely with hematoxylin and eosin followed by clearing through xylene and cover slipped over Permount medium.

For gossypol analysis, two fish were randomly selected from each dietary group (total 6 fish per treatment) and killed to collect the liver and bile samples. Three fish per each dietary group (total 9 fish per treatment) were killed for the whole-body analysis of gossypol. For gossypol analysis, faeces were collected after 16 weeks of feeding. Mineral compositions of diets and whole-body was determined by the inductively coupled plasma (ICP) emission spectrophotometric method with the use of ARI-3560 Spectrometer (Applied Research Laboratories, Valencie, CA) according to Watson and Isaac (1990).

Faeces collection and apparent digestibility test

The indirect method described by Cho and Kaushik (1990) was used to calculate the apparent 'digestibility' coefficient (ADC), with chromic oxide (0.5 g per 100 g feed on dry matter basis) as the inert indicator. The apparent 'digestibility' coefficient of protein and gossypol 'absorption' was calculated using the following formula:

$$ADC_{nutrient} = [1 - (NF/ND \times CrD/CrF)] \times 100$$

where: NF = % nutrient in faeces, ND = % nutrient in diet, CrD = % chromic oxide in diet, and CrF = % chromic oxide in faeces. Faeces were collected with a modified faecal collection system (Yamamoto et al., 1998). From the 12th week of feeding trial, fish were fed with the 0.5% chromic oxide-containing diets to facilitate the apparent 'digestibility' test until the end of the feeding trial. After 16 weeks of feeding, all fish of each treatment (three groups) were transferred to three 50 L collection tanks having a steep conical bottom connected to a faeces collecting chamber. Water flowed to the top and out at the bottom of the conical tanks at a rate of less than 0.4 l/min. Fish were prevented from stirring the faeces by a circular net placed at the base of the tank. To collect faeces, all the fish were fed their respective diets containing 0.5% chromic oxide to satiation each morning at 08:00, and again at 11:00. The faeces were collected every 40 min for 7 h, and immediately frozen at −20 °C.

The fish were fed again their respective diets at 20:00 on the same day, collection tanks cleaned, and the faeces were collected the next day at 08:00. The collected faeces were immediately frozen at −20 °C and then stored at −80 °C until analysis.

Analysis of gossypol

Gossypol in diets, liver, bile, whole-body, and faeces were determined by HPLC according to the method described by KIM and CALHOUN (1995), with some modifications. Wet liver tissue was used for analysis of (+)- and (−)-enantiomers of gossypol because preliminary assays revealed that the gossypol concentration in freeze-dried liver samples were lower than that from wet samples. The preliminary assay was conducted in triplicate to compare the two processing methods. The wet liver and freeze dried diets, whole-body, and faeces were weighed, and 5–10 volumes of complexing reagent added to obtain the 2-amino-1-propanol derivatives of (+)- and (−)-enantiomers of gossypol. The complexing reagent was composed of 2 ml 2-amino-1-propanol (Sigma Chemical, St. Louis, MO), 10 ml glacial acetic acid (Sigma Chemical) and 88 ml N, N-dimethylformamide (Sigma Chemical). The samples were homogenized in complexing reagent on ice for 10–40 s, heated at 95 °C for 30 min, cooled on ice, and then centrifuged at 1500 × *g* for 5 min. For determination of gossypol from bile, the homogenization step was omitted. After centrifugation, an aliquot of the supernatant was diluted with mobile phase to obtain a desirable concentration, centrifuged again at 1500 × *g* for 5 min, and filtered through a syringe filter (0.45 μm, Whatman Inc., Clifton, NJ) before injection to HPLC.

The HPLC system consisted of a Beckman 506 A solvent delivery system equipped with a 20 μl injection loop connected to a 4.6-mm × 150 mm Shodex C-18 column (Showa-Denko, Shoko Co. Ltd, Tokyo, Japan) packed with an octadecyl-bonded porous silica gel (5 μm), and both a UV detector (Programmable detector module 166, Beckman Instruments Inc., San Ramon, CA) set at 254 nm and an electrochemical detector (Model LC-4C; BAS, West Lafayette, IN) set applied potential at 0.75 V. The mobile phase was made of 80 ml acetonitrile and 2 mM KH_2PO_4 (final concentration) dissolved in 100 ml distilled water (HPLC grade) adjusted to pH 3.0 with H_3PO_4. Standards of (+)- and (−)-enantiomers of gossypol were provided by Dr Quezia B. Cass, Departamento de Química, Universidade Federal de São Carlos, São Carlos, Brazil (CASS et al., 1999). The retention time for (+)- and (−)-enantiomers of gossypol were 2.1 and 3.4 min, respectively, with a flow rate of 1.8 ml/min. Recovery rates were higher than 92% for both gossypol enantiomers and the detection level was 1 ng/20 μl of injection volume with a signal-to-noise ratio of 3.

Statistical analysis

Each experimental diet was fed to three groups of fish by a completely randomized design. Differences among dietary treatments were tested by one-way ANOVA, and means were compared using Tukey's multiple comparison test by the SPSS statistical package (Version 9.0, SPSS Inc., Chicago, IL). The percentage data of weight gain, specific growth rate, and hematocrit were arcsine transformed before the ANOVA analysis. Differences were considered significant at $p < 0.05$.

Results

No significant differences in fish body weight were found among all groups until week 8, but fish began to show differences in growth rates from the 12th week, followed by significant differences at the 16th week (Fig. 1). Final body weight gains and feed conversion by fish during the 16-week feeding trial are shown in Table 2. Body weight gain, feed conversion and specific growth rate of fish fed CM-TN and CM-AR diets were not significantly different compared to those of fish fed the FM-based control diet. The

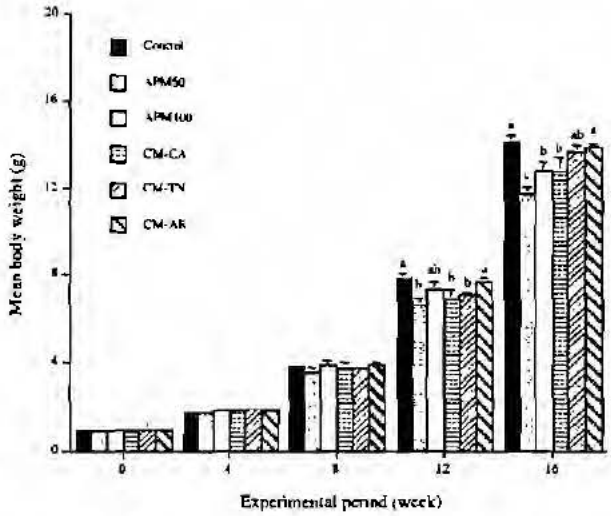

Fig. 1. Changes in mean body weight of rainbow trout fed the experimental diets for 16 weeks. Values are means ± SD of triplicate groups. Different letters (a, b) indicate significantly different values ($p < 0.05$)

Table 2. Percentage body weight gain (BWG), feed conversion (FC), protein efficiency ratio (PER), specific growth rate (SGR) and hematocrit of juvenile rainbow trout fed experimental diets for 16 weeks[1]

Diets	BWG (%)	FC	PER	SGR (%)	Hematocrit (%)
Control	1370 ± 17.00 ^{a}	1.03 ± 0.01 ^{a}	2.28 ± 0.02 ^{a}	2.40 ± 0.01 ^{a}	44 ± 1.10 ^{a}
APM50	1129 ± 14.21 ^{c}	0.87 ± 0.00 ^{c}	1.86 ± 0.00 ^{c}	2.24 ± 0.01 ^{c}	40 ± 0.73 ^{b}
APM100	1235 ± 27.23 ^{bc}	0.92 ± 0.02 ^{bc}	1.94 ± 0.04 ^{bc}	2.31 ± 0.02 ^{b}	36 ± 1.52 ^{c}
CM-CA	1235 ± 39.01 ^{bc}	0.91 ± 0.03 ^{bc}	1.92 ± 0.06 ^{bc}	2.31 ± 0.03 ^{b}	39 ± 1.04 ^{b}
CM-TN	1330 ± 16.17 ^{ab}	0.96 ± 0.01 ^{ab}	2.03 ± 0.02 ^{b}	2.37 ± 0.01 ^{ab}	39 ± 0.94 ^{b}
CM-AR	1350 ± 10.93 ^{a}	1.00 ± 0.01 ^{a}	2.18 ± 0.01 ^{a}	2.39 ± 0.01 ^{a}	39 ± 1.09 ^{bc}

[1]Means of triplicate groups; Values ± SD in the same column with different superscript are significantly different ($p < 0.05$)

protein efficiency ratio of fish fed the CM-AR diet was also not significantly different compared to that of fish fed the control diet. However, fish fed APM50, APM100, and CM-CA diets exhibited significantly lower growth performances than the fish fed the control diet. A significant decrease in hematocrit was found in fish fed diets containing CM and/or APM compared to fish fed the control diet. No differences were observed in whole-body protein and ash concentrations among all the groups (results not presented). No mortality was observed and differences in the palatability of the diets were not noticed during the 16 weeks of the feeding trial.

The ratio of (+)- and (−)-enantiomers was equal in the three CM-containing diets. Gossypol concentrations in liver, bile, whole-body, and faeces are presented in Fig. 2. No differences in gossypol concentrations were found among treatments in liver, bile and whole-body of the fish. Significantly higher concentrations of total gossypol were found in faeces of CM-TN (5.8 ± 0.4 μmol/g) and CM-AR (5.6 ± 0.6) groups than in that of CM-CA (3.7 ± 0.4) group. This trend in total gosssypol was also observed in both (+)- and

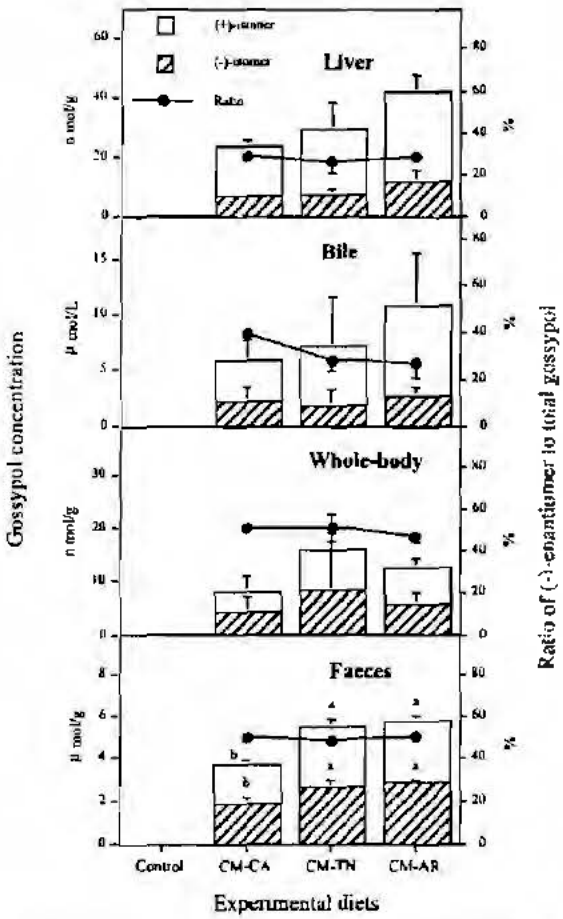

Fig. 2. Concentrations of gossypol enantiomer in liver, bile, whole-body, and excreted faeces of juvenile rainbow trout fed the control (fish meal-based) and test diets containing three different sources of cottonseed meal during 16 weeks. Values are means ± SD of triplicate groups. Different letters indicate significantly different values ($p < 0.05$)

(–)-gossypol enantiomers. A significant correlation ($r^2 = 0.85, p < 0.001$) was found between gossypol concentrations in diet and faeces. Gossypol was not detected in tissues or faeces of the fish fed the control diet. Interestingly, the ratio of (–)-enantiomers to total gossypol differed, depending on tissues or faeces. In liver and bile, the percentage ratios of (–)-enantiomers to total gossypol were less than 30% which means that over 70% of (+)-enantiomer was selectively retained in tissue. However, an approximately equal proportion of each (+)- and (–)-enantiomer were found in the whole-body and faeces of fish. No significant histopathological changes were found in liver tissues of fish examined. The colour of livers were not different between fish fed CM-containing and control diets, however, yellowish liver due to the gossypol deposition was found in tilapia, *Oreochromis* spp. fed the same amount of CM for 16 weeks in our previous study (Mbahinzireki et al., 2001).

Phosphorus and iron concentrations in diets, whole body and faeces are shown in Fig. 3. Phosphorus in faeces of the control group was significantly higher than those of other groups, and iron concentration in faeces was significantly higher only in CM-TN group

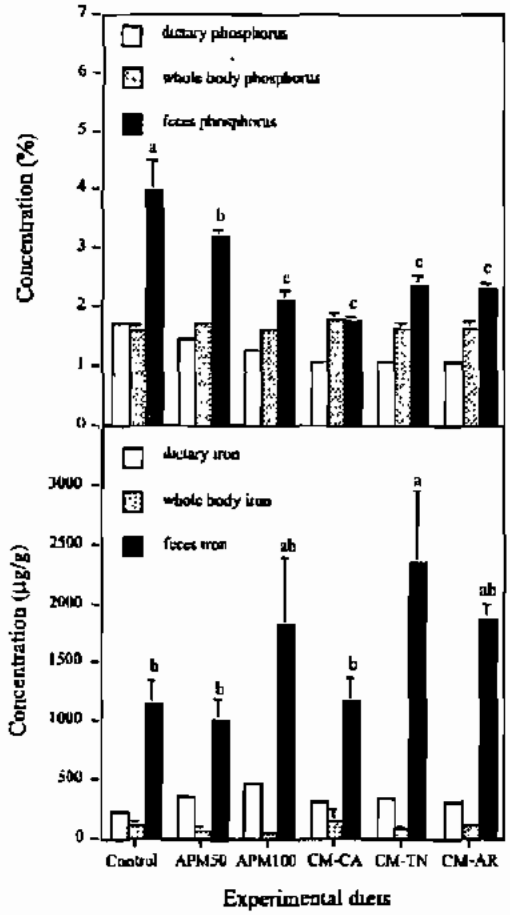

Fig. 3. Concentrations of phosphorus and iron in experimental diets, whole-body, and faeces. Values of whole-body and faeces are means ± SD of triplicate groups. Different letters are significantly different ($p < 0.05$)

compared to the control, APM50 and CM-CA groups. No significant differences were observed in whole body concentrations of phosphorus and iron among treatments. The apparent 'digestibility' of dietary protein and 'absorption' of phosphorus and gossypol after 16 weeks of feeding were different among treatments (Fig. 4). Protein 'digestibility' of diet CM-AR (89.6 ± 1.29%) was significantly higher than those of the control (85.0 ± 1.88%), APM50 (83.4 ± 0.41%), APM100 (80.6 ± 2.10%) and CM-CA (83.5 ± 1.34%) diets. The phosphorus 'absorption' was higher in diets containing CM and/or APM than in the control. The 'absorption' rate of gossypol ranged from 35 to 50% depending on the CM source, and was significantly higher in CM-TN and CM-AR groups than in CM-CA group.

Discussion

The results of the present study are significant because, to our knowledge, it is the first fish meal free and high dietary protein (45%) formulation for juvenile rainbow trout that resulted in comparable growth rate to the FM-based control diet. Furthermore, the fish fed

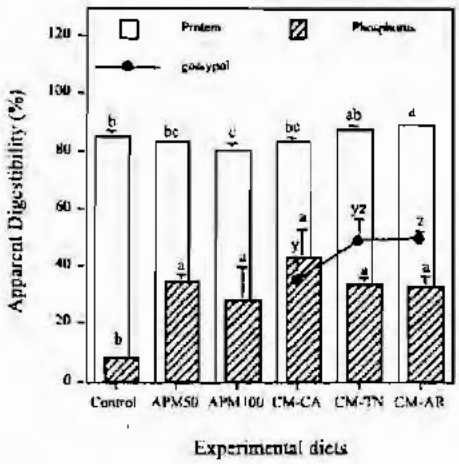

Fig. 4. Apparent 'digestibility' of dietary protein and 'absorption' of phosphorus and gossypol examined after 16 weeks of feeding trial. All the fish from the feeding trial were used for the 'digestibility' test using inert indicator, 0.5% chromic oxide. Values are means ± SD of triplicate groups. Different letters indicate significantly different values ($p < 0.05$)

the FM-based control diet (herring and menhaden FM, 1 : 1) in the present study grew at a rate comparable or higher specific growth rates (2.40 ± 0.01% per day) than indicated earlier in other studies. For instance, SKONBERG et al., (1997) used a similar size of rainbow trout and SGR of fish fed a control diet (herring FM) amounted to 1.42 and 2.56% on restricted or *ad libitum* feeding, respectively, in an 8-week-long study. Rainbow trout weighing 3 g fed a menhaden FM-based diet (*ad libitum*) showed a SGR of 2.77% after 13 weeks of feeding (WATANABE et al., 1993). The feeding period in our study was longer compared to other FM replacement studies that were conducted with juvenile rainbow trout (STICKNEY et al., 1996; YAMAMOTO et al., 1995).

HERMAN (1970) reported that in rainbow trout, growth depression did not occur until dietary free gossypol concentrations were higher than 290 mg/kg. ROBINSON and TIERSCH (1995) found no effect of 200 mg free gossypol per kg diet in channel catfish on growth, feed consumption, feed conversion ratio, and survival. Free gossypol is defined as 'acetone soluble gossypol', whereas bound gossypol can be estimated by subtracting the portion of free form from total gossypol. In the present study the free gossypol concentrations of the diets CM-CA, CM-TN, and CM-AR were 60, 150, and 225 mg/kg, respectively, based on individual CM analysis. The reason for the lower growth rate in CM-CA diet group can be attributed to the inferior nutritive value of CM rather than its gossypol concentration. The nutritive values of CM can differ among different species of cottonseeds processed (CASS et al., 1991; JAROSZEWSKI et al., 1992) and different processing methods (CHERRY et al., 1978; FORSTER and CALHOUN 1995).

Lower hematocrits were found in the group fed CM-containing diets than in the control in the present study. This result is in agreement with the results in rainbow trout broodstock (DABROWSKI et al., 2000), Nile tilapia (MBAHINZIREKI et al., 2001), swine and rats (SKUTCHES et al., 1973, 1974). The reason for the observed lower hematocrit in groups fed CM-containing diets in the present study could be the cumulative effect of gossypol and/or decreased availability of iron in CM causing increased erythrocyte fragility (COLIN-NEGRETE et al., 1996; MAKINDE et al., 1997).

ROEHM et al., (1967) reported that in rainbow trout, the liver was the main organ responsible for accumulation of gossypol and that the gossypol elimination took place

with a considerable delay in the liver. In the present study we found the percent ratio of the (–)-enantiomer to total lower in liver (26–29%) and bile (27–39%) than in the whole-body (47–52%) and faeces (49–51%). This result supported the notion that liver is the main organ for the elimination of absorbed gossypol (ROEHM et al., 1967), and was consistent with the result of tissue gossypol concentrations found in rainbow trout broodstock in our laboratory. In our previous study we observed the highest gossypol concentrations in liver compared to plasma, bile, kidney, muscle, stomach and gametes (results not published). The findings reported here may suggest that liver eliminates (–)-enantiomer more actively than the (+)-enantiomer. Several studies have shown that the (–)-enantiomer has the higher biological activities, such as antifertility, antitumour and toxicity than (+)-enantiomer (BENZ et al., 1990; JOSEPH et al., 1986; SHELLEY et al., 1999; TANPHAICHITR et al., 1988). SMITH and CLAWSON (1965) indicated that the primary pathway of gossypol excretion was via the biliary system. Our results of bile gossypol concentrations supported the hypothesis that the primary pathway of gossypol excretion is via biliary system. We found high concentrations of total gossypol in the faeces of rainbow trout (3.7–5.8 μmol/g dry matter). Heifers exposed to 1300 and 2000 mg of gossypol/kg excreted 0.58 and 2.3 μmol of total gossypol per g faeces, respectively (COLIN-NEGRETE et al., 1996). COLIN-NEGRETE et al., (1996) calculated that between 5 and 15% of gossypol was absorbed by the heifers. ABOU-DONIA and LYMAN (1970) reported that in pigs and hens, which are sensitive to gossypol toxicity, the maximum amount of radioactivity absorbed in tissues was 32.9 and 16.8% of the orally administered dose, respectively. The approach we used to determine the 'absorption' rate of gossypol in trout was to compare the gossypol concentration in diets and excreted faeces by using chromic oxide as a indirect method (CHO and KAUSHIK 1990) and to consider the total consumption and accumulation of gossypol in the whole-body throughout the feeding trial. In the present study, we found a large proportion of dietary gossypol (35–49%) absorbed by the fish in comparison to terrestrial animals (Fig. 4). We also calculated that fish consumed on average 13.1 ± 0.26 g of diet during the 16-week period, leading to a total intake of 13.7–21.4 mg of total gossypol per fish depending on the three different sources of CM-containing diets. This would then result in gossypol intake ranging from 1.07 ± 0.05 to 1.54 ± 0.02 mg per g of fish body weight. However, fish retained only 0.8–1.6 μg gossypol per g of fish body wt indicating that the amount of gossypol that was not excreted through faeces is less than 0.2% of dietary gossypol. Therefore, it may be assumed that the majority of the absorbed gossypol is metabolized to other compounds such as gossypolone, gossypolonic acid and demethylated gossic acid as proposed by ABOU-DONIA and DIECKERT (1975). However, we observed very low amounts of gossypolone in liver tissues (identified by internal standard) showing less than 1% of the total gossypol detected by HPLC (data not shown).

The result of the higher protein 'digestibility' (Fig. 4) in the fish fed CM-AR and CM-TN diets may indicate that in rainbow trout the availability of CM is comparable or a little higher than that of fish meal. Other protein sources, such as soybean, corn gluten, and poultry by-product meal had similar protein availabilities to both herring and menhaden fish meal (RICHE and BROWN 1999; SUGIURA et al., 1998). The significantly lower phosphorus 'absorption' (Fig. 4) in the control in comparison to the other groups can be explained by the significantly improved utilization of fish meal phosphorus by plant ingredients (RICHE and BROWN 1999). This result is also in agreement with the observation by SUGIURA et al., (1998) in coho salmon and rainbow trout, where phosphorus 'absorption' was inversely correlated with dietary levels of calcium and with phosphorus itself. The authors reported higher phosphorus availabilities in soybean and corn gluten meal, as well as poultry by-product and feather meal, than in both herring and menhaden fish meal. In the present study the concentrations of calcium and phosphorus in the control diet (1.67 and 1.63 g/100 g, respectively) was 1.5 and 2.3 times higher than those in CM-CA, CM-TN, and CM-AR diets (results not presented). The reason for the higher trend in

the faeces iron concentrations in CM-TN (significant) and CM-AR (not significant) compared to that of the control (Fig. 3) might be attributed to the characteristic of gossypol chelation by iron. Owing to the fact that gossypol readily reacts with iron, it was found that the reaction in the intestine resulted in the formation of an insoluble complex which is egested in the faeces (MUZAFFARUDDIN and SAXENA 1966; SKUTCHES et al., 1974).

In conclusion, this study demonstrates that CM can be used as a valuable protein source by at least 15% incorporation in diets for juvenile rainbow trout. Fish meal, a traditional protein source in fish feed, can also be completely replaced by a mixture of plant protein (CM and soybean meal) and some other animal by-product proteins. Nutritive values of CM in fish can be different depending on their origin and processing. We documented that fish excrete the (–)-enantiomer faster than the (+)-enantiomer of gossypol, and that approximately 35–50% of dietary gossypol is absorbed by fish, whereas the remaining absorbed gossypol seemed to be excreted with urine and/or through gills. This needs further study.

Acknowledgements

This study was supported by the following bodies: North Central Regional Aquaculture Center, East Lansing, MI; the National Cottonseed Products Association, Memphis, TN; the Ministry of Maritime Affairs and Fisheries through RCOID (ERC); Pukyong National University (PKNU); and the Seafood and Marine Bioresources Development Center, PKNU, Pusan, Korea. Salaries were partly provided by state and federal funds appropriated to Ohio Agriculture Research and Development Center, Wooster, OH. The authors thank the staff of the Piketon Research and Extension Center, particularly Dean Rapp and Elissa French, for their assistance in daily fish care and feeding, Przemek Bajer, for his assistance in collecting faeces, and Régis Moreau and Travis Hartman, for their critical reading of this manuscript. Quezia B. Cass, Departamento de Química, Universidade Federal de São Carlos, São Carlos, Brazil, has provided gossypol enantiomers for which we are grateful.

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Authors' address: K. DABROWSKI, School of Natural Resources, The Ohio State University, Columbus, OH 43210, USA. Tel.: + 1614 2924555; Fax: + 1614 2927432; e-mail: dabrowski.1@osu.edu

Aquaculture Research, 2001, 32 (Suppl. 1), 70–75

The patterns of plasma free amino acids after force-feeding in rainbow trout *Oncorhynchus mykiss* (Walbaum) with and without dorsal aorta cannulation

I-H Ok, S C Bai, G-J Park, S-M Choi & K-W Kim
Department of Aquaculture, Feeds and Foods Nutritional Research Center, Pukyong National University, Pusan 608-737, Korea

Correspondence: S C Bai, Department of Aquaculture, Pukyong National University, Pusan 608-737, Korea. Tel: (+82) 51 620 6137, Fax: (+82) 51 628 7430, E-mail: scbai@mail.pknu.ac.kr

Abstract

Two experiments were conducted to compare the patterns of plasma free amino acid concentrations after force-feeding in rainbow trout *Oncorhynchus mykiss* (Walbaum) with and without dorsal aorta cannulation. In the first experiment, 35 rainbow trout averaging 504 ± 7.8 g (mean ± SD) were divided into seven groups of five fish each. After 48 h starvation, a group of fish was anaesthetized and blood samples were taken at one of the following time periods: 0, 4, 8, 12, 24, 36 and 48 h after feeding. In the second experiment, five dorsal aorta cannulated rainbow trout averaging 511 ± 6.2 g (mean ± SD) were kept in a cage. After 48 h starvation, the fish were anaesthetized and blood samples were taken from the same fish at 0, 4, 8, 12, 24, 36 and 48 h after feeding. In the first experiment, the concentration of all plasma free amino acids except histidine and glycine peaked at 4 h and returned to the basal level 24 h after feeding. In the second experiment, the concentration of all plasma free amino acids except isoleucine, leucine, phenylalanine and tryptophan also peaked at 4 h and returned to the basal level 24 h after feeding. These results showed that the pattern of plasma free amino acid concentrations from fish with and without dorsal aorta cannulation were similar.

Keywords: plasma free amino acids, force feeding, rainbow trout, dorsal aorta cannulation

Introduction

Plasma free amino acid levels were measured to study amino acids metabolism and to evaluate the quality of dietary protein in trout (Nose 1972; Schlisio & Nicolai 1978; Yamada, Simpson, Tanaka & Katayama 1981; Ogata & Arai 1985; Walton & Wilson 1986; Murai, Ogata, Hirasawa, Akiyama & Nose 1987; Cowey & Walton 1988; Ash, McLean & Westcott 1989; Schuhmacher, Goldberg, Schön, Wax & Gropp 1993; Tantikitti & March 1995; Schuhmacher, Wax & Gropp 1997). In these experiments, blood samples were taken from the caudal vein and artery by needles and syringes in the anaesthetized fish randomly captured from the treatment population. The procedures of these experiments have several major limitations. First, the handling associated with the method is stressful. Second, the method does not allow repeated sampling of the same individual fish, thus requiring large numbers of fish and tanks. These limitations resulted in a large individual variation within treatment in the time course of amino acid appearance in the peripheral blood and peak level.

Procedures for dorsal aorta cannulation and repeated sampling of blood in the same resting fish are well established (Soivio, Westman & Nyholm 1972). The dorsal aorta cannulation allows repeated sampling of the same individual fish when studying changes in the levels of nutrients in the blood circulation. Therefore, the purpose of the present

study was to compare the patterns of plasma free amino acid concentrations in force-fed rainbow trout with and without dorsal aorta cannulation.

Materials and methods

Animals and husbandry

Growing rainbow trout averaging 504 ± 7.8 g (experiment I) and 511 ± 6.2 g (experiment II) mean body weight were obtained from Ewhajung trout farm in Sangju, Korea. For both experiments eight net cages (1.3 × 1.3 × 1.3 m) were placed in a flow-through concrete raceway with a water flow of 60 L min^{-1}. Aeration was provided to maintain dissolved oxygen at 7.4 ± 0.7 mg L^{-1}, and water temperature was maintained at 17 ± 0.2 °C.

Dorsal aorta cannulation

Fish were anaesthetized with 200 p.p.m MS-222 (3-aminobenzoic acid ethyl ester methansulphonate; Sigma, St Louis, MO, USA) for 3–5 min, and placed on a V-shape table, and gills were irrigated continuously with 16 °C water containing 100 p.p.m MS-222 during the operation. A 50-cm-long cannula (Clay Adams PE 50 tubing, Parsippany, NJ, USA) with a bubble about 5–6 cm from one end was washed with the heparinized Cortland saline solution (Houston 1990), and a 13-G needle was used to pierce a hole on the right nostrum (ventral side up) for the cannula to come out. A 19-G needle was used to bore a small hole in the roof of the mouth at the mid-line behind the third gill arc at a 30° angle, and a guitar wire (G) was inserted into the PE 50 as a guide. The proper insertion was indicated by a slow blood flow after the wire was withdrawn from the cannula. A 3-cm^3 syringe with a 23-G needle was used to remove air and blood clot, and the cannula was flushed with the heparin solution. The cannula was sutured behind the bubble on the roof of the mouth, led out from the right nostrum, plugged with a colour head pin, and sutured at the dorsal fin.

Experimental design, diet and force-feeding

In the first experiment, 35 rainbow trout were divided into seven groups of five fish each. After 48 h starvation a group of five fish was anaesthetized with 200 p.p.m MS-222 and blood samples were taken at one of the following time periods; 0, 4, 8, 12, 24, 36 and 48 h after force-feeding a basal diet by the stomach intubation method (Schuhmacher *et al.* 1997) at a rate of 1% body weight on a dry matter basis.

Table 1 Composition of the basal diet (% dry matter)[1]

Ingredient	Proportion (%)
Essential amino acids	17.27
Nonessential amino acids	12.36
Casein[2]	5.00
Gelatine[2]	2.00
Dextrin[2]	27.97
Dextrose[2]	5.00
Cellulose[2]	8.20
Fish oil[3]	10.00
Carboxymethyl cellulose[2]	1.00
$Ca(H_2PO_4).2H_2O$	3.00
Choline bitartrate[2]	1.20
Vitamin mixture[4]	3.00
Mineral mixture[5]	4.00

[1]Diets were neutralized with NaOH to give a final pH of 6.6.
[2]United States Biochemical (Cleveland, OH, USA).
[3]Ewha Oil Company (Pusan, Korea).
[4]Vitamin mixture (mg kg^{-1} feed unless indicated otherwise): vitamin A, 3000 IU; vitamin D3, 2400 IU; vitamin E, 120 IU; menadione sodium bisulphate, 6; vitamin B1-HCl, 15; vitamin B2, 30; vitamin B6-HCl, 15; vitamin B12, 0.06; vitamin C, 300; calcium pantothenate, 150; nicotinic amide, 150; inositol, 150; d-biotin, 1.5; choline chloride, 3000; pancreatin, 12.5.
[5]Mineral mixture (mg kg^{-1} feed): $MnSO_4$, 320; $ZnSO_4$, 270; $FeSO_4$, 750; $CuSO_4$, 60; $CoSO_4$, 7; $MgSO_4$, 17.25; K_2SO_4, 212.24; NaCl, 51.88; K_2HPO_4, 136.09; $NaSeO_3$, 0.013; KI, 0.15.

In the second experiment five dorsal aorta-cannulated rainbow trout were stocked in one cage. After 48 h starvation, the fish were anaesthetized with 200 p.p.m MS-222 and blood samples were taken from the same five fish repeatedly at 0, 4, 8, 12, 24, 36 and 48 h after force-feeding the basal diet by the stomach intubation method at a rate of 1% body weight on a dry matter basis.

The basal diet was formulated by modifying the procedure of Kim (1997), and the diet containing 29.6% crystalline amino acid mixture plus 5% casein and 2% gelatine. Ingredients and amino acid composition of the basal diet are shown in Tables 1 and 2, respectively. The basal diet mixture without oil was stored at −80 °C until used. Ingredients were mixed with 10% of fish oil and water before intubation. Fish with and without dorsal aorta

Table 2 Amino acid composition of the basal diet (% dry matter)

	From casein and gelatine	From crystalline amino acids	Total[1]
Essential amino acids			
Arginine	0 353	1 924[2]	2 277
Histidine	0 194	0 725	0 919
Isoleucine	0 252	1 674	1 926
Leucine	0 493	2.702	3.195
Lysine[2]	0 502	1 904	2 406
Methionine	0 152	1 030	1 182
Phenylalanine	0.271	1.742	2 013
Threonine	0 221	1 601	1 822
Tryptophan	0 065	0 462	0 527
Valine	0 350	1.999	2.349
Non-essential amino acids			
Alanine	0 345	1 741	2 086
Aspartic acid	0 483	3 280	3 763
Cysteine	0 019	0 172	0 191
Glycine	0 538	0 758	1 296
Glutamic acid	1 298	3 616	4 914
Proline	0 790	0 568	1 358
Serine	0 374	2 398	2 772
Tyrosine	0 270	1 335	1 605

[1]The amino acid profile was simulated with that of 35% whole chicken egg protein (Robinson, Wilson & Poe 1981).

[2]Lysine-HCl was used in crystalline amino acids mixture.

cannulated were anaesthetized with 200 p.p.m MS-222 and fed the basal diet by the stomach intubation method (diet plus 0.4 parts of distilled water per diet) using a 3-cm^3 syringe.

Sample collection and analysis

Fish were anaesthetized with 200 p.p.m MS222, and 300 μL blood was obtained from fish. In the first experiment blood samples were taken from the caudal vein using a heparinized syringe, and in the second experiment blood samples were taken from dorsal aorta using the cannula. Plasma samples were prepared by centrifugation at 3000 *g* for 10 min. For deproteinization, the plasma samples were mixed with 5-sulphosalicylic acid (458 mmol L^{-1}) in the ratio of 4 : 1, cooled on ice for 30 min and recentrifuged. The protein-free supernatant was dissolved in pH 2.2 lithium citrate sample dilution buffer in the ratio of 1 : 1, and the samples were stored at −80 °C until analysis. The plasma free amino acids were separated and quantified using a S433 amino acid analyser (Sykam, Gilching, Germany) using the ninhydrin method.

Statistical analysis

Data were subjected to ANOVA test using Statistix 3.1 (Analytical Software, St Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $P < 0.05$.

Results

Plasma free amino acid concentrations from fish without dorsal aorta cannulation are summarized in Table 3. Plasma free arginine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine, alanine and glutamic acid concentrations peaked at 4 h, returned to the basal level between 12 and 24 h, and remained constant between 24 and 48 h after feeding. Plasma free histidine concentration decreased at 8 h and peaked at 36 h after feeding. Plasma free glycine concentration decreased between 4 and 8 h, peaked at 12 h and returned to the basal level at 24 h after feeding.

Plasma free amino acid concentrations from fish with dorsal aorta cannulation are summarized in Table 4. Plasma free arginine, histidine, lysine, methionine, threonine, valine and glutamic acid concentrations peaked at 4 h, returned to near basal line level between 8 and 24 h, and remained constant between 24 and 48 h after feeding. Plasma free isoleucine, leucine, phenylalanine and tryptophan concentrations peaked at 4 h, decreased between 8 and 24 h, and remained constant thereafter. Plasma free glycine concentration decreased between 4 and 8 h, peaked at 12 h and returned to the basal level at 24 h after feeding. Plasma free alanine and aspartic acid concentrations peaked at 4 h and returned to the basal level 48 h after feeding.

Discussion

In force-fed rainbow trout with or without dorsal aorta cannulation after 48 h starvation, most of plasma free amino acids peaked approximately 4 h after feeding, and returned to the basal level at 24 h. Murai *et al.* (1987) reported that the plasma free amino acid concentrations from fish force-feeding

Table 3 Plasma free amino acid concentrations (nmol mL^{-1}) from fish without dorsal aorta cannulation after force-feeding the basal diet (experiment I)[1]

	Time (h) after force-feeding							Pooled SEM
	0	4	8	12	24	36	48	
Essential amino acids								
Arginine	$127^{c,a}$	317^{a}	217^{b}	214^{b}	90^{d}	$118^{c,d}$	$108^{c,d}$	17
Histidine	$173^{b,c,d}$	$199^{a,b}$	115^{d}	$137^{c,d}$	$142^{b,c,d}$	250^{a}	$193^{a,b}$	8
Isoleucine	103^{c}	522^{a}	379^{b}	264^{c}	146^{d}	157^{d}	$140^{c,d}$	29
Leucine	164^{d}	684^{a}	492^{b}	346^{c}	183^{d}	132^{d}	198^{d}	37
Lysine	$513^{a,b}$	663^{a}	$486^{b,c}$	$477^{b,c}$	191^{d}	$406^{b,c}$	$330^{c,d}$	27
Methionine	432^{c}	761^{a}	$604^{a,b}$	$505^{b,c}$	393^{c}	383^{c}	$378^{c,d}$	25
Phenylalanine	126^{b}	649^{a}	527^{b}	326^{c}	135^{d}	$212^{c,d}$	147^{d}	41
Threonine	$279^{c,d}$	533^{b}	364^{b}	289^{c}	181^{d}	$223^{c,d,e}$	$275^{c,d,e}$	21
Tryptophan	7^{c}	28^{a}	16^{b}	10^{c}	$12^{b,c}$	8^{c}	7^{c}	3
Valine	$189^{b,c}$	324^{a}	228^{b}	$160^{c,d}$	$126^{d,e}$	97^{e}	87^{e}	16
Non-essential amino acids								
Alanine	504^{b}	928^{a}	752^{b}	627^{c}	528^{d}	454^{e}	527^{d}	31
Aspartic acid	542^{b}	874^{a}	792^{b}	647^{c}	754^{b}	658^{c}	73^{b}	20
Glycine	674^{b}	526^{c}	432^{c}	724^{a}	$637^{b,c}$	593^{c}	427^{c}	21
Glutamic acid	324^{c}	986^{a}	842^{b}	652^{c}	317^{c}	$363^{d,e}$	401^{c}	52
Serine	275^{d}	754^{a}	693^{b}	456^{c}	435^{c}	294^{d}	324^{d}	35
Tyrosine	73^{c}	354^{a}	265^{b}	162^{c}	96^{d}	68^{d}	91^{d}	19

[1]Values are mean of five fish where the mean values in each row with a different superscript are significantly different ($P < 0.05$).

Table 4 Plasma free amino acid concentrations (nmol mL^{-1}) from fish with dorsal aorta cannulation after force-feeding the basal diet (experiment II)[1]

	Time (h) after force-feeding							Pooled SEM
	0	4	8	12	24	36	48	
Essential amino acids								
Arginine	102^{b}	668^{a}	354^{b}	245^{b}	195^{b}	145^{b}	212^{b}	36
Histidine	141^{b}	327^{a}	150^{b}	93^{b}	94^{b}	91^{b}	135^{b}	15
Isoleucine	113^{c}	654^{a}	465^{b}	327^{c}	$273^{c,d}$	216^{d}	175^{d}	33
Leucine	152^{c}	738^{a}	688^{b}	612^{c}	275^{d}	254^{d}	310^{d}	44
Lysine	$637^{b,c}$	1257^{a}	$1019^{a,b}$	$767^{a,b,c}$	$707^{b,c}$	417^{c}	$753^{b,c}$	51
Methionine	373^{b}	945^{a}	545^{b}	582^{b}	377^{b}	288^{c}	372^{b}	42
Phenylalanine	132^{c}	792^{a}	672^{b}	534^{c}	232^{c}	194^{d}	212^{d}	48
Threonine	253^{c}	641^{a}	437^{b}	392^{b}	274^{c}	252^{c}	206^{b}	27
Tryptophan	8^{c}	31^{a}	27^{b}	19^{c}	12^{b}	11^{d}	12^{b}	2
Valine	175^{c}	432^{a}	318^{b}	233^{c}	$195^{c,d}$	154^{e}	176^{d}	19
Non-essential amino acids								
Alanine	567^{c}	1105^{a}	984^{b}	642^{c}	727^{d}	682^{d}	593^{c}	38
Aspartic acid	563^{b}	1087^{a}	872^{b}	784^{c}	$813^{b,c}$	721^{d}	594^{c}	32
Glycine	653^{c}	$582^{c,d}$	472^{d}	899^{a}	724^{b}	632^{c}	474^{d}	28
Glutamic acid	351^{c}	1171^{a}	983^{b}	725^{c}	513^{d}	479^{d}	452^{d}	54
Serine	251^{c}	914^{a}	783^{b}	561^{c}	541^{c}	411^{d}	395^{d}	47
Tyrosine	81^{d}	401^{a}	195^{b}	212^{b}	105^{c}	$93^{c,d}$	87^{d}	23

[1]Values are mean of five fish where the mean values in each row with a different superscript are significantly different ($P < 0.05$).

crystalline amino acids peaked at 6 h, and returned to the basal level at 24 h. A similar result was reported by Schuhmacher *et al.* (1997). Walton and Wilson (1986) hand-fed a complete diet based on casein, and measured peak levels at 12 h. Yamada *et al.* (1981) observed that plasma free amino acid concentrations peaked between 24 and 36 h after force-feeding casein, and 12 h after force-feeding a crystalline amino acid mixture. Walton and Wilson (1986) and Cowey and Walton (1988) suggested that this lag phase was caused by an extended starvation period (7 days) and by stress owing to force-feeding. The lag phase could probably be traced back to other factors influencing amino acid uptake, i.e. dietary (nonprotein) energy source and energy content (Ogino & Takeuchi 1976; Pfeffer 1982).

Force-feeding crystalline amino acid diet leads to a peak of plasma free amino acids earlier than with force-feeding intact protein. Crystalline amino acids favour rapid intestinal amino acid uptake, which in turn leads to a fast supply of the plasma, and also an intensified amino acid catabolism (Schuhmacher *et al.* 1997; Rodehutscord, Borchert, Gregus, Michael. & Pfeffer 2000). In the case of amino acids entering plasma from intact protein, absorption of amino acids continues over a longer period of time and thereby extends the period over which the amino acids are available for protein synthesis (Tanlikitli & March 1995). Plakas, Lee, Wolke & Meade (1985) examined plasma free amino acid levels as an index of protein quality in the diet. The bioavailability of lysine in early maillard browned protein was determined by plasma free lysine response in rainbow trout.

Determination of plasma essential amino acid levels could allow accurate prediction of dietary essential amino acid needs in humans (Young, Hussein, Murray & Scrimshaw 1971), chicks (Zimmerman & Scott 1965), rats (McLaughlan & Illman 1967), pigs (Mitchell, Becker, Jensen, Harmon & Norton 1968) and rainbow trout (Kaushik 1979). Different feeding and blood sampling procedures, such as dosage, fish size, tube size, and/or texture of the material, may affect the pattern of plasma free amino acid concentrations and peak occurrence. Yamada *et al.* (1981) and Ng, Hung and Herold (1996) observed that plasma free amino acid concentrations fell 2 h after force-feeding. This may have been caused by the effect of force-feeding. Stress leads to a net catabolism of peripheral proteins, elevation of plasma free amino acid levels and increased amino acid metabolism in several fish species (van der Boon, van den Thillart & Addink 1991; Vijayan, Ballantyne & Leatherland 1991).

The present experiment results show that the patterns of most plasma free amino acid concentrations from force-fed rainbow trout with and without dorsal aorta cannulation were similar, and the dorsal aorta cannulation did not affect the pattern of plasma free amino acid concentrations in rainbow trout. These result indicate that force-fed rainbow trout might recover from the stress of dorsal aorta cannulation within 48 h of the operation, and the dorsal aorta cannulation would allow repeated sampling on the same individual fish to study nutrient metabolism in the blood circulation. This is in line with findings in rainbow trout (Brown, Eales & Hara 1986) and channel catfish *Ictalurus punctatus* (Ratinesque) (Mazik, Plakas & Stehly 1994) subjected to dorsal aorta cannulation. The pattern of plasma free amino acid concentrations from force-fed rainbow trout with dorsal aorta cannulation may be useful in determining the optimum blood sampling time and evaluating protein quality and essential amino acid requirements. However, concentrations of most plasma free essential amino acids in rainbow trout with dorsal aorta cannulation were higher than those without dorsal aorta cannulation. This may have been caused by the stress effects of repeated blood samplings. Thus, further studies should be done to refine the optimum blood sampling time and to confirm the stress effects of anaesthetization in rainbow trout with dorsal aorta cannulation.

Acknowledgments

This research was supported in part by the funds of the Feeds and Foods Nutrition Research Center, Korea Sea Grant Program, and Brain Korea 21 program at Pukyong National University (Pusan, Republic of Korea).

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Journal of Aquaculture 63:109–117, 2001
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Replacement of Fish Meal by a Mixture of Animal By-Products in Juvenile Rainbow Trout Diets

KYE-ONG-JUN LEE, KONRAD DABROWSKI,* AND JOOST H. BLOM

School of Natural Resources,
Ohio State University
Columbus, Ohio 43210, USA

SUNGCHUL C. BAI

Department of Aquaculture,
Pukyong National University,
Pusan 608-737, Korea

Abstract.—A mixture of animal by-products (MAB) was tested to replace fish meal (FM) in diets for juvenile rainbow trout *Oncorhynchus mykiss*. Fish averaging (±SD) 0.96 ± 0.07 g were divided into 15 groups, and 3 groups were fed one of five isonitrogenous diets replacing 0, 20, 40, 60, or 100% of FM protein with similar percentages of MAB (control, MAB20, MAB40, MAB60, or MAB100, respectively). The MAB consisted of 25% meat and bone meal, 24.5% leather meal, 20% squid liver powder, 15% feather meal, 7.5% blood meal (spray-dried), 7.5% poultry by-product meal, and 0.25% each methionine and lysine. After 16 weeks of feeding, fish fed diets MAB40, MAB60, and MAB100 exhibited significantly lower growth performance than the fish fed the control and MAB20 diets. Apparent digestibility estimates of protein with different collection times (every 40 min and after 12 and 48 h) did not show, in a feces collection system, protein leaching into the water for up to 48 h. The MAB substitution of up to 60% of FM protein in diets did not show differences in apparent protein digestibility (85.8 ± 1.05% for MAB20, 83.1 ± 0.45% for MAB40, and 82.7 ± 2.67% for MAB60) compared with the control (85.0 ± 1.86%), whereas in the MAB100 group digestibility (77.7 ± 4.42%) was significantly lower than in the other groups. The apparent phosphorus absorption of test diet groups was significantly higher (36.9 ± 11.12% for MAB20, 24.0 ± 6.20% for MAB40, 57.1 ± 5.22% for MAB60, and 57.4 ± 5.34% for MAB100) than that of the control (8.3 ± 0.15%). Concentrations of protein and ash in the whole body increased as MAB substitution in diets increased. The findings suggest that MAB could replace up to 28% of FM protein in diets for juvenile rainbow trout for 16 weeks without adverse growth effects.

Fish meal (FM) has been a major ingredient in fish diets because of its high protein quality and palatability. Substituting less expensive protein sources for high-price FM in salmonid feeds is one way to lower production costs (Hardy 1996). For this reason, many studies have been conducted to replace or reduce its inclusion in fish diets by various less expensive alternative animal and vegetable protein sources; however, each candidate has characteristics that make it inferior in some respect to high-quality FM (Hardy 1996; Mambrini et al. 1999).

Feather meal, meat and bone meal, poultry by-product meal, and blood meal, the supplementary protein sources commonly incorporated at low levels (5–15%) in practical fish feeds (Murai 1992), have individually been studied as an animal protein replacements for FM. These terrestrial animal by-product meals have a high protein level and favorable essential amino acids profiles, but they are deficient in one or more of the essential amino acids (NRC 1993). If the proper combinations of these by-products are used in fish diets, the quality of the diet is likely to be improved (Davies et al. 1989). Few studies that used combinations of feed ingredients as an FM replacement have been reported. Yamamoto et al. (1995) reported that combinations of meat meal and malt protein flour effectively improved the growth of and protein utilization by fingerling rainbow trout *Oncorhynchus mykiss* compared with the replacement of FM by each ingredient alone. El-Sayed (1998) reported that in Nile tilapia *Oreochromis niloticus* a blood meal and meat and bone meal mixture resulted in lower growth performances compared with a FM-based diet.

* Corresponding author: dabrowski.1@osu.edu
† Present address: Piketon Research and Extention Center, Ohio State University, Piketon, Ohio 45661, USA.

Received April 7, 2000; accepted October 5, 2000

TABLE 1.—Proximate composition (%) of fish meal, mixture of animal by-products (MAB), and feed ingredients used for MAB (dry-matter basis).

Ingredient	Protein	Lipid	Ash	Moisture
Fish meal (herring)	75.7	10.0	10.4	5.71
Fish meal (menhaden)	65.6	10.2	19.0	6.21
MAB	73.1	14.4	12.3	5.96
Leather meal	68.5	16.7	7.75	4.11
Meat and bone meal	55.5	18.0	23.0	4.39
Feather meal	88.7	8.20	3.45	9.79
Squid liver powder	51.2	20.6	6.60	10.1
Poultry by-product meal	68.0	16.7	15.1	3.63
Blood meal	90.5	0.74	2.30	8.23

Because of their high protein levels and economic impacts, leather meal and squid liver powder can also be good candidates for replacing FM in fish feeds. Leather meal is produced from the tanning process, which generates much greater quantities of by-products and wastes than leather product (Simeonova and Dalev 1996). One ton of wet salted hides yields only 200 kg of leather but over 680 kg of solid by-product, and the chemical composition of the by-products varies according to the processing method (Cabeza et al. 1998). Squid liver meal is one of the traditional protein sources in diets for penaeid shrimp because of its high level of lipid and n-3 highly unsaturated fatty acid fraction (n denotes the position of the first double bond from the methyl end). Merican and Shim (1995) reported that squid liver meal is characterized by high digestibility of dry matter and total lipid in diets for adult *Penaeus monodon*.

Therefore, it is important to study the nutritional values of combinations of animal by-products in order to replace FM in commercial fish diets without compromising growth and feed efficiency. The purpose of this study was to evaluate juvenile rainbow trout fed diets containing graded substitutions of a mixture of animal by-products (MAB) for FM protein, specifically by examining their growth and feed utilization, the whole body composition of minerals, and apparent protein and phosphorus digestibility. As part of the digestibility estimation we tested whether leaching of nitrogen occurred from sedimentary feces by comparing digestibility estimates based on feces collected at different times.

Methods

Diets.—Five experimental diets were formulated to be isonitrogenous and isocaloric in terms of crude protein (45%) and gross energy (4,180 kcal/kg). The energy value of each diet was estimated on the basis of mammalian physiological fuel values, i.e., 4 kcal/g protein or carbohydrate and 9 kcal/g lipid (Lee and Putnam 1973; Garling and Wilson 1977). A mixture of animal by-products (MAB) used in this experiment was obtained from Pukyong National University, Pusan, Korea, and consisted of 25% meat and bone meal, 24.5% leather meal, 20% squid liver powder, 15% feather meal, 7.5% blood powder, 7.5% poultry by-product meal, and 0.25% each methionine and lysine (Table 1). The ratio of each ingredient for MAB was based on protein contents and amino acid compositions and cost analysis for economic benefits. For the experimental diets (Table 2), FM was replaced by MAB on the basis of crude protein as follows: control diet = 100% FM; MAB20 diet = 80% FM: 20% MAB; MAB40 diet = 60% FM: 40% MAB; MAB60 diet = 40% FM: 60% MAB; and MAB100 diet = 100% MAB. To balance for phosphorus, calcium phosphate (monobasic) was added. The calculated essential amino acid concentrations in the experimental diets met or exceeded those recommended by NRC (1993). The experimental diets were pelleted, freeze dried, and stored at −20°C until used. For digestibility tests, 0.5% chromic oxide was included in the diets.

Fish, facility, and feeding trial.—The feeding trial was performed at the Piketon Research and Extension Center aquaculture facility with juvenile rainbow trout averaging (±SD) 0.96 ± 0.07 g initial weight. Prior to the feeding trial, fish were fed a commercial diet for 2 weeks to allow for adjustment to the experimental conditions. Fish were randomly distributed into groups of 30 in each of 3 tanks per treatment. Each experimental diet was fed to triplicate groups of fish; feeding rates ranged from 4% of fish weight at the beginning to 2% at the end of the feeding trial (NRC 1993). The fish were fed three times per day, 7 d per week. The feeding trial was conducted for 16 weeks in 50-L flow-through circular fiberglass tanks supplied with well water at a flow rate of 1.8–2.0 L/min.

TABLE 2.—Composition (%) of control and experimental diets; MAB = mixture of animal by-products.

Ingredient or characteristic	Control	MAB20	MAB40	MAB60	MAB100
Fish meal, menhaden	20.00	16.00	12.00	8.00	0.00
Fish meal, herring	20.00	16.00	12.00	8.00	0.00
MAB[a]	0.00	7.73	15.46	23.19	38.65
Krill meal[b]	5.00	5.00	5.00	5.00	5.00
Wheat middlings	28.00	28.00	28.00	28.00	28.00
Corn gluten meal	11.60	11.60	11.60	11.60	11.60
Brewer's yeast	6.00	6.00	6.00	6.00	6.00
Calcium phosphate	0.00	0.50	1.00	1.50	2.50
Vitamin mixture[c]	0.50	0.50	0.50	0.50	0.50
Mineral mixture[d]	0.50	0.50	0.50	0.50	0.50
Vitamin C[e]	0.05	0.05	0.05	0.05	0.05
Choline	0.10	0.10	0.10	0.10	0.10
Fish oil, menhaden	8.00	7.80	7.60	7.40	7.06
Cellulose	0.25	0.22	0.19	0.16	0.04
Proximate composition					
Crude protein	45.3	45.1	44.8	44.9	43.8
Phosphorus	1.63	1.60	1.64	1.58	1.52
Lipid	16.9	16.7	16.9	16.8	17.5
Energy (Kcal/kg)[f]	4,180	4,178	4,177	4,176	4,180

[a] Composed of 25% meat and bone meal, 24.5% leather meal, 20% squid liver powder, 15% feather meal, 7.5% blood meal, 7.5% poultry by-product meal, and 0.25% each methionine and lysine.

[b] Krill hydrolysates; Specialty Marine Products, Ltd., West Vancouver, British Columbia, Canada.

[c] Roche Performance Premix composition (per gram of vitamin mixture): vitamin A = 2,646 IU; vitamin D_3 = 221 IU; vitamin E = 66.1 IU; vitamin B_{12} = 13 μg; riboflavin = 13.2 mg; niacin = 61.7 mg; D-pantothenic acid = 22.1 mg; menadione = 1.32 mg; folic acid = 1.76 mg; pyridoxine = 4.42 mg, thiamin = 7.95 mg, d-biotin = 0.31 mg (Hoffman-La Roche, Inc., Nutley, New Jersey).

[d] Five mg Se in the form of sodium selenite per kilogram Bernhart Tomarelli salt mixture (ICN Pharmaceuticals, Inc., Irvine, California).

[e] Phospitan C (Mg-L-ascorbyl-2-phosphate); Showa Denko K. K., Tokyo, Japan.

[f] Calculated based on the compositions of the ingredients used (NRC 1993).

Supplemental aeration was also provided to maintain dissolved oxygen at levels near saturation. Water temperature increased gradually from 8°C to 15°C during the experiment and a diurnal light: dark cycle was regulated at 12 h: 12 h. Total fish weight in each tank was monitored every 4 weeks to check growth and adjust feeding rate. Feeding was stopped 24 h prior to weighing.

Feces collection and protein and phosphorus absorption.—The indirect method was used to calculate the apparent digestibility coefficient (ADC) with 0.5% chromic oxide as an inert indicator (Cho and Kaushik 1990). The apparent digestibility coefficients for protein and phosphorus were calculated using the following formula:

$$ADC_{nutrient} = [1 - (NF/ND \times CrD/CrF)] \times 100,$$

where: NF = percent nutrient in feces, ND = percent nutrient in diet, CrD = percent chromic oxide in diet, and CrF = percent chromic oxide in feces. Feces were collected with a modified fecal collection system after 16 weeks of the feeding trial (Yamamoto et al. 1998). From week 12 of the feeding trial, fish were fed the 0.5% chromic oxide diets to facilitate the digestibility test. After 16 weeks of feeding trial, three groups of 20 fish per diet treatment were transferred to three 50-L collection tanks having a steep conical bottom connected to a feces collection chamber. Water flowed to the top and out at the bottom of the conical tanks at a rate of less than 0.4 L/min. Fish were prevented from stirring the feces by a circular net placed at the base of the tank. To test the apparent nutrient digestibilities of the five experimental diets, the fish were fed their last respective meal at 2000 hours, and the feces were collected the next day at 0800 hours. To compare apparent protein digestibility with different times of fecal collection (every 40 min, after 12 h, and 48 h), the feces of the control fish fed the FM-based diet were collected every 40 min for 7 h and again at 48 h after feeding. The collected feces were immediately frozen at −20°C and then stored at −80°C until analyzed.

Sample collection and analysis.—Analyses of crude protein, moisture, and ash in diets and whole

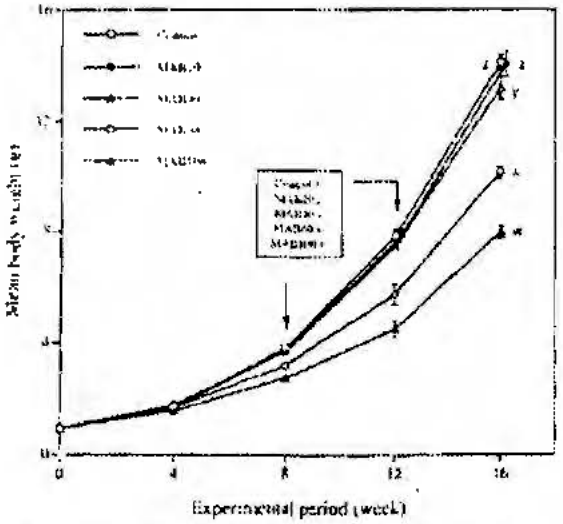

FIGURE 1.—Changes in mean body weight of juvenile rainbow trout fed five different diets (see Tables 1 and 2 for descriptions) for 16 weeks. Values are means ± SD of triplicate groups. Diets having different letters (z–w) are significantly different ($P < 0.05$); the boxed inset indicates statistical comparisons between groups at 8 and 12 weeks.

bodies were performed by standard procedures (AOAC 1995). Dietary lipid was determined according to Folch et al. (1957). At the end of the feeding trial, all fish were weighed and counted to calculate percent weight gain (body weight gain × 100/initial body weight), feed efficiency (body weight gain × 100/dry feed consumed), protein efficiency ratio (body weight gain/protein intake), specific growth rate ([$\log_e$ final body weight − $\log_e$ initial body weight] × 100/days), and survival. Diets and fish whole body were dry-ashed to determine mineral compositions by the inductively coupled plasma (ICP) emission spectrometry method (ARI-3560 Spectrometer; Applied Research Laboratories, Valencie, California) according to Watson and Isaac (1990). For chromium analysis, samples were digested with perchloric acid, then treated with the same method as other minerals.

Statistical analysis.—Data were subjected to one-way analysis of variance (ANOVA) using the SPSS statistical package (Version 9.0, SPSS Inc.). Percentage data were arcsine-transformed before analysis. A Fisher least significant difference (LSD) test was used to compare treatment means with $P = 0.05$. The possible replacement level of FM protein by MAB was determined by broken-line regression analysis (Robbins et al. 1979).

Results

Growth Performance and Whole Body Minerals

Average body weights of fish fed experimental diets during the 16-week period are shown in Figure 1. Fish growth rates began to differ in week 8 and became distinctly different between weeks 12 and 16. After the final week, fish fed diets containing 40, 60, and 100% MAB protein (diets MAB40, MAB60, and MAB100) exhibited significantly lower weight gain, feed efficiency, protein efficiency ratio, and specific growth rate compared with fish fed the control and MAB20 diets (Table 3). The regression equation for the broken-line analysis technique was $Y = 1558 - 8.527X$ ($r = 0.96$) and $Y = 1316$. The breakpoint at 28.3% MAB substitution level produced the least mean square error, and this value was determined as the acceptable substitution level of MAB. Interestingly, the whole body protein levels of fish increased as the MAB substitution in diets increased. No mortality was observed during the 16 weeks of the feeding trial. Lower diet acceptance was noticed in the fish fed MAB100 after the week 12.

TABLE 3.—Weight gain (WG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), and whole body protein (WBP) of juvenile rainbow trout fed experimental and control diets for 16 weeks. Values are means ± SE of triplicate groups by diet; values in the same row followed by different letters are significantly different ($P < 0.05$). The acronym MAB = mixture of animal by-products.

Characteristic	Control	MAB20	MAB40	MAB60	MAB100
WG (%)[a]	1370 ± 17.0 z	1365 ± 26.9 z	1275 ± 23.5 y	959 ± 3.89 x	734 ± 5.59 w
FE (%)[b]	103 ± 1.09 z	104 ± 1.79 z	94.9 ± 1.70 y	80.3 ± 1.15 x	68.8 ± 0.67 w
SGR (%)[c]	2.40 ± 0.0 z	2.40 ± 0.02 z	2.34 ± 0.02 y	2.11 ± 0.00 x	1.89 ± 0.01 w
PER[d]	2.28 ± 0.02 z	2.31 ± 0.04 z	2.09 ± 0.04 y	1.78 ± 0.03 x	1.57 ± 0.01 w
WBP (%)[e]	56.1 ± 0.78 x	56.0 ± 0.70 x	56.5 ± 0.43 x	58.5 ± 0.58 y	60.4 ± 0.26 z

[a] Weight gain (%) = [(final weight − initial weight) × 100]/initial weight.
[b] Feed efficiency (%) = [body weight gain (g) × 100]/dry feed intake (g).
[c] Specific growth rate (%) = [($\log_e$ final weight − $\log_e$ initial weight) × 100]/d.
[d] Protein efficiency ratio = body weight gain (g)/protein intake (g).
[e] Whole body protein (%) = (protein concentration × 100)/dry whole body weight (g).

TABLE 4.—Total ash and mineral composition of the whole body of fish fed experimental and control diets for 16 weeks. MAB = mixture of animal by-products. Values are means ± SE of triplicate groups by diet; values in the same row without a letter in common are significantly different ($P < 0.05$).

Mineral	Initial	Control	MAB20	MAB40	MAB60	MAB100
Ash (%)		9.87 ± 0.23 yx	9.43 ± 0.50 x	9.93 ± 0.24 yx	10.4 ± 0.30 zy	11.3 ± 0.15 z
P (%)	1.47	1.59 ± 0.04 x	1.53 ± 0.03 x	1.64 ± 0.02 yx	1.72 ± 0.04 x	1.89 ± 0.08 z
K (%)	1.37	1.20 ± 0.01 y	1.21 ± 0.02 y	1.23 ± 0.05 y	1.28 ± 0.03 zy	1.36 ± 0.08 z
Ca (%)	1.52	1.76 ± 0.06 yx	1.61 ± 0.07 x	1.77 ± 0.05 yx	1.88 ± 0.06 zy	2.11 ± 0.12 z
Na (%)	0.38	0.30 ± 0.01 x	0.31 ± 0.01 x	0.33 ± 0.02 yx	0.35 ± 0.003 y	0.40 ± 0.02 z
Mg (%)	0.09	0.107 ± 0.00 y	0.107 ± 0.00 y	0.113 ± 0.00 zy	0.113 ± 0.001 zy	0.120 ± 0.00 z
Fe (μg/g)	227	117 ± 20.6 z	63.6 ± 5.36 y	99.6 ± 20.33 zy	73.3 ± 9.68 zy	67.0 ± 1.31 y
Mn (μg/g)	61.0	21.6 ± 6.48 z	7.02 ± 0.31 yx	13.5 ± 2.66 zy	11.2 ± 1.04 y	10.7 ± 1.23 y
Cu (μg/g)	1.23	1.72 ± 0.20 x	1.84 ± 0.16 x	2.61 ± 0.32 yx	3.44 ± 0.26 zy	3.87 ± 0.53 z
Zn (μg/g)	91.5	61.4 ± 0.81 yx	58.9 ± 2.35 x	65.2 ± 2.25 yx	68.3 ± 0.26 y	76.5 ± 3.70 z

Whole body ash levels increased as MAB substitution in diets increased and were significantly higher in MAB100 groups compared with the control (Table 4). All the individual mineral levels, except for Fe and Mn, followed the same trend as whole body ash. However, the levels of ash and individual minerals in feces collected 12 h after last feeding decreased as dietary ash and individual mineral levels decreased (Table 5), as related to increased MAB substitution in diets (Table 6).

Apparent Protein and Phosphorus Availability

After 16 weeks of feeding, apparent protein and phosphorus availabilities were determined in the feces collected 12 h after a meal (Figure 2). The apparent protein digestibility of diets containing MAB20 (85.8 ± 1.05%), MAB40 (83.1 ± 0.45%), and MAB60 (82.7 ± 2.67%) were not significantly different from that of the control diet (85.0 ± 1.88%), even though they produced lower growth rates than the control. However, apparent protein digestibility of the MAB100 diet (77.7 ± 4.41%) was significantly lower ($P < 0.05$) than that of the control diet. Contrary to the observed trend of apparent protein digestibility, test diets containing MAB exhibited significantly higher apparent phosphorus digestibility than the control diet. Apparent protein digestibility determined at fecal collection times of 12 h (85.0 ± 1.88%) and 48 h (87.4 ± 1.16%) did not differ significantly from that of feces collected every 40 min (86.0 ± 2.52%).

Discussion

Growth Performance and Whole Body Minerals

Based on the broken-line analysis, we found that approximately 30% of FM protein could be replaced by MAB, which would lead to growth rates in juvenile rainbow trout comparable to FM-based diets. This is significant because it is the first time, to our knowledge, that two new animal protein sources, leather meal and squid liver powder, have been shown to be effective MAB components in replacing FM in fish diets. Fish fed the FM-based control diet (1 herring: 1 menhaden) grew very well, showing comparable or higher specific growth rates (SGR, percent growth rate 47 d, 2.40 ± 0.01) than those reported in the literature. For instance, in an other rainbow trout study, Skonberg et al. (1997) found that similarly sized fish (initial

TABLE 5.—Ash and mineral composition of feces of the fish fed the experimental and control diets for 16 weeks; MAB = mixture of animal by-products. Values are means ± SE of triplicate groups; values in the same row without a letter in common are significantly different ($P < 0.05$).

Mineral	Control	MAB20	MAB40	MAB60	MAB100
Ash (%)	27.7 ± 0.33 z	22.7 ± 0.96 y	23.0 ± 1.16 y	17.0 ± 0.00 x	13.3 ± 0.33 w
P (%)	3.95 ± 0.08 z	3.39 ± 0.19 y	3.41 ± 0.18 y	2.11 ± 0.02 x	1.50 ± 0.04 w
K (%)	0.04 ± 0.007 zy	0.05 ± 0.009 z	0.04 ± 0.001 zy	0.03 ± 0.001 y	0.03 ± 0.007 zy
Ca (%)	6.45 ± 0.12 z	6.08 ± 0.34 z	5.96 ± 0.37 z	4.53 ± 0.12 y	3.43 ± 0.20 x
Na (%)	0.30 ± 0.02 yx	0.39 ± 0.01 zy	0.42 ± 0.03 z	0.33 ± 0.02 yx	0.26 ± 0.04 x
Mg (%)	0.74 ± 0.03 z	0.51 ± 0.03 x	0.66 ± 0.02 y	0.25 ± 0.02 w	0.20 ± 0.003 w
Fe (μg/g)	2,996 ± 473 z	815 ± 10.2 x	1,926 ± 187 y	1,029 ± 92.5 x	1,215 ± 85.6 yx
Mn (μg/g)	3,694 ± 614 z	435 ± 24.1 y	861 ± 16.6 y	429 ± 34.3 y	295 ± 22.0 y
Cu (μg/g)	24.0 ± 0.32 zy	17.4 ± 0.80 x	24.9 ± 1.76 z	23.5 ± 0.63 zy	21.9 ± 0.43 y
Zn (μg/g)	233 ± 4.73 z	225 ± 11.5 zy	217 ± 13.8 zy	201 ± 5.30 y	155 ± 6.70 x

TABLE 6.—Mineral composition of the experimental and control diets; MAB = mixture of animal by-products.

Mineral component	Control	MAB20	MAB40	MAB60	MAB100
P (%)	1.63	1.59	1.64	1.58	1.52
K (%)	0.96	0.92	0.92	0.88	0.81
Ca (%)	1.67	1.58	1.56	1.40	1.19
Na (%)	0.43	0.41	0.41	0.38	0.34
Mg (%)	0.32	0.30	0.30	0.29	0.26
Fe (μg/g)	235	259	297	336	404
Mn (μg/g)	103	101	105	103	102
Cu (μg/g)	16.5	14.9	14.7	17.3	17.7
Zn (μg/g)	89.3	84.3	86.6	84.9	81.4

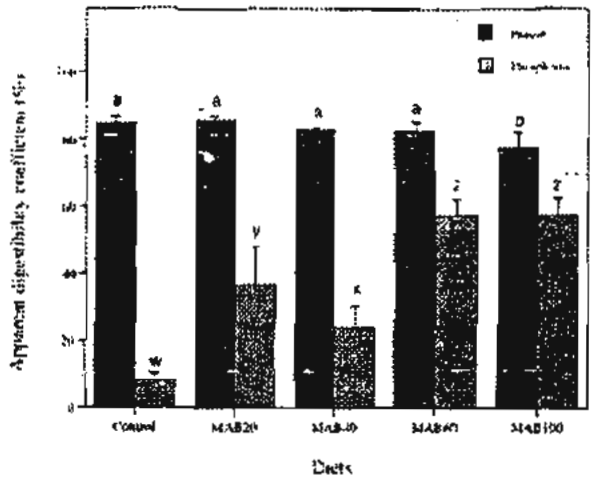

FIGURE 2.—Apparent digestibility coefficients for protein and phosphorus in juvenile rainbow trout fed experimental diets for 16 weeks. Feces were collected 12 h after the last feeding in the digestibility test. Values are means ± SD of triplicate groups. Bars having different letters (a–b for protein, z–w for phosphorus) are significantly different ($P < 0.05$).

weight, 1.8 g) fed a control diet (herring FM-based) for 8 weeks had an SGR of 1.42% SGR for restricted feeding (3–4%/ d) and 2.56% for ad libitum feeding at temperatures of 15–21°C. Rainbow trout, weighing 3 g, fed a menhaden FM-based diet ad libitum had an SGR of 2.77% after 13 weeks of feeding (Watanabe et al. 1993). Furthermore, the feeding period in our study was relatively long, compared with many other FM replacement studies of juvenile rainbow trout, because of rapid growth rates in the juveniles (Watanabe et al. 1993; Yamamoto et al. 1995; Stickney et al. 1996).

Most individual animal protein sources, such as meat and bone meal, feather meal, blood meal, and poultry by-product meal, have been able to replace less than 50% of FM in diets of salmonids. Fowler (1991) found that poultry by-product meal could compose 20% of a practical diet for chinook salmon *Oncorhynchus tshawytscha*, with concurrent reduction of the FM by 50%, without impairing growth and feed efficiency. Feather meal was reported as a minor replacer for FM in diets for coho salmon *O. kisutch* (Higgs et al. 1979), chinook salmon (Fowler 1982), and Nile tilapia (Bishop et al. 1995). High levels of meat and bone meal in fish diets showed different results in different fish species. Blood meal was also studied in rainbow trout (Luzier et al. 1995), eel (Lee and Bai 1997), and tilapia (Lee and Bai 1998). When FM is replaced with combined ingredients, the interpretation of results is difficult because many interactions between nutrients may be involved in nutrient metabolism. In our study, approximately 30% of MAB protein was able to replace FM protein in juvenile rainbow trout diets, showing that in the group with 40% MAB the growth rate decrease was less than 7% of the control. The lower growth performance is probably related to the inferior quality of the animal protein sources, but feather meal and squid liver powder seem to have potential as animal protein sources in rainbow trout feed.

Sugiura et al. (1998) reported that the levels of Ca and ash in salmonid diets formulated with animal by-product sources were inversely correlated to the percentage of net absorption of Ca, Fe, and Mn, indicating possible antagonistic interactions of Ca with these minerals. Our results were similar to that of Sugiura et al. (1998). In our study, ash and some mineral whole body concentrations (Table 4) were inversely correlated with those of the experimental diets (Table 6). The whole body concentrations of Ca, K, Na, and Mg were inversely correlated with dietary levels of Ca ($r = -0.89$), K ($r = -0.98$), Na ($r = -0.98$), and Mg ($r = -0.91$; $P < 0.05$ for all). However, the levels of ash and Ca in feces showed a positive correlation with the dietary ash ($r = 0.94$; $P < 0.05$) and Ca ($r = 0.99$; $P < 0.01$). A similar trend was observed in other mineral levels between diet and feces, even though the positive correlations were not significant ($P > 0.05$). This result confirmed data of Sugiura et al. (1998), who reported that fecal nutrient losses were positively correlated ($P < 0.05$) to nutrient intake, except for protein, Na, K, and Zn.

Apparent Protein and Phosphorus Availability

Apparent protein digestibility was not significantly different for up to 60% replacement of FM protein compared with that of the control diet, even

though weight gain was lower in the 40% and 60% replacement groups than in the control. This result may indicate that apparent protein digestibility of MAB is slightly lower than that of fish meal in juvenile trout. Although FM and MAB were not the only protein sources in the diets, the other protein sources (e.g., wheat middlings, corn gluten meal, yeast, and krill meal) were incorporated in all the diets at the same levels. Watanabe and Pongmaneerat (1991) reported that rainbow trout had lower protein digestibility of meat and bone meal (78%) than of white (92%) and brown (90%) FM. Robaina et al. (1997) mentioned that poor digestibility of meat and bone meal in diets of gilthead seabream *Sparus aurata* was due to its high ash content. The reason for the lower apparent protein digestibility of MAB in our study might be attributed to the poor amino acid profile rather than the high ash content of meat and bone meal (the major ingredient in MAB) because the ash content of MAB was lower than that of FM (Table 1).

Higher apparent phosphorus availabilities were found in the test diet groups than the control group (Figure 2). The reason for this seems to be due to supplementation of highly absorbable monobasic calcium phosphate in the test diets (NRC 1993). The availability of phosphorus from FM, which is primarily of bone origin, is generally lower than that of certain other high-protein feed ingredients, such as casein and yeast. Therefore, our findings suggest that using a mixture of animal by-products might improve phosphorus availability in juvenile trout diets. Furthermore, an MAB could have an advantage in reducing phosphorus release into the water, as indicated in feces phosphorus concentrations (Table 5). This is an important consideration because of the effect of aquacultural phosphorus discharge on stream eutrophication (Hardy 1999).

Feces collection frequently results in nutrients leaching into the water and leads to overestimation of digestibility: approximately 5–8% higher values than those determined by using fecal stripping or dissection methods (Brown 1993; Hajen et al. 1993; Watanabe et al. 1996; Allan et al. 1999). However, Allan et al. (1999) reported that stripping was not a suitable method for collecting digesta from juvenile silver perch *Bidyanus bidyanus* smaller than 10 g, and dissection methods exhibited considerably lower values than collection methods with rapid settlement. In our study, we used a collection method employing rapid settlement (Yamamoto et al. 1998) and determined apparent protein digestibility from feces collected at three different schedules (every 40 min, 12 h, and 48 h). Feces collected every 40 min retained their original bound form. However, we found some loosened of fecal form at 12 h and more at 72 h collections, but the related average values of protein digestibility were not statistically significant. This is supported by Allan et al. (1999), who reported similar digestibility from feces collected at different times (2, 6, 12, and 18 h), and by Satoh et al. (1992), who reported minimal differences in protein and lipid digestibility derived from feces collected at 3, 6, 9, 12, and 15 h after rapid settlement in a fecal collection chamber. Therefore, our findings suggests that, with rapid feces settlement systems, fecal collection times of 40 min to 48 h do not result in different protein digestibility values.

Based on the wholesale market price of 1998, fish meal (menhaden:herring, 1:1) used in the present study was US$ 2.51/kg and the estimated cost for MAB was 0.56/kg (Jack Bardall, OARDC, Wooster, Ohio, personal communication). Therefore, we expect that at least $ 0.22/kg of juvenile rainbow trout feed could be saved if 30% of FM protein is replaced by MAB. Use of MAB in fish diets as a FM replacement could be more cost-effective in the future if the price of FM continues to rise.

In conclusion, our findings suggest that MAB could replace approximately 30% of FM protein in diets for juvenile rainbow trout without adverse growth effects for up to 16 weeks. Also, fecal collections within 48 h of last feedings should not significantly overestimate apparent protein digestibility values. Further research will be required to determine the feasibility of using MAB composed of different combinations of ingredients and examine affects on larger sizes of fish.

Acknowledgments

The authors thank the staff of the Piketon Research and Extension Center, especially Dean Rapp and Elissa French, for their assistance in daily care and feeding of fish, Przemek Bajer for his assistance in collecting feces, and Travis Hartman for his critical reading of this manuscript. This study was supported by the North Central Regional Aquaculture Center, East Lansing, Michigan; the Ministry of Maritime Affairs and Fisheries through RCOID (ERC), Pukyong National University (PKNU); and Seafood and Marine Bioresources Development Center, PKNU, Pusan, Korea. Salaries were partly provided by state and fed-

eral funds appropriated to Ohio Agriculture Research and Development Center, Wooster, Ohio.

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ANEXO 5: CURRICULUM VITAE DEL EQUIPO DOCENTE

Se deberá incluir en esta sección el curriculum vitae completo y fotocopia de los certificados de títulos (si corresponde) de cada uno de los integrantes del equipo docente de la actividad de formación o capacitación. Especificar función y responsabilidad en la ejecución de la propuesta.

Se debe completar la ficha que sigue a continuación **para cada uno de los integrantes** del equipo docente.

FICHA DE ANTECEDENTES PERSONALES RESUMIDA	
ANTECEDENTES PERSONALES (Obligatorio para todos los participantes)	
Nombre completo	Bai Sungchul Charles
RUT	
Fecha de Nacimiento	23 de Febrero de 1954
Nacionalidad	Koreana
Dirección particular (indicar comuna y región)	Feeds and Foods Nutrition Research Center Pukyong National University Busan 608-737, Korea
Fono particular	(82-51) 620-6137/6874
Celular	
E-mail	scbai@pknu.ac.kr
Banco y número de cuenta corriente personal	
Género (Masculino o femenino)	Masculino
Indicar si pertenece a alguna etnia (mapuche, aymará, rapa nui, atacameña, quechua, collas, alacalufe, yagán, huilliche, pehuenche)	
Nombre y teléfono de la persona a quien avisar en caso de emergencia	

ACTIVIDAD PROFESIONAL Y/O COMERCIAL (ACTUAL)	
Nombre de la Institución o Empresa a la que pertenece	Feeds and Foods Nutrition Research Center Pukyong National University, Pusan
Rut de la Institución o Empresa	
Nombre y Rut del Representante Legal de la empresa	
Cargo	Investigador/Docente
Profesión	Dr. en Nutricion Animal
Dirección comercial (Indicar comuna y región)	Feeds and Foods Nutrition Research Center Pukyong National University Busan 608-737, Korea
Fono y Fax comercial	
E-mail	
Clasificación de público o privado	
Banco y número de cuenta corriente de la institución	
ACTIVIDAD COMO AGRICULTOR (ACTUAL) (Completar sólo si se dedica a esta actividad)	
Tipo de Agricultor (pequeño, mediano o grande)	
Nombre de la propiedad en la cual trabaja	
Cargo (dueño, administrador, etc.)	
Superficie Total y Superficie Regada	
Ubicación (detallada)	
Rubros a los que se dedica (incluir desde cuando se trabaja en cada rubro) y niveles de producción en el rubro de interés	
Resumen de sus actividades	
Organizaciones (campesinas, gremiales o	

empresariales) a las que pertenece y cargo, si lo ocupa	

CURRICULUM VITAE
Brief to December, 2005.

NAME: Sungchul Charles Bai

CURRENT TITLE: Undergraduate and Graduate Faculty
Professor/Department of Aquaculture
Director/Feeds and Foods Nutrition Research Center
Pukyong National University
Busan 608-737, Korea (82-51) 620-6137/6874
E-mail: scbai@pknu.ac.kr or sccbai@empal.com
Fax (82-51) 628-6873/6875 Homepages: www.ffnrc.com

DATE OF BIRTH: February 23, 1954 (Korea)

EDUCATION : B.S., Animal Husbandry, Kon-Kuk University, Seoul, 1980
M.S., Animal Nutrition, California State University, Fresno, 1984
Ph.D., Nutrition/Physiological Chemistry, School of Veterinary Medicine, University of California, Davis, 1990

PROFESSIONAL AND ACADEMIC APPOINTMENTS:

President-elect, World Aquaculture Society, 2006 -Present
President, Korean Eel Aquaculture Research Association, 2003 – present
President, Vision 21 Korean Aquaculture Forum, 2003 - Present
Board of Director, World Aquaculture Society (WAS), 2003 – present
Editor, Aquaculture (The official Journal of Korean Aquaculture Society) 2002 – present
Associate Editor, The Journal of World Aquaculture Society (JWAS), 2001 - present
Editorial Board Member, Aquaculture Research, Blackwell Science Ltd., 2000 – present
Editorial Board Member, Journal of Asian Fisheries Society, 2005 - Present
Director, Feeds and Foods Nutrition and Research Center, Busan, Korea, 2000 – present
Visiting Professor, Dept. of Animal Sciences, Univ. of California Davis, Davis, Ca, 1999-2000
Chairman, Dept. of Aquaculture, PKNU, Busan, Korea, 1996 – 1998
Professor, Dept. of Aquaculture, PKNU, Busan, Korea, 1993-Present (promotion in 2005)
Visiting Assistant Professor, Sch. of Nat. Resources, The Ohio State University, USA, 1993-93
Postdoctoral Research Associate, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, 1990-1992
Postgraduate Researcher and Graduate Research Assistant, Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, CA, 1986-1990.

TEACHING AND ACADEMIC ACTIVITIES:

Current teaching Subjects: Feeds and Nutrition; Feed Technology; Fish Nutrition; Nutritional Biochemistry; Aquaculture Internship; Research.
Department Undergraduate Advisor, and 15 Undergraduates
Current Undergraduate, Graduate Students and Staffs: 16 studdents and 8 full time staffs.
Member, Faculty of Aquaculture and Fisheries Biology for Graduate School Faculty of Aquaculture for Graduate School of Industry

RESEARCH INTERESTS:

Basic nutrition of fishes and shellfishes, with primary emphasis on species cultured for human consumption. Research activities include determining dietary requirements for and metabolism of various nutrients. Consideration is given to such aspects as nutritional biochemistry, nutritional energetics, and nutrition-disease interactions of fish as it relates to human health. Research is coordinated to provide a comprehensive basic fish nutrition program which are targeted to improve production efficiency in aquaculture and enhance the quality of resulting products, to develop fishmeal analog and microencapsulated larval feeds, and to develop the computer feed formulations and the analytical methodology in aquaculture and fisheries managements.

CURRENT GRANT and Other SUPPORTs:

Ministry of Marine Affairs and Fisheries, $100,000 (2006. 3 – 2007. 3)
Binex, Doha Industry and other industry projects, $40,000 (2004. 10 – 2006. 7)
FFNRC with 8 staffs made annually $100,000 in 2005 and expected $150,000 in 2006.

PROFESSIONAL MEMBERSHIP :

American Society of Nutritional Sciences (ASNS).
Korean Aquaculture Society.
Korean Fisheries Society.
Korean Society of Animal Nutrition & Feedstuffs.
New York Academy of Sciences
Sigma Xi, The Scientific Research Society.
The Korean Society for Chitin and Chitosan.
Vision 21 Korean Aquacultue Forum
World Aquaculture Society (WAS)

SELECTED PUBLICATIONS:

Books	Journal papers and Thesis		Conference Proceedings, Research Reports, Academic Essays, etc.		Patents	
	Domestic	Foreign	Domestic	Foreign	Registered	Applied
13	42	61	144	135	4	6

ANEXO 6: PRECIOS Y COTIZACIONES

Se deberán adjuntar en este Anexo los precios de referencia y las cotizaciones que avalen los cálculos de costos realizados.

TARIFA E ITINERARIO MR. BAI

FECHA	VUELO	RUTA		SALE	LLEGA
14 Julio 06.	Korean A. 1402	Pusan	Seoul	07.00 hrs.	08.00 hrs.
14 Julio 06.	Korean A. 017	Seoul	Los Angeles	15.00 hrs.	10.20 hrs.
14 Julio 06.	Lan 601	Los Angeles	Santiago	14.05 hrs.	06.05 hrs.
23 Julio 06.	Lan 600	Santiago	Los Angeles	21.10 hrs.	07.40 hrs.
24 Julio 06.	America 1936	Los Angeles	San Francisco	13.50 hrs.	15.05 hrs.
13 Agosto 06.	Korean 024	San Francisco	Seoul	14.40 hrs.	18.40 hrs.
14 Agosto 06.	Korean 1405	Seoul	Pusan	20.05 hrs.	21.20 hrs.

VALOR : USD 3.680.-

NOTAS :

- Reservas confirmadas ida y regreso.
- Estadía mínima 05 días y máxima 3 meses.
- Reserva y emisión simultanea.
- Cambios antes de la partida NO permite.
- Cambios después de la partida con multa de USD 300.-

ANEXO 3. VALORES VIÁTIVOS ALOJAMIENTO Y ALIMENTACIÓN
(Para aplicar en los cálculos de cotos, aporte FIA y aporte de contraparte)

País	Ciudad	Viático (US$/día) Incluido alojamiento
CHILE		93