USSEC 2nd Asian Aquaculture Feed Formulation Database Workshop

Day 2:

Educational Modules



Agenda

0900-0915 Introduction and Outline of Aquaculture Formulation Approach

0915-1015 Introduction to Formulation with Bestmix:

- •Ingredients selection
- Nutritional specifications selection
- Least-cost formulation

1015-1030 Introduction to Feed Formulation Exercises

1045-1145 Feed Formulation Exercise: Formulating for a species

1145-1245 Educational Module #1: Ingredient Composition and Nutritive Value

- •Adjusting the composition of ingredients on Bestmix
- Development of equations for predicting parameters
- Variability in the nutritive value of ingredients: Nutritional principles

1345-1445 Educational Module #2 Nutritional Specifications

- •Nutritional specifications How they are developed, adjusted, updated
- Meeting essential fatty acids and minor lipids requirements
- Effectively meeting phosphorus requirement

1445-1530 Feed Formulation Exercise (continued)

1545-1645 Presentation of feed formulations

1645-1715 Wrap-up, certificate presentation and group picture

Educational Module #1: Ingredient Composition and Nutritive Value (1h)

Adjusting the composition of ingredients on Bestmix

Development of equations for predicting parameters

Variability in the nutritive value of ingredients:
 Nutritional principles

Educational Module #2 Nutritional Specifications (1h)

Nutritional specifications – How they are developed, adjusted, updated

Meeting essential fatty acids and minor lipids requirements

Effectively meeting phosphorus requirement

Educational Module #3: Dietary Energy: Definitions and Requirements (30 min)

- Energy Partitioning Scheme
- Dietary Energy
 - Gross energy
 - Digestible energy
 - Metabolizable energy
- Bioenergetics Model
 - Energy Requirement Estimations
 - Theoretical feed requirement and feed conversion ratio

Educational Module #1: Ingredient Composition and Nutritive Value (1h)

 Adjusting the composition of ingredients on Bestmix

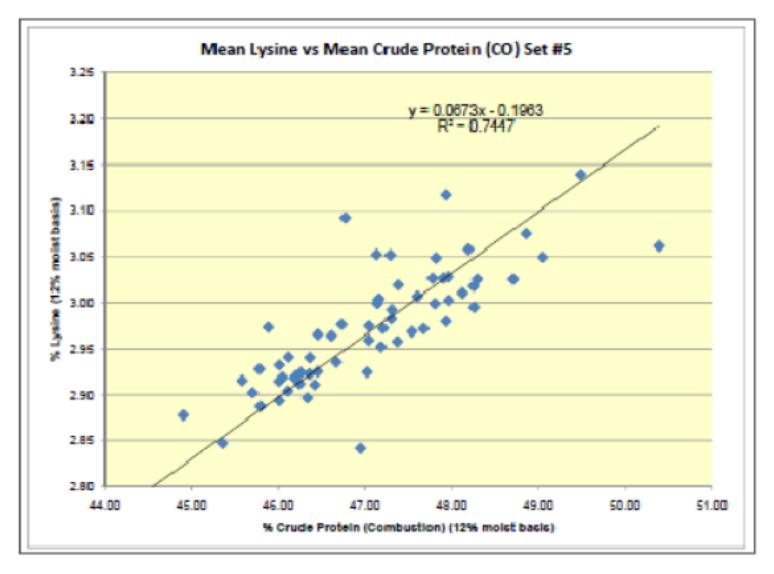
Development of equations for predicting parameters

Variability in the nutritive value of ingredients:
 Nutritional principles

Adjusting the Composition of Ingredients

- Adjusting the composition of ingredients in AAFFD and Bestmix
 - Done on the basis of coefficients that relate amino acid, fatty acid, and mineral (Ca, P) compositions to protein, lipid or ash
 - Coefficients are specific per ingredients or ingredient types
 - Example of equation in BestMix:
 - If Nutrients.Amino acid coefficients.Arg Coeff <>0 Then Nutrients.Amino acids.Arginine = Round (Nutrients.Proximate analysis.Crude Protein *Nutrients.Amino acid coefficients.Arg Coeff /100,2) End If

Variability of Lysine Concentration in Relation to Crude Protein Content of US Soybean Meal Samples



Gross Energy

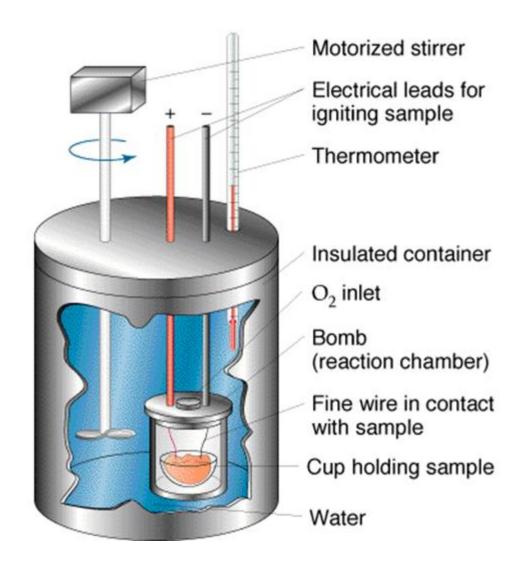
Gross energy (GE) is the commonly used term for enthalpy (Δ H) of combustion in nutrition. However, as opposed to enthalpy, GE is generally represented by a + sign. GE content of a substance is usually measured by its combustion in a heavily walled metal container (bomb) under an atmosphere of compressed oxygen. The method is referred to as bomb calorimetry.

The GE content of an ingredient or a compounded diet depends upon its chemical composition.

The mean values of GE of carbohydrates, proteins and lipids are 17.2, 23.6 and 39.5 kJ/g, respectively (Blaxter, 1989).

Minerals (ash) have no GE because these components are not combustible.

Bomb Calorimeter



Gross Energy (MJ/kg) =

Crude Protein (kg/kg) x 23.6 MJ/kg + Lipids (kg/kg) x 39 MJ/kg + Total Carbohydrate (kg/kg) x 17 MJ/kg

Gross Energy (MJ/kg) = 0.32 * 23.6 + 0.06 * 39 + 0.4 * 17 For a 32% CP, 6% fat and 40% total CHO feed

Gross energy (MJ/kg)*1000 / 4.184 = Gross energy (kcal/kg)

Digestible Energy (MJ/kg) =

Gross energy (MJ/kg) x ADC Gross Energy

<u>or</u>

Species specific Carnivorous vs. omnivorous vs. carp vs. shrimp?

Crude Protein (kg/kg) x 23.6 MJ/kg x ADC Crude Protein

+

Lipids (kg/kg) x 39 MJ/kg x ADC Lipids

+

Total Carbohydrate (kg/kg) x 17 MJ/kg x ADC Total CHO

Species specific
Carnivorous vs. carp vs. shrimp?

Digestible Energy (MJ/kg) =

Crude Protein (kg/kg) x 23.6 MJ/kg x ADC Crude Protein

ADC crude protein: 0.85 to 0.9

+

Lipids (kg/kg) x 39 MJ/kg x ADC Lipids

ADC Lipids = 0.85-0.95

+

Total Carbohydrate (kg/kg) x 17 MJ/kg x ADC _{Total CHO}

ADC total CHO = 0.4 to 0.7 (depends on fiber level, heat processing, species)

Digestible Energy (MJ/kg) (Starch +sugars) =

Crude Protein (kg/kg) x 23.6 MJ/kg x ADC Crude Protein

+

Lipids (kg/kg) x 39 MJ/kg x ADC Lipids

+

Starch+ Sugars (kg/kg) x 17 MJ/kg x ADC Total Starch+Sugars

Species specific Carnivorous vs. carp vs. shrimp?

Crude Protein (kg/kg) x 23.6 MJ/kg x ADC Crude Protein

ADC crude protein: 0.85 to 0.9

+

Lipids (kg/kg) x 39 MJ/kg x ADC Lipids

ADC Lipids = 0.85-0.95

+

Total Starch + Sugars (kg/kg) x 17 MJ/kg x ADC Total Starch+sugars

ADC total starch+sugars = 0.6 to 0.95 (depends on heat processing, species)

Equations in AAFFD and BestMix

- 'Energy calculations
- Nutrients.Energy Aqua.Gross Energy -MJ = Round (23.6*Nutrients.Proximate analysis.Crude Protein /100+ 39*Nutrients.Proximate analysis.Crude Lipids /100 + 17*Nutrients.Proximate analysis.Total CHO /100 ,1)
- Nutrients.Energy Aqua.Gross energy -Kcal = Round (Nutrients.Energy Aqua.Gross Energy -MJ * 238.85,0)
- Nutrients.Energy Aqua.DE Carp = Round ((23.6*Nutrients.Proximate analysis.Crude Protein /100*Nutrients.App Diges Coeff Prox Aqua.ADC DM -fish /100 + 39*Nutrients.Proximate analysis.Crude Lipids /100*Nutrients.App Diges Coeff Prox Aqua.ADC CP -fish /100 + 17*Nutrients.Proximate analysis.Total CHO /100*Nutrients.App Diges Coeff Prox Aqua.ADC GE fish /100)* 238.85 ,0)
- Nutrients.Energy Aqua.DE Fish Carni = Round ((23.6*Nutrients.Proximate analysis.Crude Protein /100*Nutrients.App Diges Coeff Prox Aqua.ADC DM -fish /100 + 39*Nutrients.Proximate analysis.Crude Lipids /100*Nutrients.App Diges Coeff Prox Aqua.ADC CP -fish /100 + 17*Nutrients.Proximate analysis.Total CHO /100*Nutrients.App Diges Coeff Prox Aqua.ADC GE fish /100)* 238.85 ,0)
- Nutrients.Energy Aqua.DE Fish Omni = Round ((23.6*Nutrients.Proximate analysis.Crude Protein /100*Nutrients.App Diges Coeff Prox Aqua.ADC DM -fish /100 + 39*Nutrients.Proximate analysis.Crude Lipids /100*Nutrients.App Diges Coeff Prox Aqua.ADC CP -fish /100 + 17*Nutrients.Proximate analysis.Total CHO /100*Nutrients.App Diges Coeff Prox Aqua.ADC GE fish /100)* 238.85 ,0)
- Nutrients.Energy Aqua.DE Shrimp = Round ((23.6*Nutrients.Proximate analysis.Crude Protein /100*Nutrients.App Diges Coeff Prox Aqua.ADC DM -fish /100 + 39*Nutrients.Proximate analysis.Crude Lipids /100*Nutrients.App Diges Coeff Prox Aqua.ADC CP -fish /100 + 17*Nutrients.Proximate analysis.Total CHO /100*Nutrients.App Diges Coeff Prox Aqua.ADC GE fish /100)* 238.85 ,0)

Variability in the Nutritive Value of Ingredients: Nutritional Principles

- Most variability in nutritive value is associated with chemical damage/ degradation of proteins and lipids in the feed ingredients
- Damage can occur due to heat treatment, chemical reaction, oxidative rancidity and microbial action
- Some natural variability exists and mainly related to variability in raw material composition and seasonal variability, affecting nutrient levels (fatty acid, amino acids, minerals) and levels of anti-nutritional factors and contaminants
- Differences between species are probably minor, except for starch digestibility and fermentation of soluble fiber components and ability to use starch and sugars,

Digestibility - Direct method (Total Collection Method)

Requires:

Very accurate estimate of feed consumption (e.g. over 24-72h)

Total collection of fecal material produced (e.g. over 24-72h)

	Feed g/fish	Feces g/fish	Digestibility
Dry matter	100	25	100-25 75% 100
Protein	40	4	40-4 90% 40
Lipid	20	1	20-1 95% 20

Digestibility – Indirect method

Requires:

- Use of digestion indicator (marker) = 100% indigestible
- Collection of representative samples fecal material produced

Apparent Digestibility Coefficient (ADC) = $1-(F/D \times Di/Fi)$

	Feed %	Feces	Digestibility	%
Dry matter		95	1-(95/95 x 1/4)	75
Protein	40	8	1-(8/40x 1/4)	95
Lipid	20	6	1-(6/20 x 1/4)	92.5
Marker	1	4	1-(4/1 x 1/4)	0

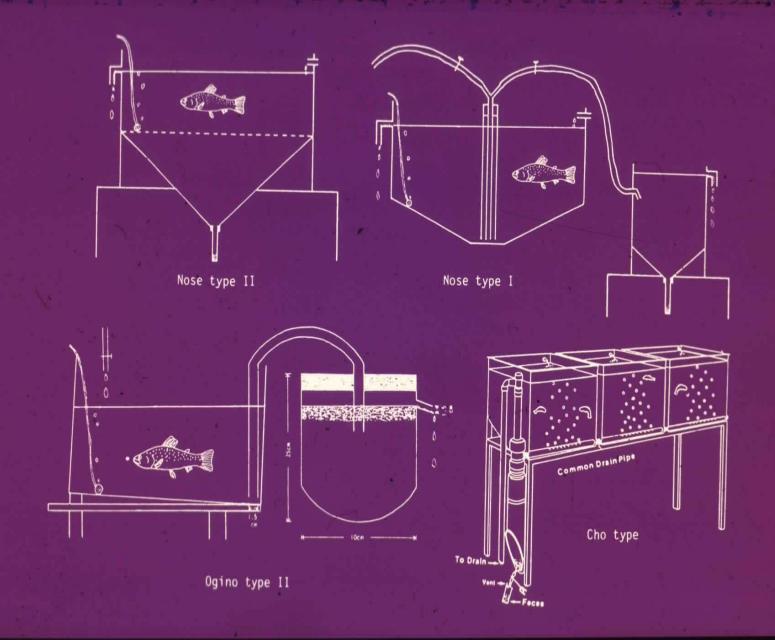
Measuring Digestibility in Fish

Several Methods:

Stripping, dissection, siphoning

Three passive collection methods believed to be more reliable:

TUF Column (Japan)
St.-Pee System (France)
Guelph System (Canada)

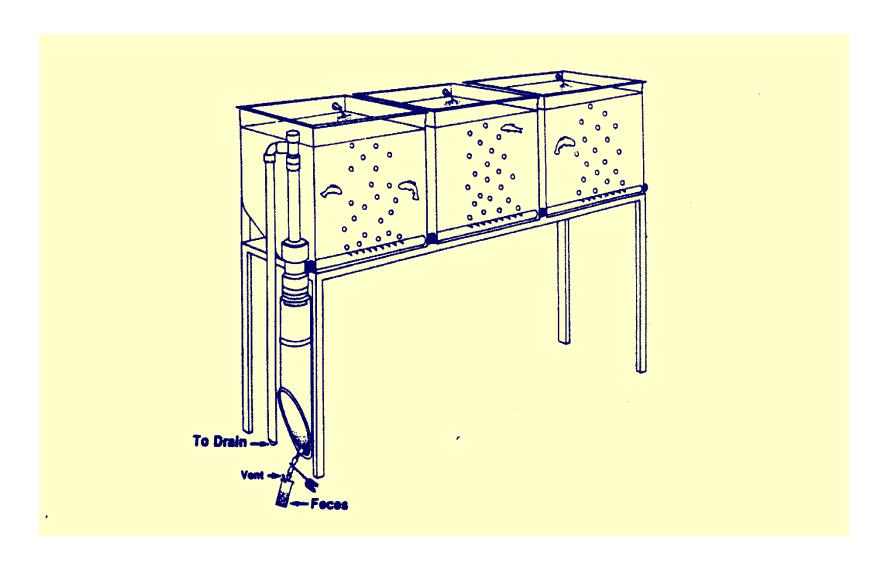


St-Pée System (INRA, St-Pée-sur-Nivelle, France)



Choubert, Luquet, and de la Noue (1979)

Guelph System (Cho et al., 1982)



The Guelph System













Digestibility of Single Ingredients

Most ingredients cannot be fed alone

Acceptance (palatability)

Pelletability

Nutritional quality

Test diet

70% Reference diet

30% Test ingredient

Reference Diet	%
Fish meal	30
Corn gluten meal	13
Soybean meal	17
Wheat middlings	27
Vitamin premix	1
Mineral premix	1
Fish oil	10
Digestion indicator	1
	100

Equation - Digestibility

$$ADC_{ingr} = ADC_{test} + ((1-s)D_{ref}/sD_{ingr}) (ADC_{test}-ADC_{ref})$$

ADC_{ingr}= Apparent digestibility coefficient test diet

ADC_{ref}= Apparent digestibility coefficient reference diet

D_{ref}= Nutrient content of reference diet

D_{ingr}= Nutrient content of ingredient

S = Level of incorporation of ingredient in test diet (e.g. 30%)

Apparent digestibility coefficients (%)

Ingredients	Dry Matter	Crude Protein	Lipid	Energy
Alfalfa meal	39	87	71	43
Blood meal				
ring-dried	87	85	-	86
spray-dried	91	96	-	92
flame-dried	55	16	-	50
Brewer's dried yeast	76	91	-	77
Corn yellow	23	95	-	39
Corn gluten feed	23	92		29
Corn gluten meal	80	96	-	83
Corn distiller dried soluble	46	85	71	51
Feather meal	77	77	-	77
Fish meal, herring	85	92	97	91
Meat and bone meal	70	85	-	80
Poultry by-products meal	76	89	-	82
Rapeseed meal	35	77	-	45
Soybean, full-fat, cook.	78	96	94	85
Soybean meal, dehulled	74	96	-	75
Wheat middlings	35	92	-	46
Whey, dehydrated	97	96	-	94
Fish protein concentrate	90	95	-	94
Soy protein concentrate	77	97	_	84

Feather Meal

	A)	DC
Guelph System	Protein	Energy
Cho et al. (1982)	58%	70%
Sugiura et al. (1998)	82-84%	N/A
Bureau (1999)	81-87%	76-80%
Stripping	HCl hydrolyz	ed feather meal
Pfeffer et al. (1995)	83%	81%

Poultry By-Products Meal

	ADC	
Guelph System	Protein	Energy
Cho et al. (1982)	68%	71%
Hajen et al. (1993)	74-85%	65-72%
Sugiura et al. (1998)	96%	N/A
Bureau et al. (1999)	87-91%	77-92%

ISSUES (in order of importance)

- 1. Aquatic System Fecal Collection System
- 2. Experimental Design Experimental Dietary Design
 - 1. Focus on individual ingredient
 - 2. Focus on complete feed
- 3. Chemical Analyses
 - 1. Digestion indicator analysis
 - 2. Proximate, energy and chemical analysis
- 4. Digestibility Equations Mathematical & statistical issues
- 5. Factors

- 1. Batch variability for ingredients
- 2. Environmental factors
- 3. Species and lifestages differences

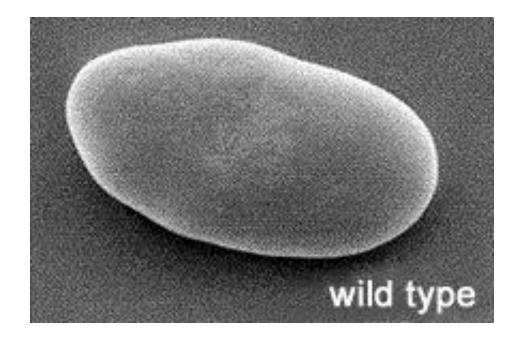
Factors Affecting Digestibility of Nutrients?

Processing / Chemical Damage

Digestibility of Starches from Various Botanical Origins

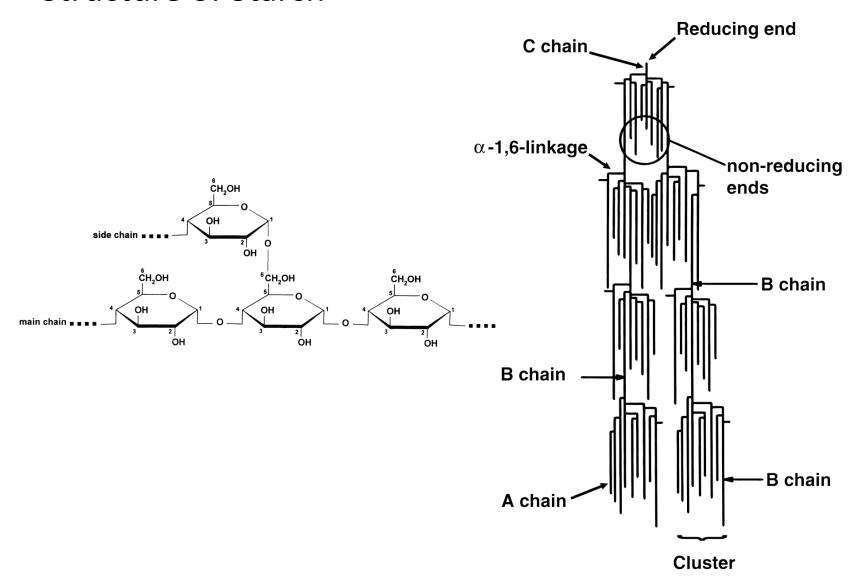
Starch type (at 30% of diet)	ADC starch (%)	
Corn, raw	33	
Corn, raw (65% amylose)	19	
Corn, "Waxy", raw (99% amylopectin)	54	
Corn, extruded	96	
Corn, gelatinized	96	
Wheat	54	
Rice	39	
Manioc	16	
Potato	3	

Starch Granule from a Pea Seed



http://www.jic.bbsrc.ac.uk/staff/cliff-hedley/Starch.htm

Structure of Starch



http://www.jic.bbsrc.ac.uk/staff/cliff-hedley/Starch.htm

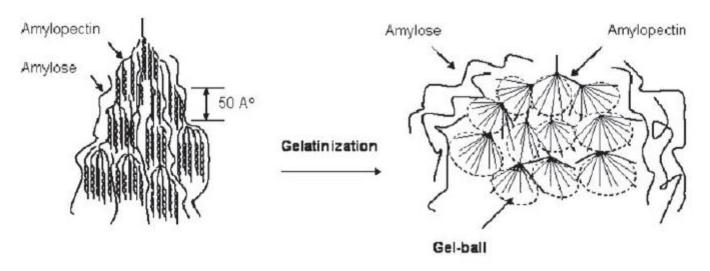


Fig. 5. Schematic representation of microstructure and phase transition of starch during gelatinization.





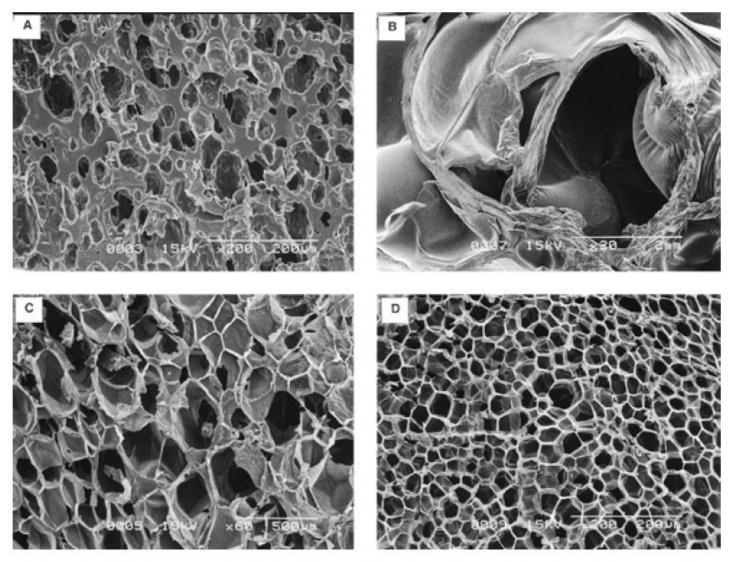


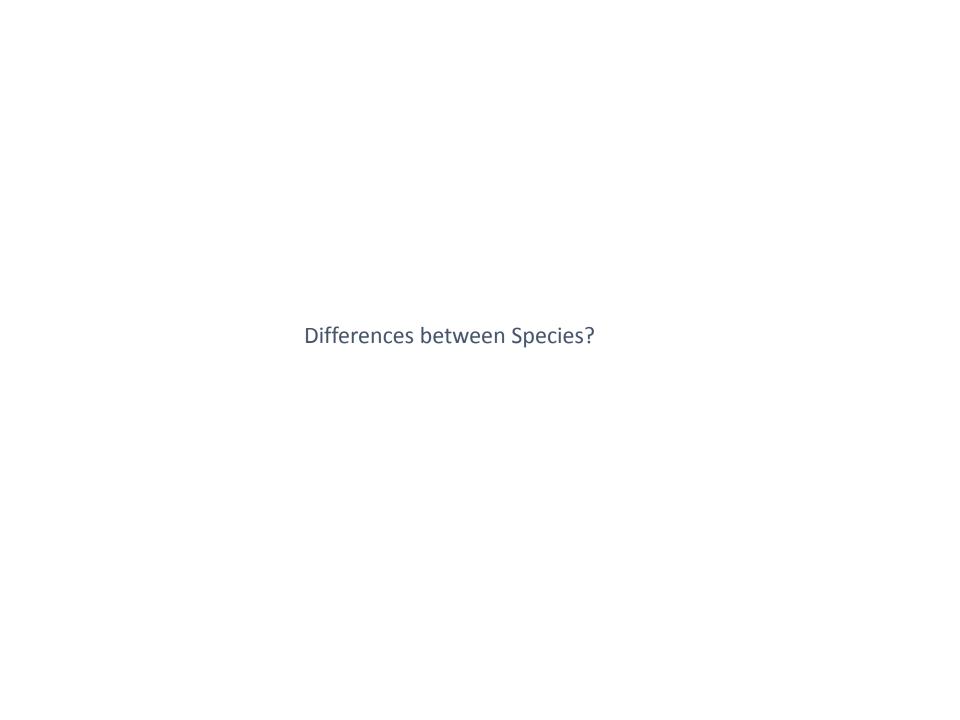
Fig. 3. Scanning electron micrographs of cross-sections of microwave-puffed products at different temperatures and water feeding rates: A, 70°C and 42 g/min (×200); B, 110°C and 42 g/min (×20); C, 90°C and 63 g/min (×200); D, commercial popcorn (×200).

Blood Meal

	AD	ADC		
Guelph System	Protein	Energy		
Spray-dried	96-99%	92-99%		
Ring-dried	85-88%	86-88%		
Steam-tube dried	84%	79%		
Rotoplate dried	82%	82%		
<u>†</u>	_	4 (4000)		

Different drying technique

Bureau et al. (1999)



Blood Meal

	AD	ADC		
Guelph System	Protein	Energy		
Spray-dried	96-99%	92-99%		
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Different drying technique

Bureau et al. (1999)

Feather Meal



75-85% Crude Protein

Rich in:

- Arginine (5.8%)
- Cystine (3.8%)
- Threonine (3.9%)

Poor in:

- Lysine: (1.8%)
- Histidine: (0.7%)
- Tryptophan: (0.55%)

High variability in nutritional value!!!

Great Variability in Digestibility of Feather Meals from Various Origins by Rainbow Trout

Author	ADC		
_	DM	СР	GE
		(%)	
Cho et al. (1982)	75	58	70
Cho and Kaushik (1990)	81	77	77
Bureau et al. (1999)	79	81	76
Bureau et al. (1999)	80	81	80
Bureau et al. (1999)	82	81	83
Bureau et al. (1999)	84	87	80
Cheng et al. (2004)	80	77	77
Gaylord et al. (2008)	-	87	88

Variability of raw materials







Variability processing equipment

Batch Pressure Cooker



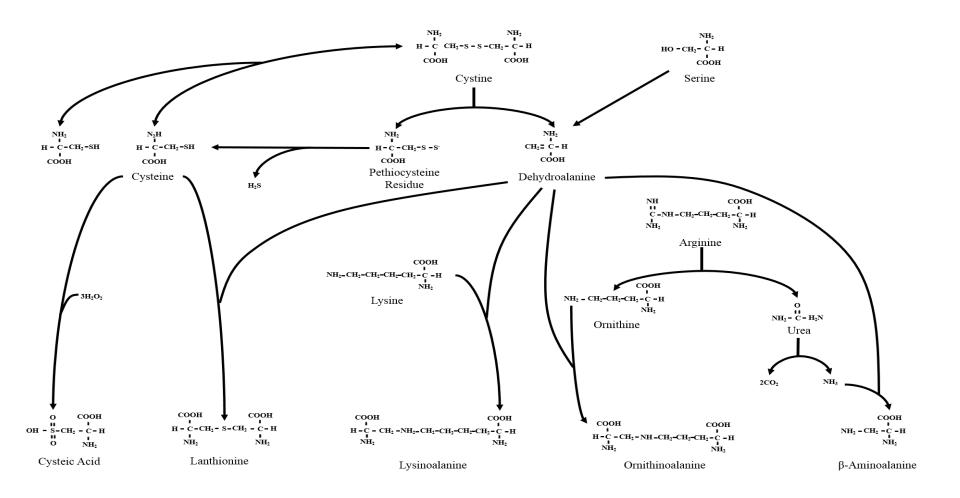
Ring dryer with full manifold for vital wheat gluten

Variability of the processing conditions affects available amino acid content and level of cross-linked amino acid of no nutritive value.

	Pressure (kPa)				
Amino acids	207	310	414	517	SEM
Methionine	0.43	0.37	0.39	0.31	0.02*
Cystine	3.99	2.44	2.21	1.48	0.24**
Lysine	1.37	1.08	1.02	0.93	0.08**
Threonine	3.54	3.16	3.22	2.97	0.06**
Arginine	5.46	4.99	5.31	5.00	0.13
Valine	5.98	5.70	5.92	5.48	0.11*
Isoleucine	4.07	3.88	4.07	3.80	0.05*
Leucine	6.62	6.28	6.60	6.13	0.10*
Aspartate	4.20	3.08	3.25	2.95	0.19**
Glutamate	7.82	6.66	6.86	6.34	0.21**
Serine	9.30	8.37	8.73	8.05	0.17**
Lanthionine	0.33	0.72	0.90	0.96	0.03**
TA Cys equivalent ¹	4.53	3.16	3.05	2.29	0.26**
TME	3.51	2.97	3.03	2.95	0.11*

Moritz and Latshaw (2001)

Formation of Cross-Linked Amino Acids



Disulfide Bonds

Very stable (heat) & indigestible

Cys-Cys (Cystine)

Certain natural proteins, such as keratins and lysozymes, contain many disulfide bonds

Raw feather and hair (>90% keratins) Apparent digestibility coefficient = 0%

(Steam hydrolyzed, pressure cooked)

Feather treated with keratinase Apparent digestibility coefficient > 70% (enzyme-treated)

Moist heat + pressure break disulfide bonds

Overheated proteins (dried at high temperature) = creation of disulfide bonds

Flame-dried (drum) blood meal Spray-dried blood meal

Apparent digestibility coefficient = 16% Apparent digestibility coefficient = 99%

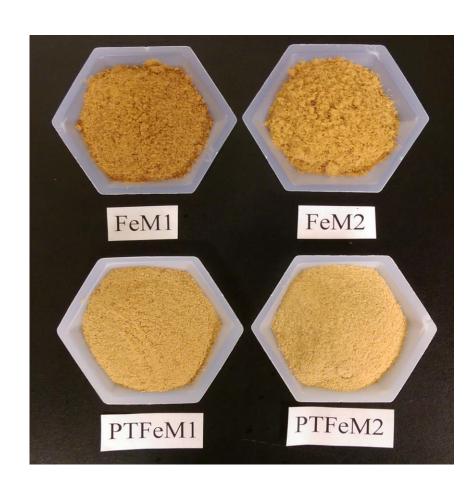
Slope-Ratio assay to assess the bioavailability of PTFEMs

Processing of two feather meals

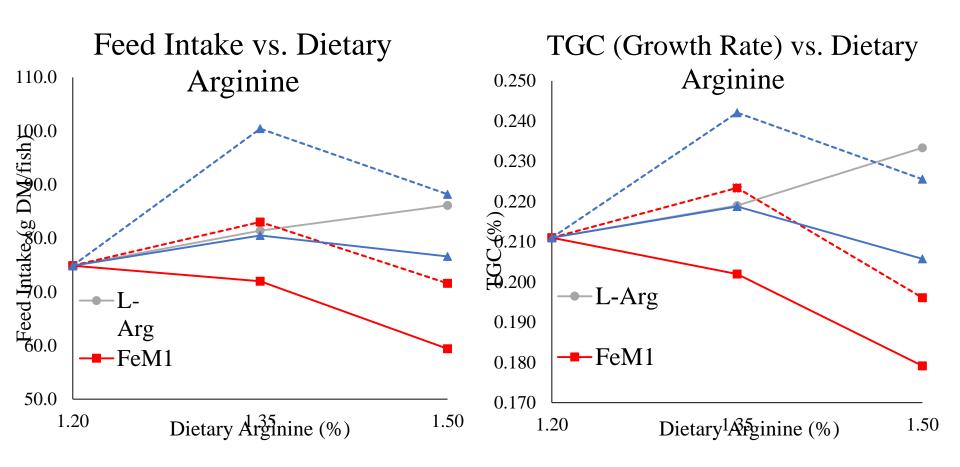
- 2% sodium sulfite
- 0.05% bacterial enzyme
- 2:1 water:FeM ratio
- 24h incubation

Slope-ratio assay carried out using the protocol of Poppi et al 2010.

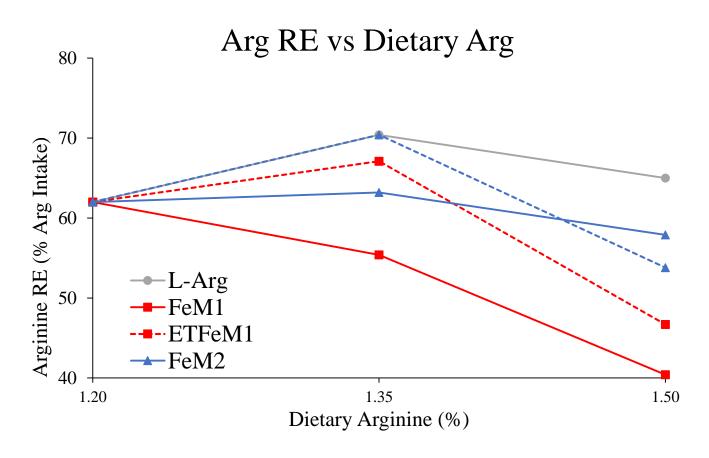
- 12 diets
- 1 basal diet deficient in arginine (1.2%)
- 10 diets were formulated to contain 1.35% or 1.5% arginine by adding increasing amounts of L-Arg, FeMs, or PTFeMs
- 1 Control diet with fish meal (20%)



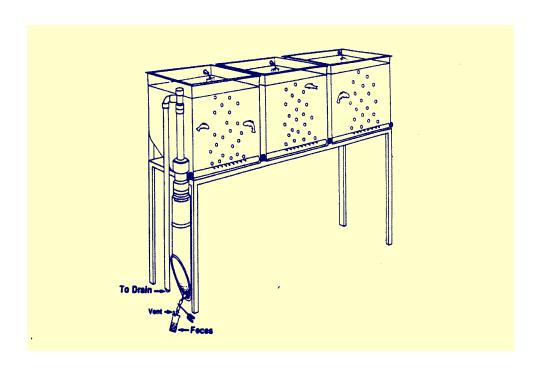
Results of slope-ratio assay



Results of slope-ratio assay



Assessing the apparent digestibility coefficient of the 12 diets



$$ADC_{ingr} = ADC_{test} + ((1-s)D_{ref}/sD_{ingr}) (ADC_{test}-ADC_{ref})$$

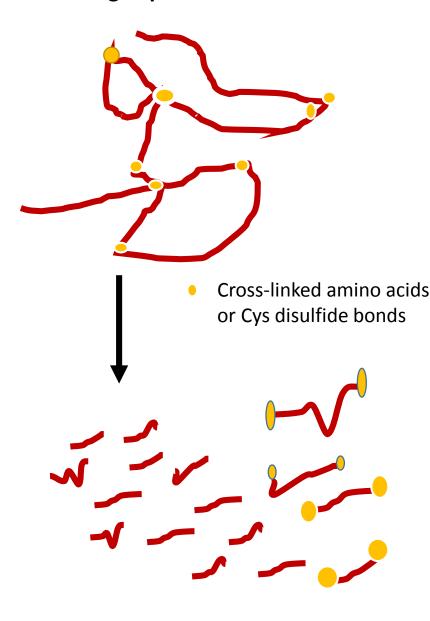
Results of Digestibility Trial

ADC - (- 1 - 1 - 1 - 1 - 1		l
ADC of nutrients,	gross energy and	i arginine
, LD C OI II G CI I CI I CO,	Aloos chicky and	

	Source	DM	CP	GE	Arg
		%	%	%	%
1.2% Arginine					
Diet 1	-	77.3	93.9	81.6	94.3
1.35% Arginine					
Diet 2	L-Arg	77.3	93.7	81.7	95.1
Diet 4	FeM1	74.1	91.4	77.0	90.5
Diet 6	PTFeM1	78.5	94.6	82.1	94.8
Diet 8	FeM2	74.4	90.8	78.5	87.1
Diet 10	PTFeM2	78.8	94.6	82.7	93.3
1.5% Arginine					
Diet 3	L-Arg	78.3	94.2	82.4	95.3
Diet 5	FeM1	74.4	89.6	77.9	83.7
Diet 7	PTFeM1	74.8	92.0	78.2	91.7
Diet 9	FeM2	75.2	88.2	78.5	80.9
Diet 11	PTFeM2	76.6	93.5	80.6	94.3
Diet 12	FM	69.1	86.9	75.4	85.2

Native, undamaged protein

Damaged protein



Educational Module #2 Nutritional Specifications (1h)

Nutritional specifications – How they are developed, adjusted, updated

Meeting essential fatty acids and minor lipids requirements

Effectively meeting phosphorus requirement

Nutritional Specifications

- Nutritional specifications are guidelines. The are defined carefully, reviewed occasionally, and generally quite strictly followed by feed formulators to ensure consistency of nutritional quality of feeds
- Nutrient restrictions are "practical" values taking into account :
 - Requirements of the animal
 - Production objectives
 - Ex: Minimizing cost of formula while obtaining maximum performance
 - Uncertainties
 - Ex: Uncertainties around estimate of nutritional composition, nutritional requirements or potential losses of nutrients requiring use of certain safety margin

Ingredient Restrictions

- Generally driven by practical considerations and "gaps" in knowledge
- Considerations:
 - Effect on processing (handling limitations, effect on pellet quality, etc.)
 - Chemical and/or nutritional characteristics not easily or not adequately addressed through the current nutritional specifications
 - Logistical, risk management and market issues (limited availability, contamination, variability, final product characteristics, customer concerns, export regulations, etc.)
- In general, the more we characterize the animals and the ingredients, the less important the ingredient specifications.
 However, some logistical considerations still always play a role

Specifications are sometime highly related / redundant but the formulation program can't deal with this

Least Cost Feed Formulation = Linear Programming

Program solving a series of linear (additive) equations to achieve a certain objective (i.e. minimize cost)

Solving dozens of independent equations until all equations are "true"

No real linkage / feedback loop between equations

Some nutritional specifications are interrelated but the program doesn't know this.

Digestible Lysine content >= 2.4%
Digestible Methionine content >= 0.7%
Digestible TSAA content > = 1.1%
A-Linolenic Acid Content > = 1.0%
Total n-3 fatty acid content > = 1.0% ←
EPA content >= 0.2%
DHA content >= 0.4%
EPA+DHA Content >= 0.6%
Total Phosphorus content
Digestible Phosphorus content

Adequately and Cost-Effectively Meeting Requirements

Key Strategies:

1- Determining nutrient requirements across life stages

Effective approach: Fine characterization of nutrient requirements

Research trials / review of literature

Use of nutritional models

2- Cost-effectively meeting nutrient requirements

Effective approach: Fine chemical characterization of ingredients

Digestibility trials, in vitro lab analysis

Use nutritional models (digestible nutrients)

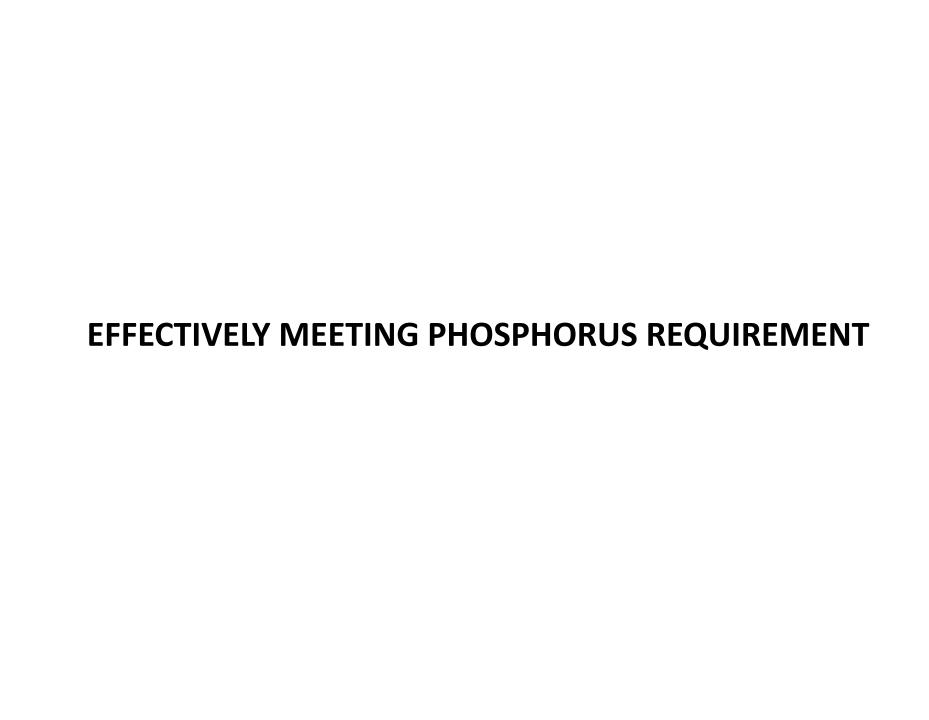
Use additives and processing techniques

3- Verifying if predictions correspond to commercial reality

Effective approach: Benchmarking / production modeling

Investment in Research & Development (R&D)

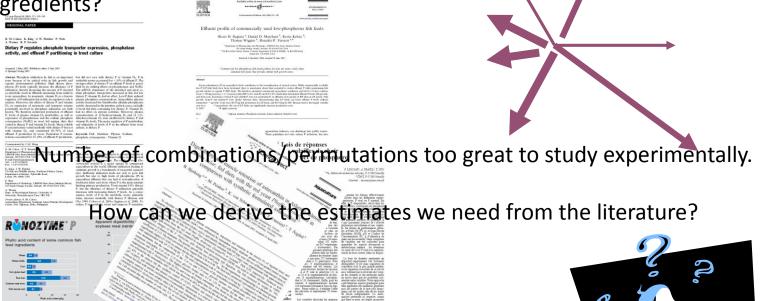
Never be satisfied with status quo



Challenge:

Predicting digestible nutrient (e.g. lipids, phosphorus) contents of balanced feeds formulated to widely different digestible nutrient levels and made with a great variety





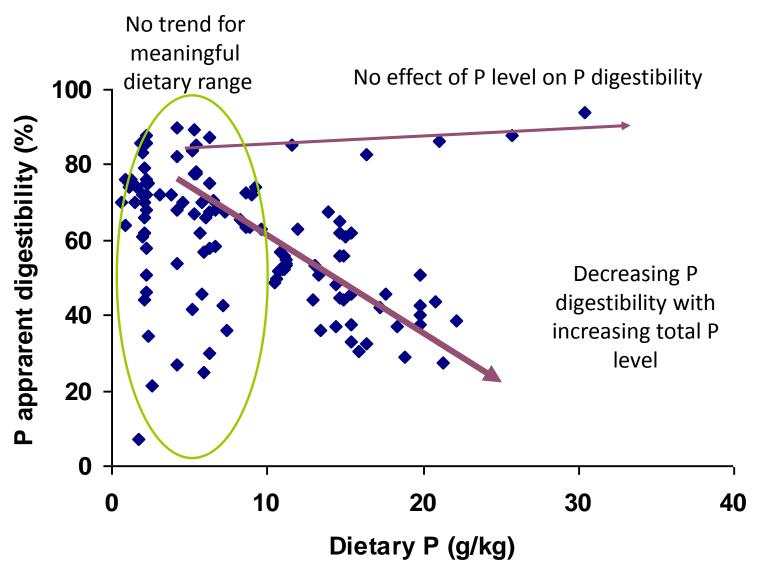
1. y acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis growing juveniles of gilthead sea bream (Sparus aurata L.): J.H. Robin a.*, C. Regosta, J. Arzela, S.J. Kaushikh



It is not sufficient to know different factors have effects. You also need to be able to quantify the combined effects of these different factors

etworking of systemic and local components of GH/IGF axis

Example: Dietary Phosphorus Digestibility



Dataset: 137 treatments from 22 studies with rainbow trout

The answer is organizing the information at hand in a sensible way!

Modelling can be a very effective way of achieving this.



Before After

P Content of Common Fish Feed Ingredients

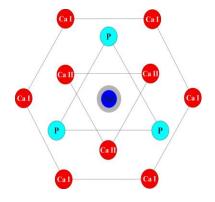
Ingredients	P content (%)
Fish meal	1.08 - 4.19
Meat and bone meal	2.49 - 7.08
Poultry by-product meal	1.65 - 3.45
Blood meal	0.08 - 1.71
Feather meal	0.54 - 1.26
Corn gluten meal	0.44 - 0.55
Soybean meal	0.64 - 0.85
Wheat middling	0.97 - 1.17

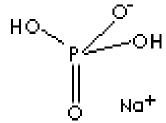
Summarized from various sources in literature

P Forms Present in Feed

1. Inorganic P

- Bone P: hydroxyapatite Ca₁₀(OH)₂(PO₄)₆
- Pi supplement:
 - Monobasic: NaH₂PO₄, Ca(H₂PO₄)₂
 - Dibasic: CaHPO₄



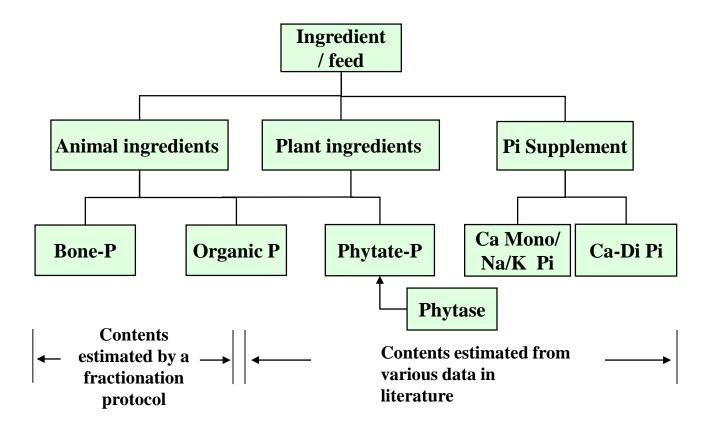


P Forms Present in Feed

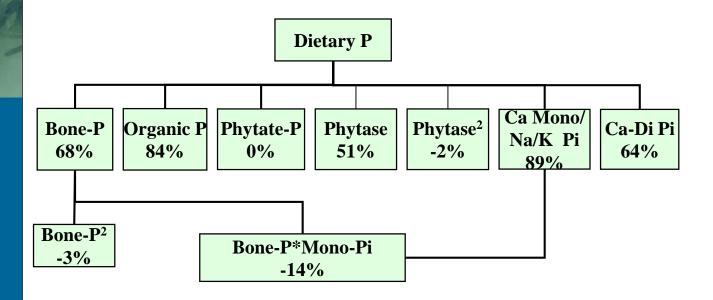
2. Organic P

- Phospholipids, e.g. phosphatidyl choline
- Phosphoproteins, e.g. casein
- Phosphosugars, e.g. Glucose-6-P
- Phytate: account for 60 80% of total P in plant ingredients

Classification and Content of P Compounds

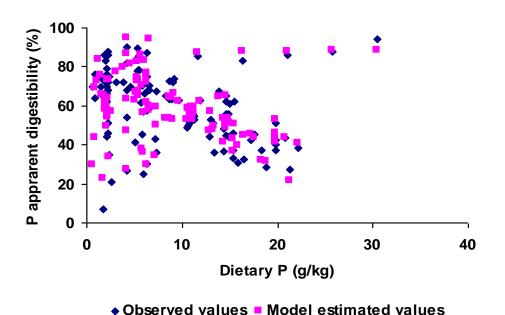


Results: Parameter Estimates From Multiple Regression



P Digestibility Model

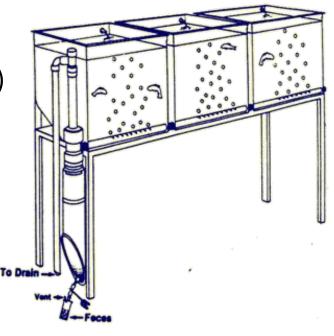
• The model explained 96% of the variance of the data and well described the observations of the dataset



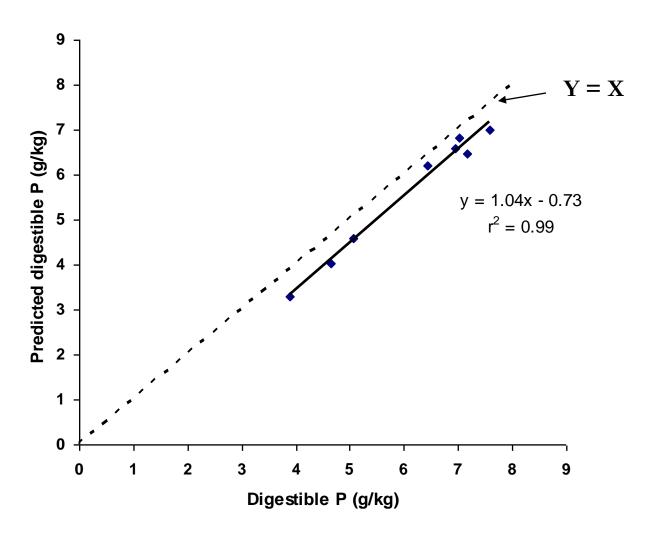
Experimental Validation by Digestibility Trial

 Digestibility trial conducted with the Guelph system using the protocol of Cho et al. (1982)

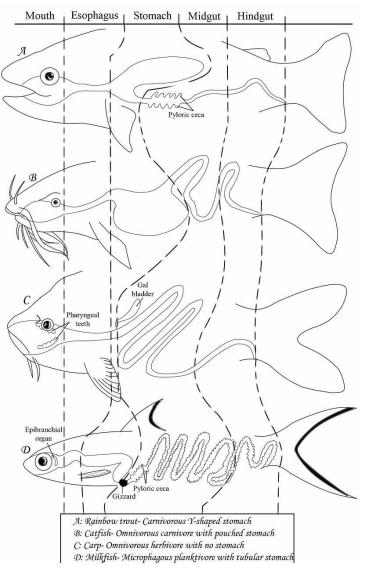
- Reference diet:
 - Fish meal/corn gluten meal-based diet
- Test diets:
 - 2 fish meals (high vs. low ash)
 - 1 meat and bone meal
 - 2 poultry by-products meals (high vs. low ash)
 - 2 soy protein concentrates (regular vs. dephytinized)



Results of Experimental Validation



Differences between fish species in terms of mineral digestibility?

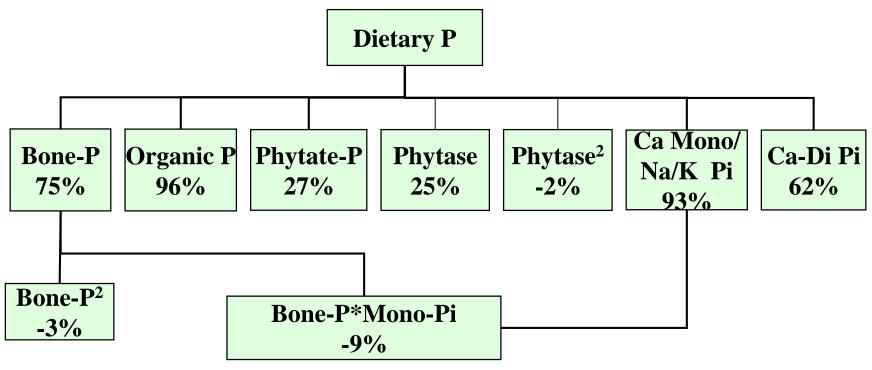


Short GI tract

Effect of absence of true stomach?

Effect of very long and/or very acid GI tract?

P Digestibility Model for Tilapia



```
Digestible P = 0.75 bone-P

+ 0.27 phytate-P

+0.95 organic P

+0.93 Ca monobasic /Na/ K Pi supplement

+0.62 Ca dibasic Pi supplement

+0.25 phytase/phytate

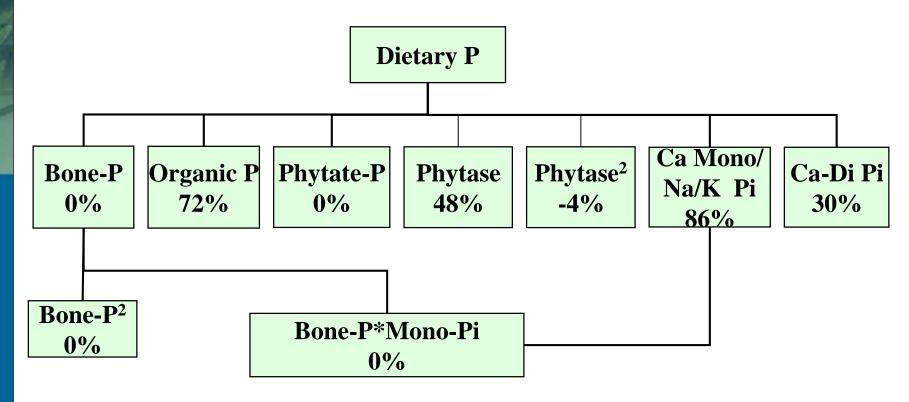
- 0.02 (phytase/phytate)<sup>2</sup>

- 0.03 (bone-P)<sup>2</sup>

- 0.09 bone-P

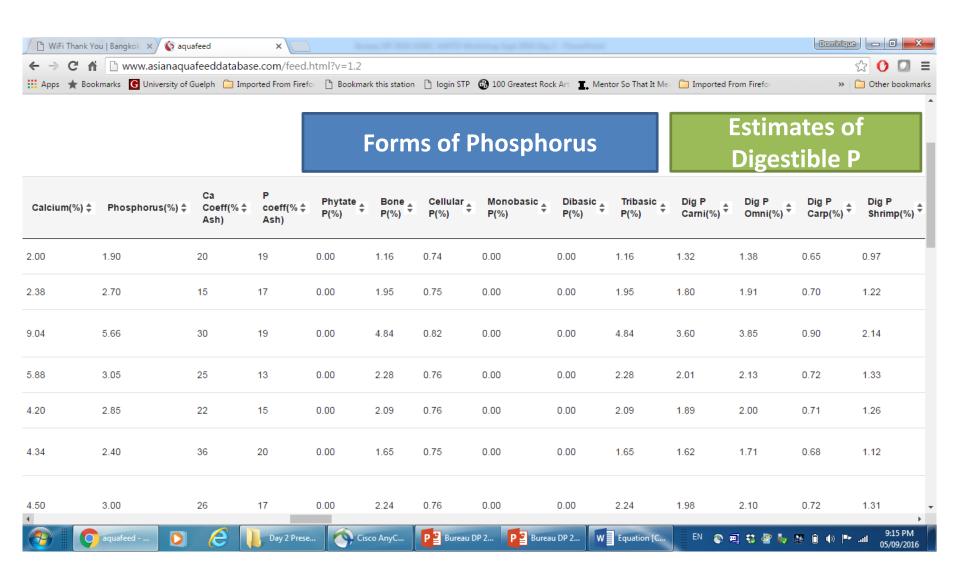
× *Ca monobasic /Na/ K Pi supplement
```

P Digestibility Model for Common carp



```
Digestible P = 0 bone – P + 0 phytate – P + 0.72 organic P
+ 0.86 Ca monobasic /Na/ K Pi supplement
+ 0.30 Ca dibasic Pi supplement
+ 0.48 phytase/phytate – 0.04 (phytase/phytate)<sup>2</sup>
```

Forms of Dietary P and Estimation of Digestible P



Equations in AAFFD and BestMix

- 'Digestible Phosphorus calculation, Aqua
- Nutrients.Minerals.Dig P Carni = Nutrients.Minerals.Bone P * 68/100 +Nutrients.Minerals.Cellular P * 84/100 + Nutrients.Minerals.Monobasic P * 89/100 + Nutrients.Minerals.Dibasic P * 64/100
- Nutrients.Minerals.Dig P Omni = Nutrients.Minerals.Cellular P * 72/100 + Nutrients.Minerals.Monobasic P * 86/100 + Nutrients.Minerals.Dibasic P * 30/100
- Nutrients.Minerals.Dig P Carp = Nutrients.Minerals.Bone P * 75/100 +Nutrients.Minerals.Cellular P * 95/100 + Nutrients.Minerals.Monobasic P * 90/100 + Nutrients.Minerals.Dibasic P * 62/100
- Nutrients.Minerals.Dig P Shrimp = Nutrients.Minerals.Bone P * 70/100 +Nutrients.Minerals.Cellular P * 85/100 + Nutrients.Minerals.Monobasic P * 85/100 + Nutrients.Minerals.Dibasic P * 60/100

Educational Module #2 Nutritional Specifications (1h)

Nutritional specifications – How they are developed, adjusted, updated

Meeting essential fatty acids and minor lipids requirements

Effectively meeting phosphorus requirement

Outstanding Issue – Independent recommendations that are interrelated

Least Cost Feed Formulation = Linear Programming

Program solving a series of linear (additive) equations to achieve a certain objective (i.e. minimize cost)

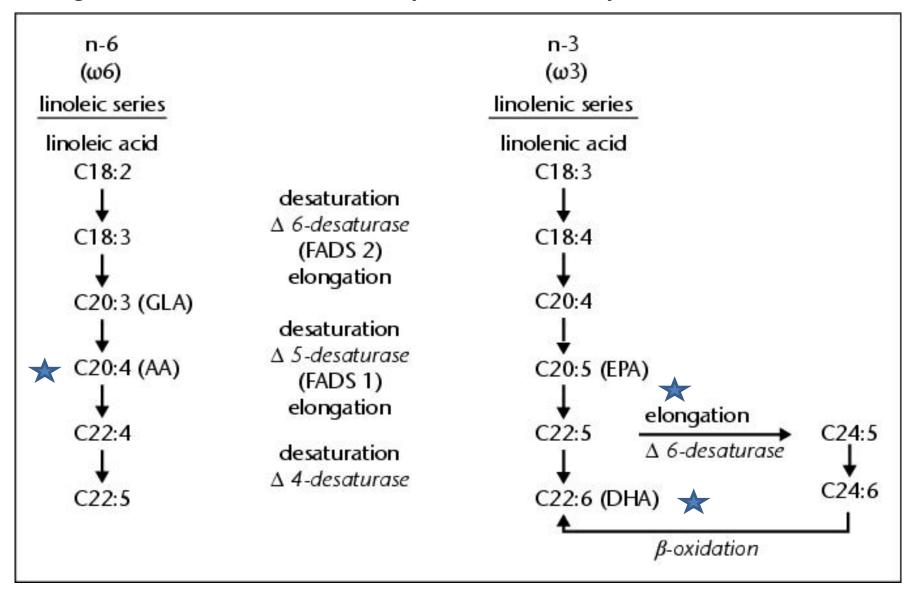
Solving dozens of independent equations until all equations are "true"

No real linkage / feedback loop between equations

Some nutritional specifications are interrelated but the program doesn't know this.

Digestible Lysine content >= 2.4%
Digestible Methionine content >= 0.7%
Digestible TSAA content > = 1.1%
A-Linolenic Acid Content > = 1.0%
Total n-3 fatty acid content > = 1.0%
EPA content >= 0.2%
DHA content >= 0.4%
EPA+DHA Content >= 0.6%
Total Phosphorus content
Digestible Phosphorus content

Elongation and Desaturation of Polyunsaturated Fatty Acids



Determining What Species Needs What and How Much?

- A little more complicated than for other nutrients
 - Synthesis / bioconversion plays an important role but efficiency of conversion depends on species and life stages
 - ALA (18:3 n-3) = precursor of 20:5 n-3 and 22:6 n-3
 - LA (18:2 n-6) = precursor of 20:4 n-6
 - Substitution issues = "physically" and metabolically one fatty acid can partly replace another one
 - Deficiency is thus not always very overtly seen
 - Metabolic needs can be very small (ng) and body reserve large (mg or g)

TABLE 6-1 Reported Quantitative Essential Fatty Acid (EFA) Requirements of Juvenile and Subadult Freshwater and Diadromous Species of Finfish^a

Species	Scientific Name	EFA	Requirement (% Dry Diet)	Reference
Arctic charr	Salvelinus alpinus	18:3n-3	1.0-2.0	Yang et al. (1994)
Atlantic salmon	Salmo salar	18:3n-3 n-3 LC-PUFA	1.0 0.5–1.0	Ruyter et al. (2000a) Ruyter et al. (2000b)
Ayu	Plecoglossus altivelus	18:3n-3 or EPA	1.0	Kanazawa et al. (1982)
Channel catfish	Ictalurus punctatus	18:3n-3	1.0-2.0	Satoh et al. (1989)
Cherry salmon	Oncorhynchus masou	18:3n-3 or n-3 LC-PUFA	1.0	Thongrod et al. (1990)
Chum salmon	Oncorhynchus keta	18:2n-6 and 18:3n-3	1.0 of each	Takeuchi et al. (1979)
Coho salmon	Oncorhynchus kisutch	18:2n-6 and 18:3n-3	1.0 of each	Yu and Sinnhuber (1979)
Common carp	Cyprinus carpio	18:2n-6 18:3n-3	1.0 0.5–1.0	Takeuchi and Watanabe (1977) Takeuchi and Watanabe (1977)
Grass carp	Ctenopharyngodon idella	18:2n-6 18:3n-3	1.0° 0.5	Takeuchi et al. (1991) Takeuchi et al. (1991)
Japanese eel	Anguilla japonicus	18:2n-6 and 18:3n-3	0.5 of each	Takeuchi et al. (1980)
Milkfish	Chanos chanos	18:2n-6 and 18:3n-3	0.5 of each	Bautista and de la Cruz (1988)
Rainbow trout	Oncorhynchus mykiss	18:3n-3 n-3 LC-PUFA	0.7-1.0 0.4-0.5	Castell et al. (1972) Takeuchi and Watanabe (1976)
Sheatfish	Silurus glanis	18:3n-3	1.0	Borgut et al. (1998)
Striped bass	Morone chrysop × Morone saxatilis	n-3 LC-PUFA	1.0	Gatlin et al. (1994)
Tilapia	Tilapia zilli Oreochromis nilotica Oreochromis niloticus × Oreochromis aureus	18:2n-6 18:2n-6 n-3 required	1.0 0.5 ?	Kanazawa et al. (1980) Takeuchi et al. (1983) Chou and Shiau (1999)
Whitefish	Coregonus laveratus	18:3n-3 n-3 LC-PUFA	> 1.0 0.5-1.0	Thongrod et al. (1989) Watanabe et al. (1989)

[&]quot;Based on Tocher (2010).

TABLE 6-3 Reported Quantitative Essential Fatty Acid (EFA) Requirements of Juvenile and Subadult Marine Species of Finfish^a

Species	Scientific Name	EFA	Requirement (% Dry Diet)	Reference
European sea bass	Dicentrarchus labrax	n-3 LC-PUFA	1.0	Coutteau et al. (1996a)
Gilthead sea bream	Sparus aurata	n-3 LC-PUFA n-3 LC-PUFA DHA:EPA	0.9 (DHA:EPA = 1) 1.9 (DHA:EPA = 0.5) 0.5	Kalegeropoulos et al. (1992) Ibeas et al. (1994) Ibeas et al. (1997)
Grouper	Epinephelus malabaricus	n-3 LC-PUFA, DHA > EPA	1.0	Wu et al. (2002)
Japanese flounder	Paralicthys olivaceus	n-3 LC-PUFA	1.4	Takeuchi (1997)
Korean rockfish	Sebastes schlegeli	n-3 LC-PUFA EPA or DHA	0.9	Lee et al. (1993) Lee et al. (1994)
Red drum	Sciaenops ocellatus	n-3 LC-PUFA EPA + DHA	0.5-1.0 0.3-0.6	Lochman and Gatlin (1993) Lochman and Gatlin (1993)
Red sea bream	Pagrus major	n-3 LC-PUFA or EPA EPA DHA	0.5 1 0.5	Yone (1978) Takeuchi et al. (1990) Takeuchi et al. (1990)
Silver bream	Rhabdosargus sarba	n-3 LC-PUFA	1.3	Leu et al. (1994)
Starry flounder	Paralichthys stellatus	n-3 LC-PUFA	0.9	Lee et al. (2003)
Striped bass	Morone chrysop × Morone saxatilis	n-3 LC-PUFA	1.0	Gatlin et al. (1994)
Striped jack	Pseudocaranx dentex	DHA	1.7	Takeuchi et al. (1992c)
Turbot	Psetta maxima	n-3 LC-PUFA ARA	0.8 - 0.3	Gatesoupe et al. (1977) Castell et al. (1994)
Yellowtail flounder	Pleuronectes ferrugineus	n-3 LC-PUFA	2.5	Whalen et al. (1999)
Yellowtail/Kingfish	Seriola spp.	n-3 LC-PUFA	2.0-2.4	Deshimaru et al. (1982)

[&]quot;Based on Tocher (2010).

A-Linolenic Acid Content > = 1.0%

Total n-3 fatty acid content > = 1.0%

EPA content >= 0.2%

DHA content >= 0.4%

EPA+DHA Content >= 0.6%

Evidence that for some species DHA is the essential fatty acid and that EPA doesn't have to same efficacy.

Table 1
Requirement of docosahexaenoic acid (DHA) and its efficacy in comparison to eicosabentaenoic acid (EPA) for larval marine fish fed on enriched *Artemia*

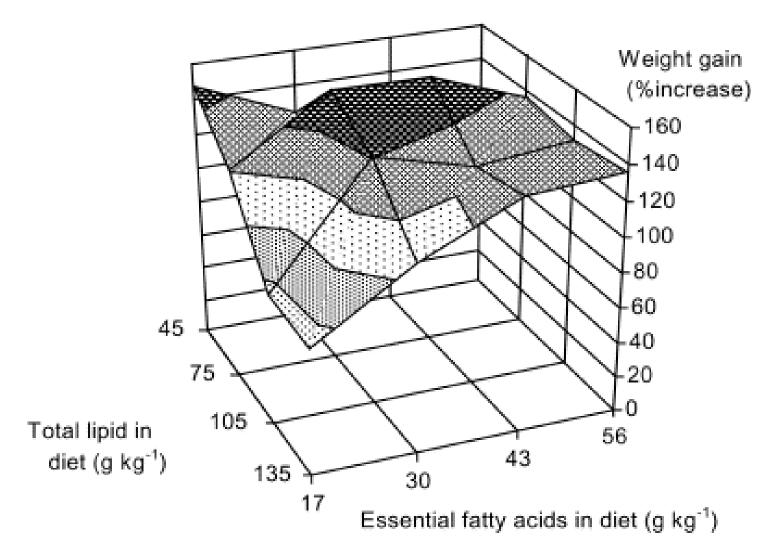
Species	Requirement of	Relative efficacy	Relative efficacy		
	DHA (%)	Growth	Survival rate	Vitality test ^a	
Japanese flounder	1.0-1.6	EPA = DHA	EPA = DHA	$EPA \leq DHA^b$	
Red sea bream	1.0-1.6	EPA = DHA	EPA = DHA	EPA < DHA	
Cod	1.6-2.1	$EPA \leq DHA$	$EPA \leq DHA$	EPA < DHA	
Striped jack	1.6-2.2	$EPA \leq DHA$	EPA < DHA	EPA < DHA	
Yellowtail	1.4-2.6	EPA < DHA	EPA < DHA	EPA < DHA	

^aSurvival at the 24th h after fish were held in air for 30–60 s by a scoop net and moved to a culture tank.

This is a lot more informative and accurate than "fish oil replacement value"

^bSalinity tolerance test (65% for 120 min) was employed for flounder.

Combined Response of Shrimp to Dietary Lipid and Essential Fatty Acid Contents

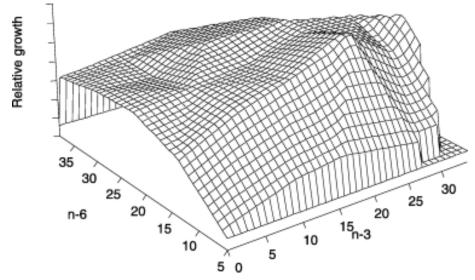


B. D. Glencross, D. M. Smith, M. R. Thomas and K. C. Williams. 2002. Optimising the essential fatty acids in the diet for weight gain of the prawn, *Penaeus monodon*. *Aquaculture 204*, 85-99.

GLENCROSS, D.M. SMITH, M.R. THOMAS & K.C. WILLIAMS. 2002.

The effect of dietary n-3 and n-6 fatty acid balance on the growth of the prawn *Penaeus monodon* B. Aquaculture Nutrition 8, 43

Dietary n-3 and n-6 fatty acid balance



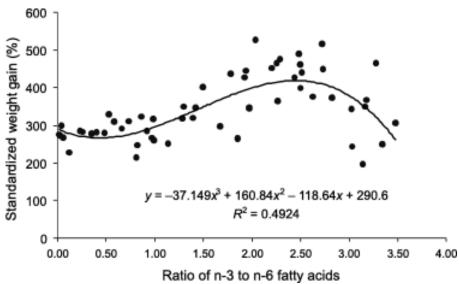
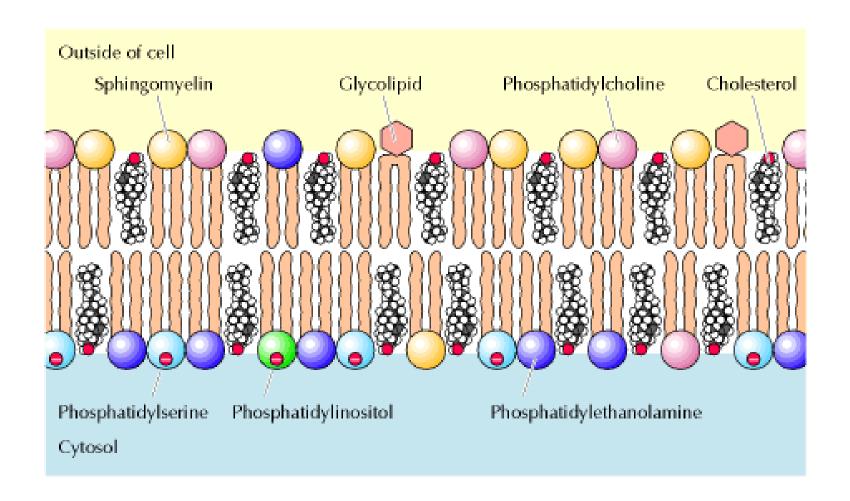


TABLE 6-5 Reported Phospholipid Requirements in Juvenile and Larval Shrimp Species

Species	Requirement	Reference
Tiger shrimp (Penaeus monodon [juvenile])	1.0-1.5%	Paibulkichakui et al. (1998)
P. monodon	80% pure soybean PC	Chen (1993)
Marine shrimp (Penaeus penicillatus)	80% pure soybean PC	Chen and Jenn (1991)
Pacific white shrimp (Litopenaeus vannamei)	1.5% PC (from soybean) 6.5% deoiled soybean lecithin	Coutteau et al. (1996b)
Kuruma prawn (Marsupenaeus japonicus [juvenile])	1.0% (PC + PE)	Kanazawa et al. (1979c)
M. japonicus (juvenile)	3.0% soybean (lecithin) PE and PI	Teshima et al. (1986a,b)
M. japonicus (larvae)	3.0% soybean lecithin	Kanazawa (1983)
M. japonicus (larvae)	0.5 to 1.0% (PC and Pl)	Kanazawa et al. (1985)
Banana shrimp (Fenneropenaeus merguiensis)	2.5% pure soybean lecithin	Thongrod and Boonyaratpalin (1998)

TABLE 6-6 Reported Quantitative and Qualitative Phospholipid Requirements of Finfisha

Species	Developmental Stage	Phospholipid Supplement ^h and Levels Studied ^c	Optimal Requirement and Criteria Used ^d	Feeding Period	Reference
Atlantic salmon	Juvenile (180 mg)	0, 2, 4, 6, and 8% SL/CPL	6% (G)	14 weeks	Poston (1991)
(Salmo salar)	Juvenile (180 mg)	0 and 4% SL	4% (G)	16 weeks	Poston (1990b)
	Juvenile (1.0 g)	0 and 4% SL	4% (G)	12 weeks	Poston (1990b)
	Juvenile (1.7 g)	0 and 4% SL	4% (G)	12 weeks	Poston (1990b)
	Juvenile (7.5 g)	0 and 4% SL	0% (no requirement)	12 weeks	Poston (1990b)
Ayu	Larvae	0 and 3% SL or EL	3% (G,S,M)	20 days	Kanazawa et al. (1981)
(Plecoglossus altivelus)	Larvae	0, 1, 3, and 5% SL	3% (M), 5% (G,S)	50 days	Kanazawa et al. (1983a)
C	Larvae	0 and 3% EL or BPL	3% (G,S,M)	50 days	Kanazawa et al. (1983a)
	Juvenile	0 and 3% SL or BPL	3% (G)	33 days	Kanazawa et al. (1981)
	Juvenile	0, 1, 3, and 5% EL.	3% (G)	33 days	Kanazawa et al. (1981)
Common carp	Larvae	0 and 2% EL	2% (G,S)	25 days	Geurden et al. (1995a)
(Cyprinus carpio)	Larvae	0 and 2% PL	2% (G,S)	21 days	Geurden et al. (1995a)
,	Larvae	0 and 2% SPC, SPI, or EL	2% (G,S,M except EL)	25 days	Geurden et al. (1997a)
European sea bass	Larvae	3, 6, 9, and 12% SL	12% (G,S,M)	40 days	Cahu et al. (2003)
(Dicentrarchus labrax)	Juvenile	0 and 3% SL	3% (G)	40 days	Geurden et al. (1995b)
	Juvenile	0 and 2% EPC or SPC	2% (G)	40 days	Geurden et al. (1995b)
Gilthead sea bream (Sparus aurata)	Larvae	9.11, and 15% SL	> 9% (G,S)	23 days	Seiliez et al. (2006)
Japanese flounder	Larvae	0, 3, 5, and 7% SL	7% (G,S)	30 days	Kanazawa (1993)
(Paralichthys olivaceus)	Juvenile	0, 3, 5, and 7% SL	7% (G)	30 days	Kanazawa (1993)



Source: Cooper, G.M. 2000.The Cell: A Molecular Approach. 2nd Ed. Sinaeur Associate Inc., Sunderland, Mass. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=cooper

TABLE 6-7 Reported Cholesterol/Sterol Requirements of Shrimp and Other Crustaceans

Species	Requirement	Reference	
Cholesterol alone			
Pacific white shrimp (Litopenaeus vannamei)	0.35%	Gong et al. (2000)	
Kuruma prawn (Marsupenaeus japonicus)	0.50%	Kanazawa et al. (1971)	
M. japonicus (larval)	1.00%	Teshima et al. (1983)	
M. japonicus	0.20%	Shudo et al. (1971)	
M. japonicus	2.00%	Deshimaru and Kuroki (1974)	
American lobster (Homarus americanus)	0.50%	Kean et al. (1985)	
H. americanus	0.50%	Castell et al. (1975)	
Signal crayfish (Pacifastacus leniusculus)	0.40%	D'Abramo et al. (1985b)	
Cholesterol + phospholipid			
L vannamei	0.14% (1.5% deoiled soybean lecithin)	Gong et al. (2000)	
L vannamei	0.13% (3.0% deoiled soybean lecithin)	Gong et al. (2000)	
H. americanus	(8% soybean lecithin)	D'Abramo et al. (1985a)	

Take Home Message

Freshwater fish:

Require either n-3 or n-6 fatty acids

(probably all fish require both types)

Elongate & desaturate shorter chain fatty acids but requirement= 5 to 10x

Marine fish:

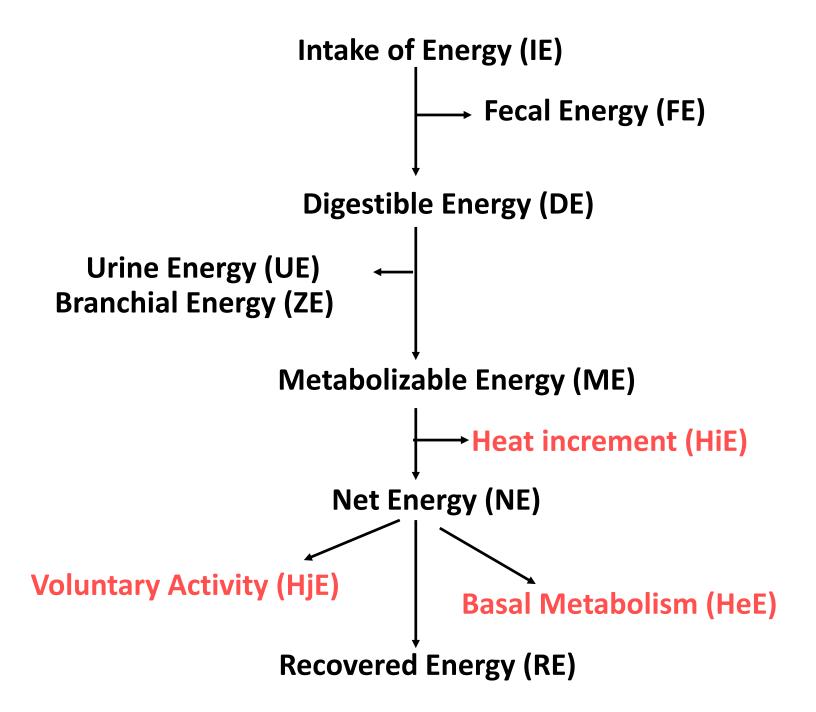
Generally require n-3 fatty acids and small amount of n-6 fatty acids Very limited ability to elongate (and desaturate) shorter chain fatty acids Basically require EPA, DHA and AA (20:4 n-6)

Marine crustaceans:

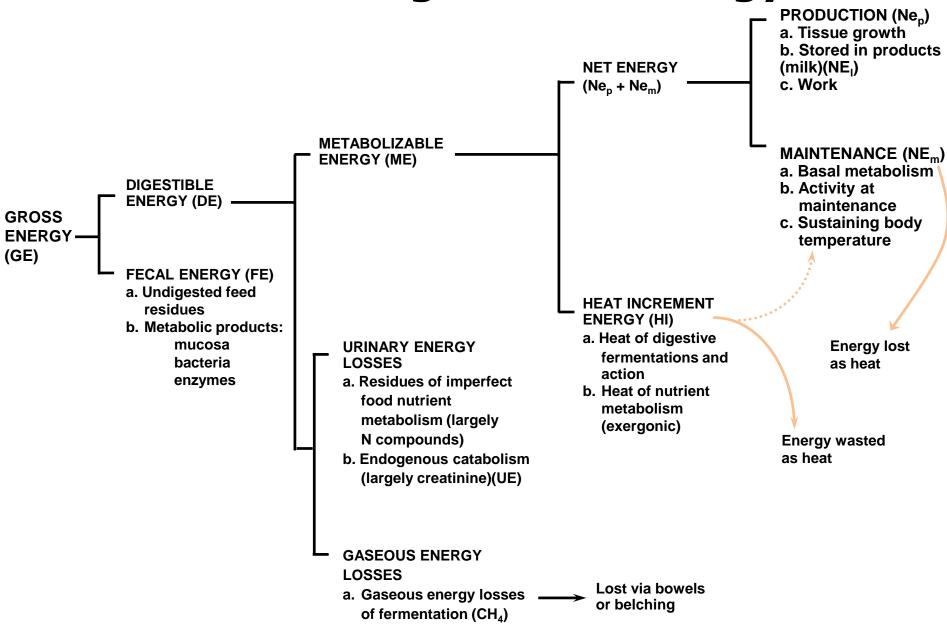
Generally require n-3 fatty acids and small amount of n-6 fatty acids Very limited ability to elongate (and desaturate) shorter chain fatty acids Basically require EPA, DHA and AA (20:4 n-6) Require phospholipids Require cholesterol (or sterols)

Educational Module #3: Dietary Energy: Definitions and Requirements (30 min)

- Energy Partitioning Scheme
- Dietary Energy
 - Gross energy
 - Digestible energy
 - Metabolizable energy
- Bioenergetics Model
 - Energy Requirement Estimations
 - Theoretical feed requirement and feed conversion ratio



Partitioning of Feed Energy



Growth Most important parameter in aquaculture

Affected by: Feed (quantity and quality)

Temperature, environment

Genetics

Rearing practices

Nutrient deposition:

Growth is the result of nutrients deposition (water, protein, lipid, minerals, etc.)

Energy deposited = "average nutrient deposition"

Energy deposited + cost of living and cost of depositing energy
= Digestible energy requirement
= Feed requirement

Determining Energy and Feed Requirements

1- Predict or describe growth

Need appropriate growth model

2- Determine nutrient / energy gains

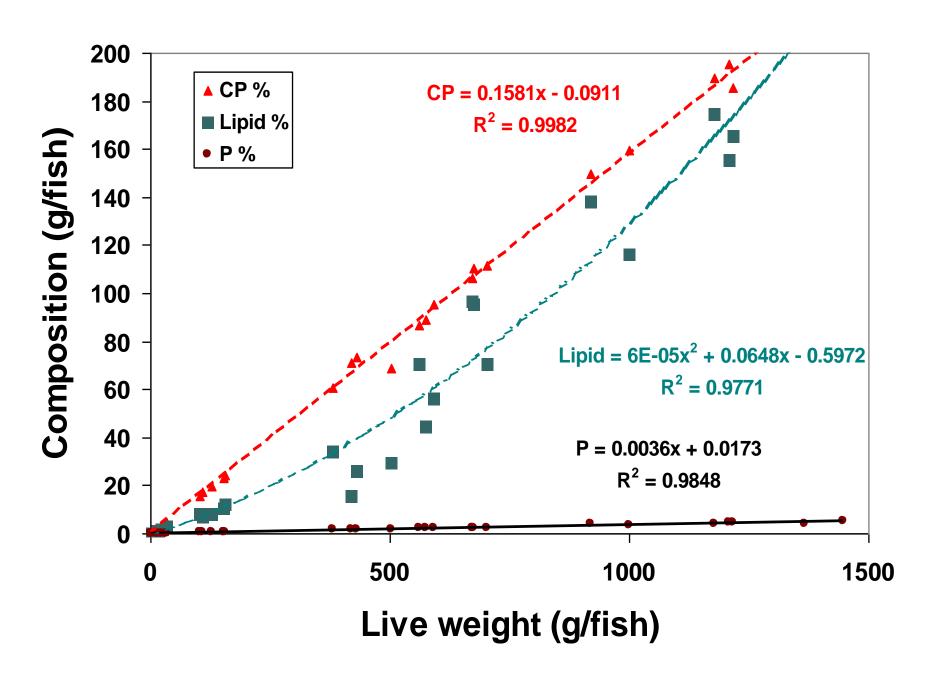
Carcass composition x growth

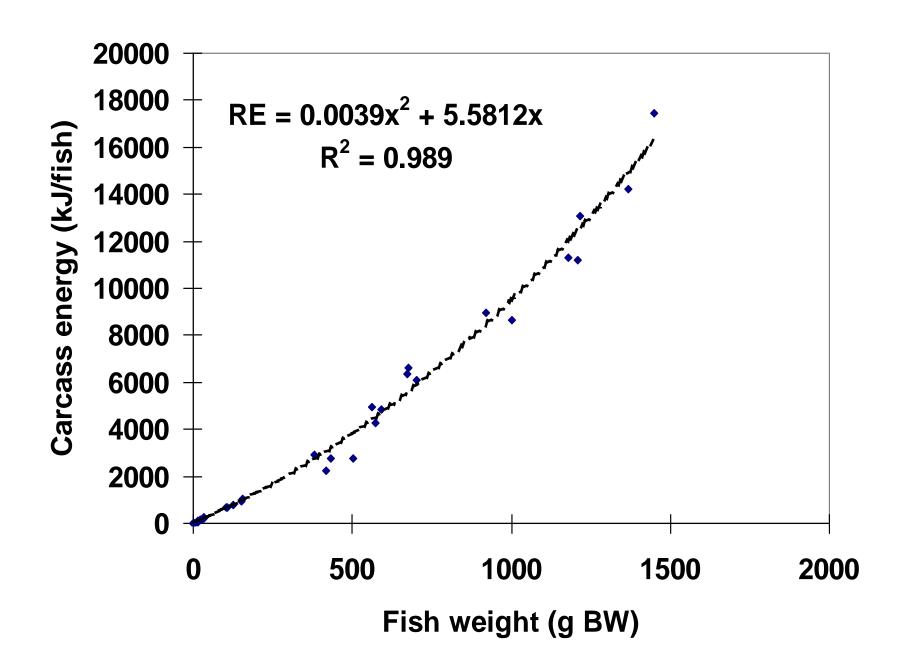
3- Estimate heat and metabolic losses

Maintenance (HeE) + Heat increment (HiE) + Non-fecal losses (UE+ZE)

4- Digestible energy requirement = sum

$$DE = RE + HeE + HiE + (UE+ZE)$$





Estimate of basal metabolism (HeE)

Rainbow trout:

 $HeE = -0.01 + 3.26T - 0.05T^2 \text{ kJ kg}^{-1} \text{ MBW d}^{-1}$

where MBW =

Metabolic body weight = live weight (kg) 0.82

Rainbow trout: 36 kJ kg^{0.82} 15°C

Homeotherms: 270 kJ kg^{0.75} at 37°C

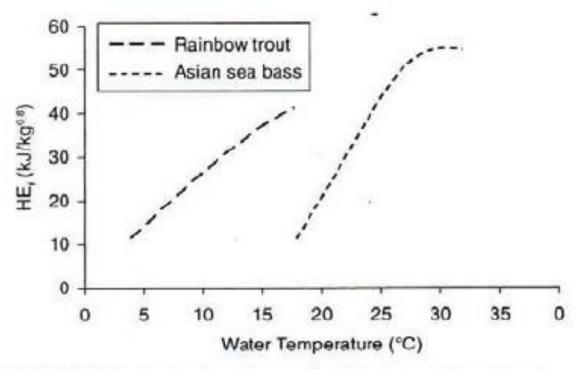
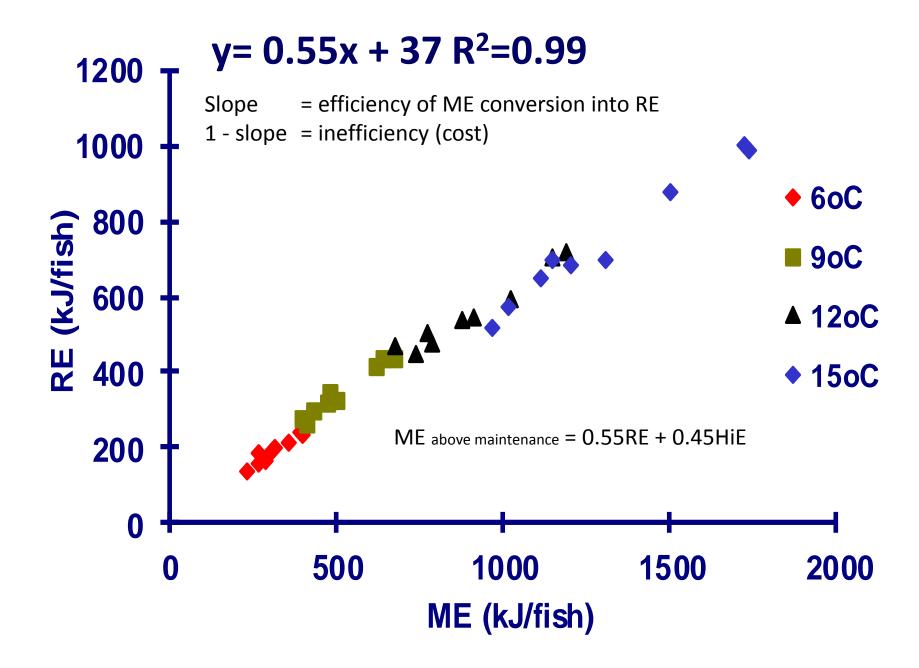


FIGURE 4-2 Fasting heat losses of rainbow trout, Oncorhynchus mykiss, and Asian sea bass, Lates calcarifer (expressed as HE_p kJ per kg⁰⁸ per day and as a function of water temperature).



Efficiency of ME Utilization & Estimates of HiE

Results from various energy budget using regression RE as a function of MEI

ME above maintenance =
$$0.61RE + 0.39HiE$$

or
 $HiE = 0.64 RE$

Azevedo et al. (1998)

ME above maintenance =
$$0.68RE + 0.32HiE$$

or
 $HiE = 0.47 RE$

Rodehutscord and Pfeffer (1999)

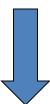
ME above maintenance =
$$0.64RE + 0.36HiE$$

or
 $HiE = 0.56 RE$

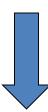
Bureau et al. (2006)

Efficiency of ME utilization not significantly affected by water temperature or feeding level. Nutrient composition (starch level) may affect ME utilization.

Digestible Energy Requirement/ Digestible Energy of Feed



Theoretical Feed Requirement/kg weight gain



Theoretical Feed Conversion Ratio

Energy Requirements of Asian Sea Bass (Lates calcarifer).

Live Weight g/fish	Growth Rate g/d	<u>RE</u>	HeE M	HiE+(UE+ZE) J/kg gain	DE Req
10	1.1	4.5	1.2	3.1	8.9
50	2.2	5.7	2.3	3.9	11.9
100	3.0	6.3	2.9	4.3	13.6
250	4.4	7.2	4.1	4.9	16.3
500	5.9	8.0	5.4	5.4	18.8
1000	8.0	8.8	7.0	6.0	21.8
2000	10.7	9.7	9.0	6.6	25.4
3000	12.7	10.3	10.5	7.0	27.8

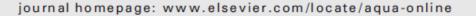
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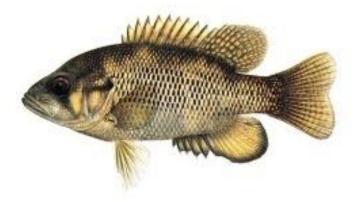


Bioenergetics-Based Factorial Model to Determine Feed Requirement and Waste Output of Tilapia Produced under Commercial Conditions

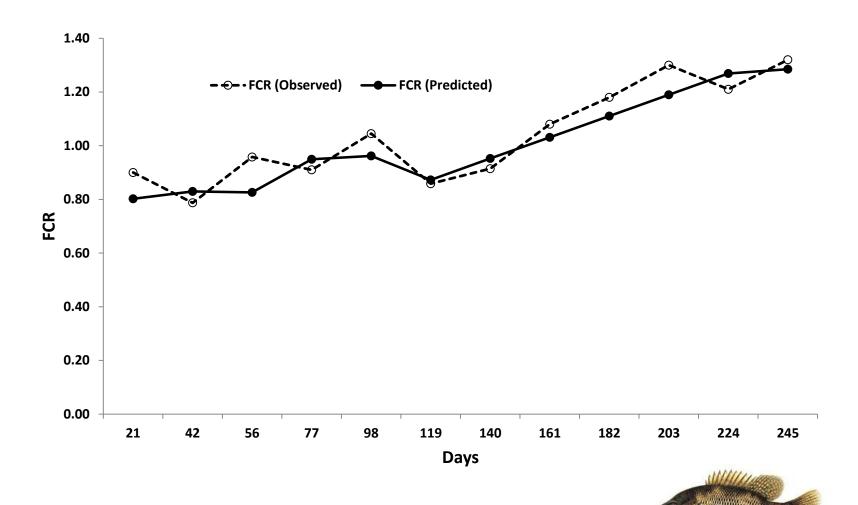


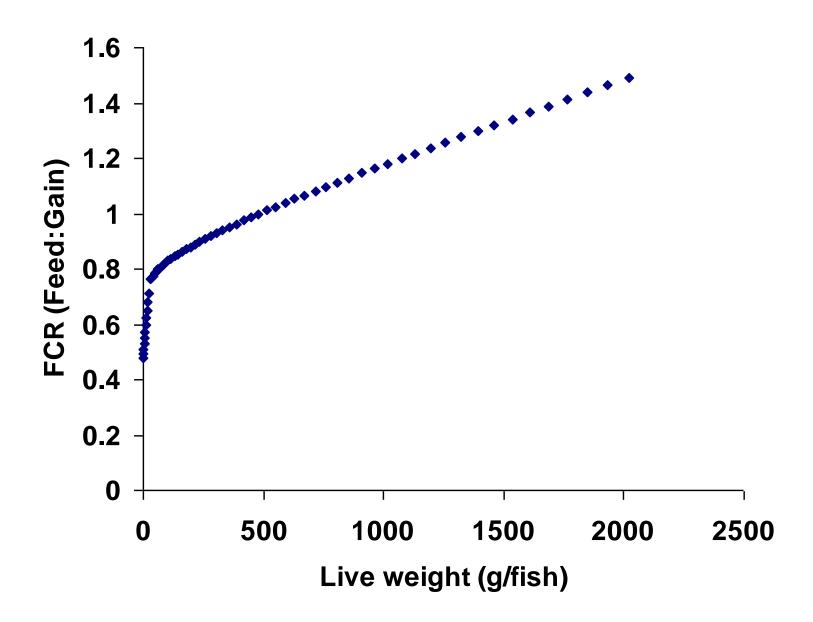
M.A. Kabir Chowdhury a,*, Sohail Siddiqui b, Katheline Hua c, Dominique P. Bureau a

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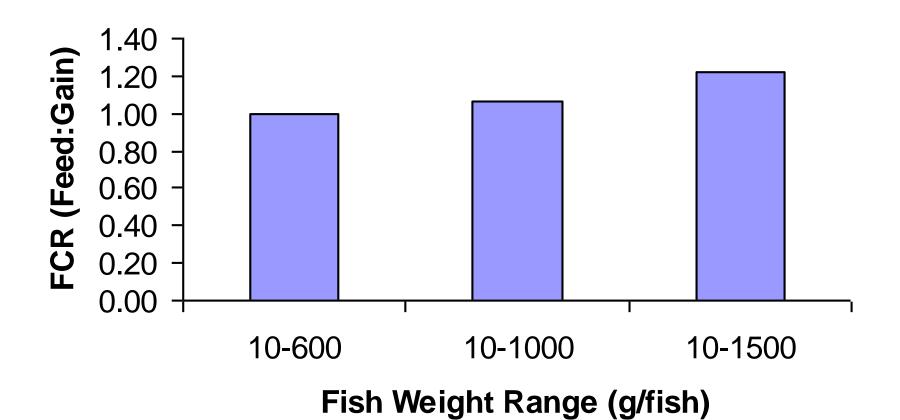


Observed and predicted evolution of feed conversion ratio (feed:gain) of Nile tilapia during a pilot-scale trial





Expected FCR of fish reared to different harvest weights



Feed: 37% DP, 20 MJ DE