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IFFO
THE MARINE INGREDIENTS ORGANISATION

UNIVERSITY of GUELPH

In Focus
IFFO Webinar Series

SEARCHING FOR UNKNOWN GROWTH FACTORS IN FISHMEAL

ONLINE – 10th August 2022

Unknown Unknown's

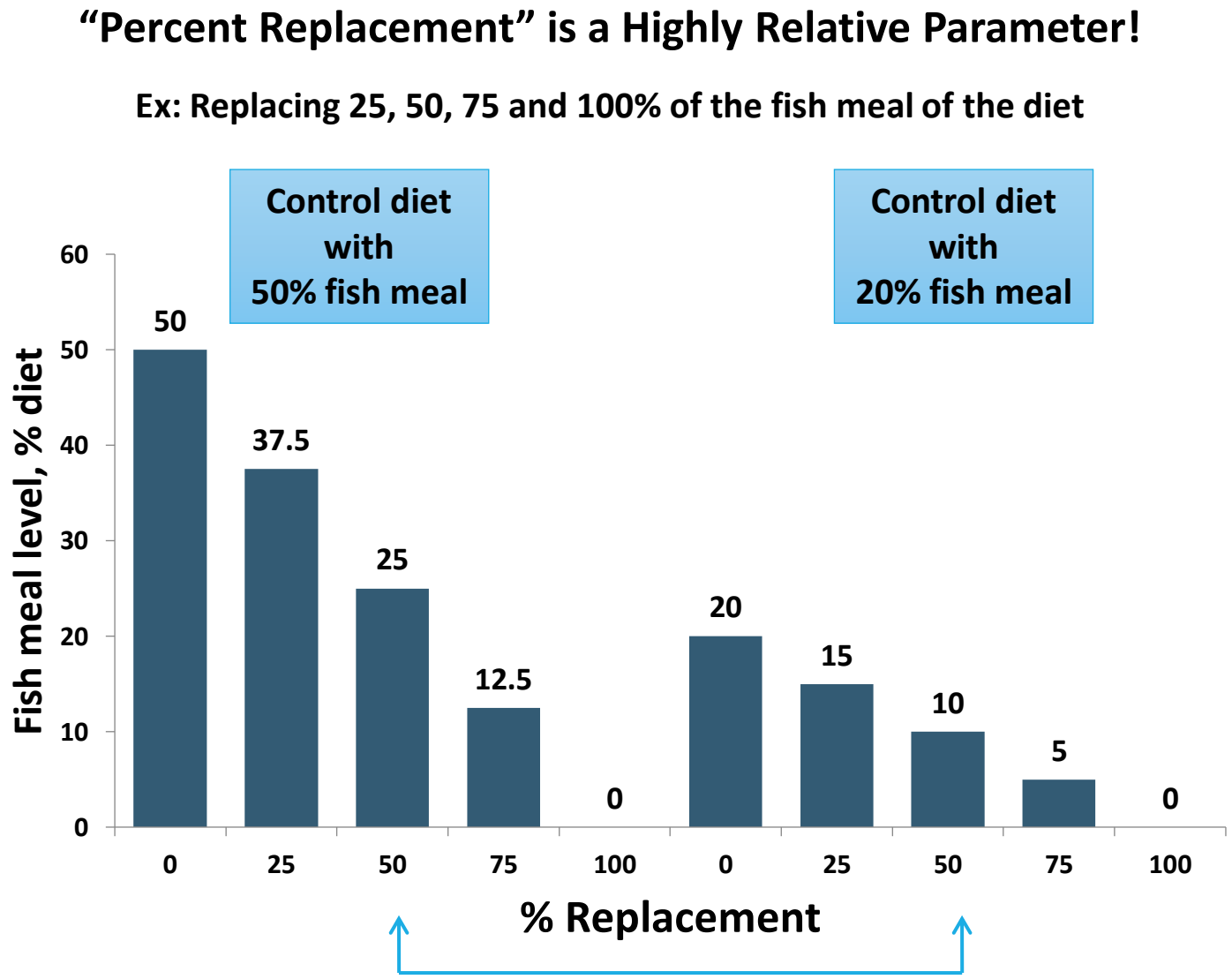
"...because as we know, there are known known's; there are things that we know that we know. We also know there are known unknown's; that is to say we know there are some things we do not know. But there are also unknown unknown's, the ones we don't know we don't know."

Donald Rumsfeld
United States Secretary of Defense
February 2002

The Fish Meal Story

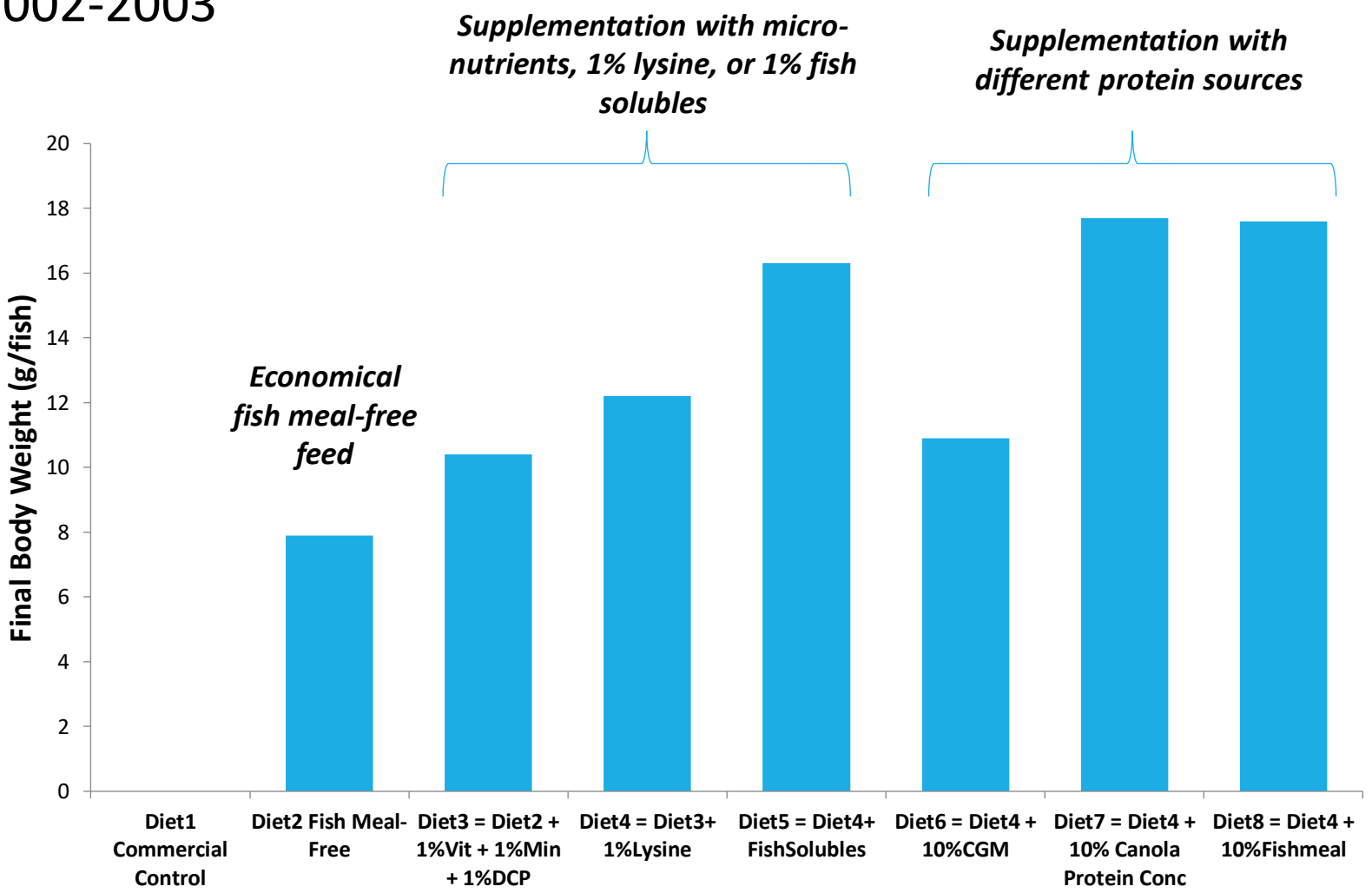
- In the past, a “central” ingredient in aquaculture feed formulation, in terms of weight and supply of nutrients
- Today, used a much lower levels but still an important component of most feeds as a “strategic”, “functional” ingredient
- Many studies to completely replace fish meal are often failing (at least for a number of species)
- What are we missing or not considering?
  - Some conventional nutrients?
  - Some overlooked nutrients/components?
  - Palatability?
  - Putative growth factors?

#1. Let’s get rid of inappropriate terminology, please!



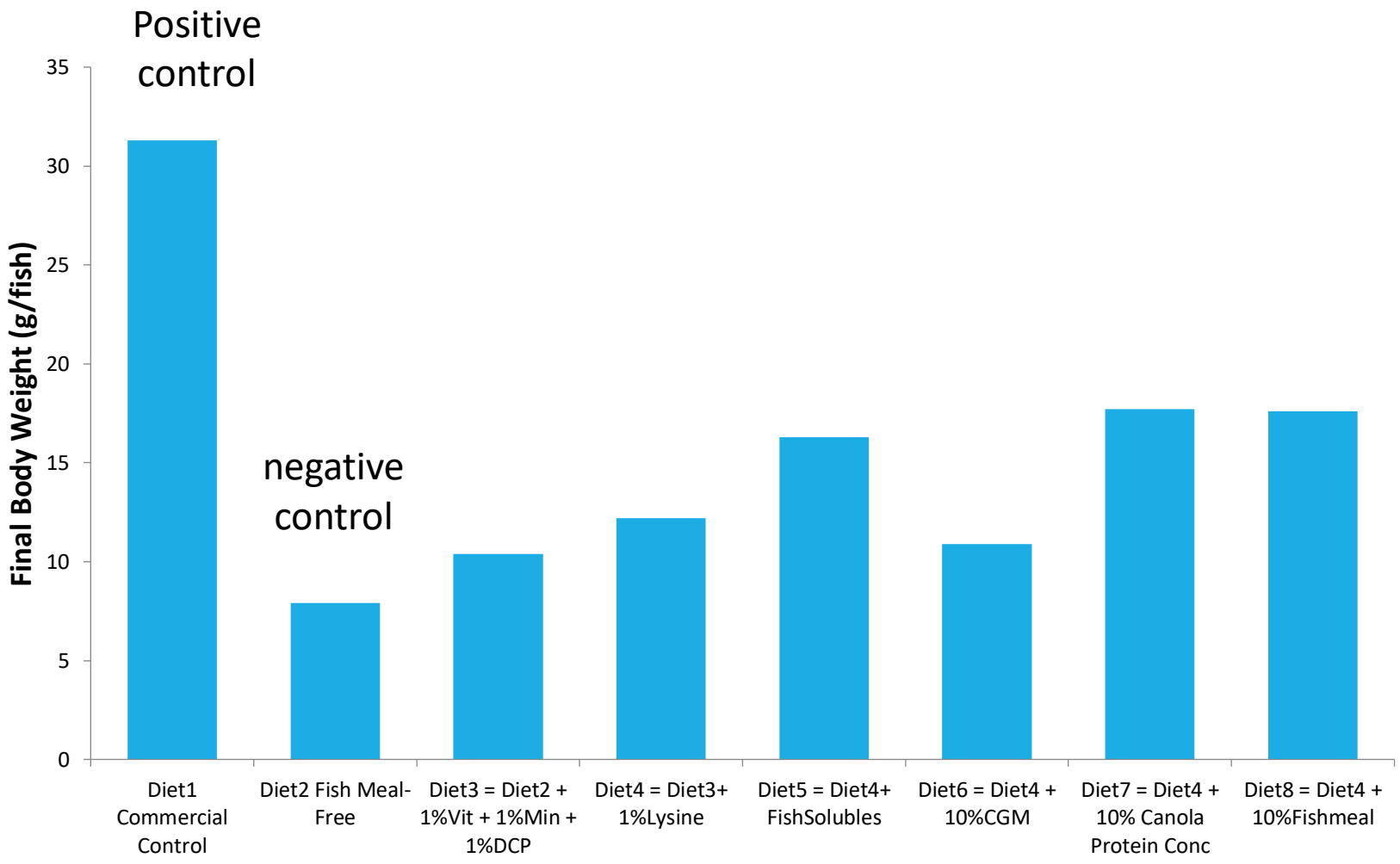
Simple Supplementation Experiment to All-Plant Protein Feed Fed to Rainbow Trout

2002-2003

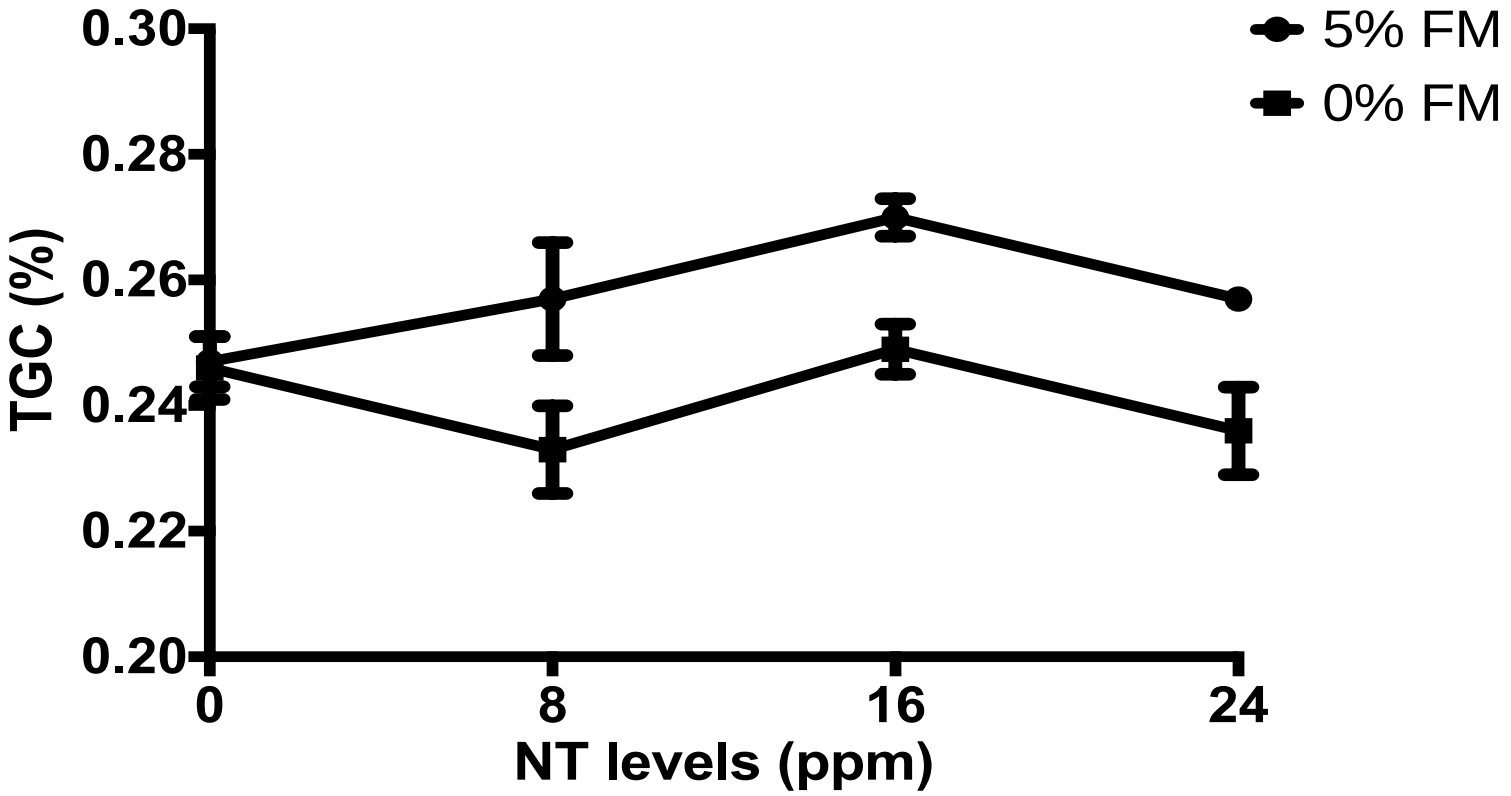


12-week feeding trial with rainbow trout (initial weight = 1.3 g/fish)

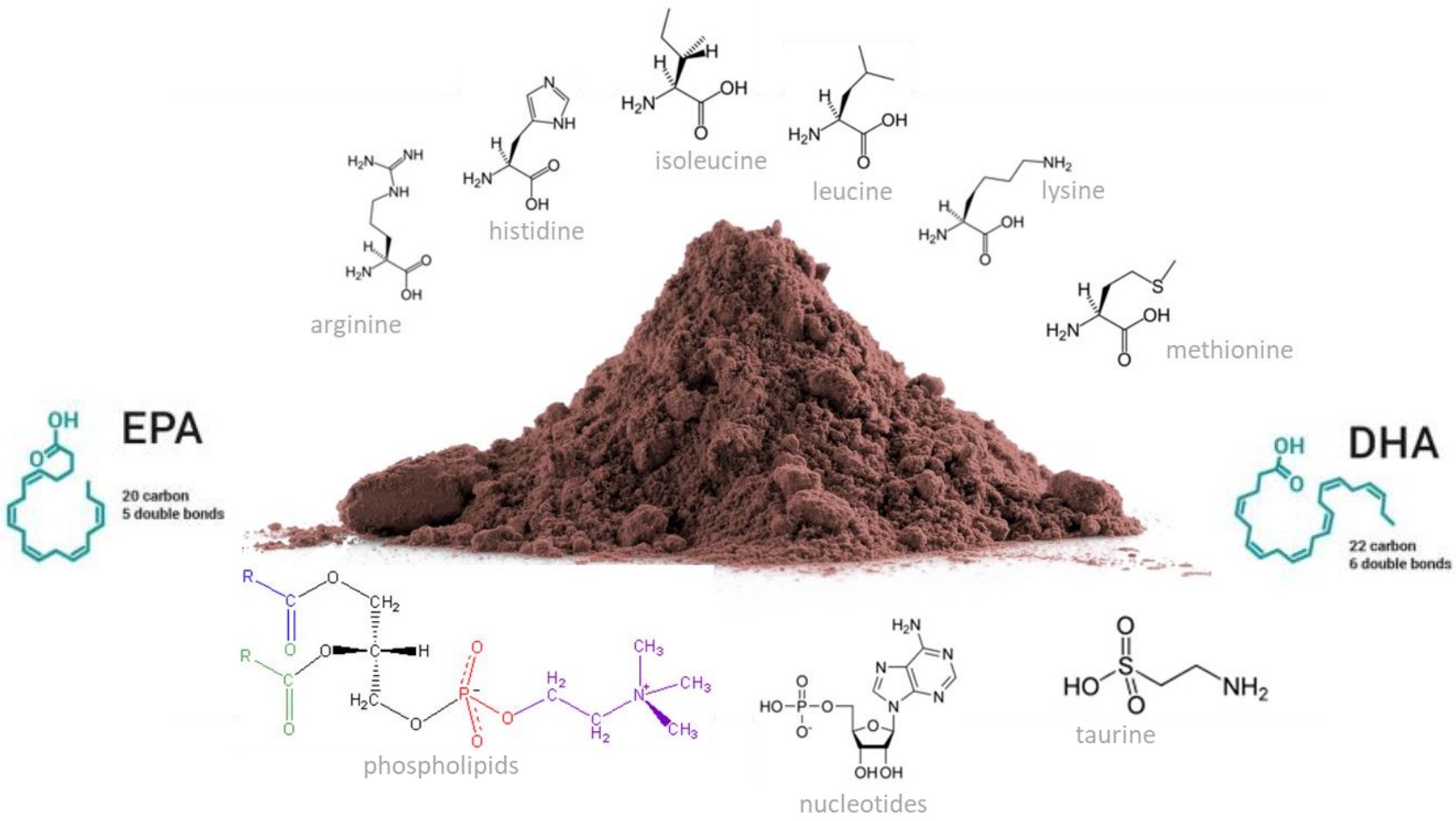
The Importance of a Proper Yardstick or Performance Benchmarking!



Growth rate of rainbow trout in response to being fed experimental diets containing increasing nucleotide levels with different fishmeal inclusion levels.



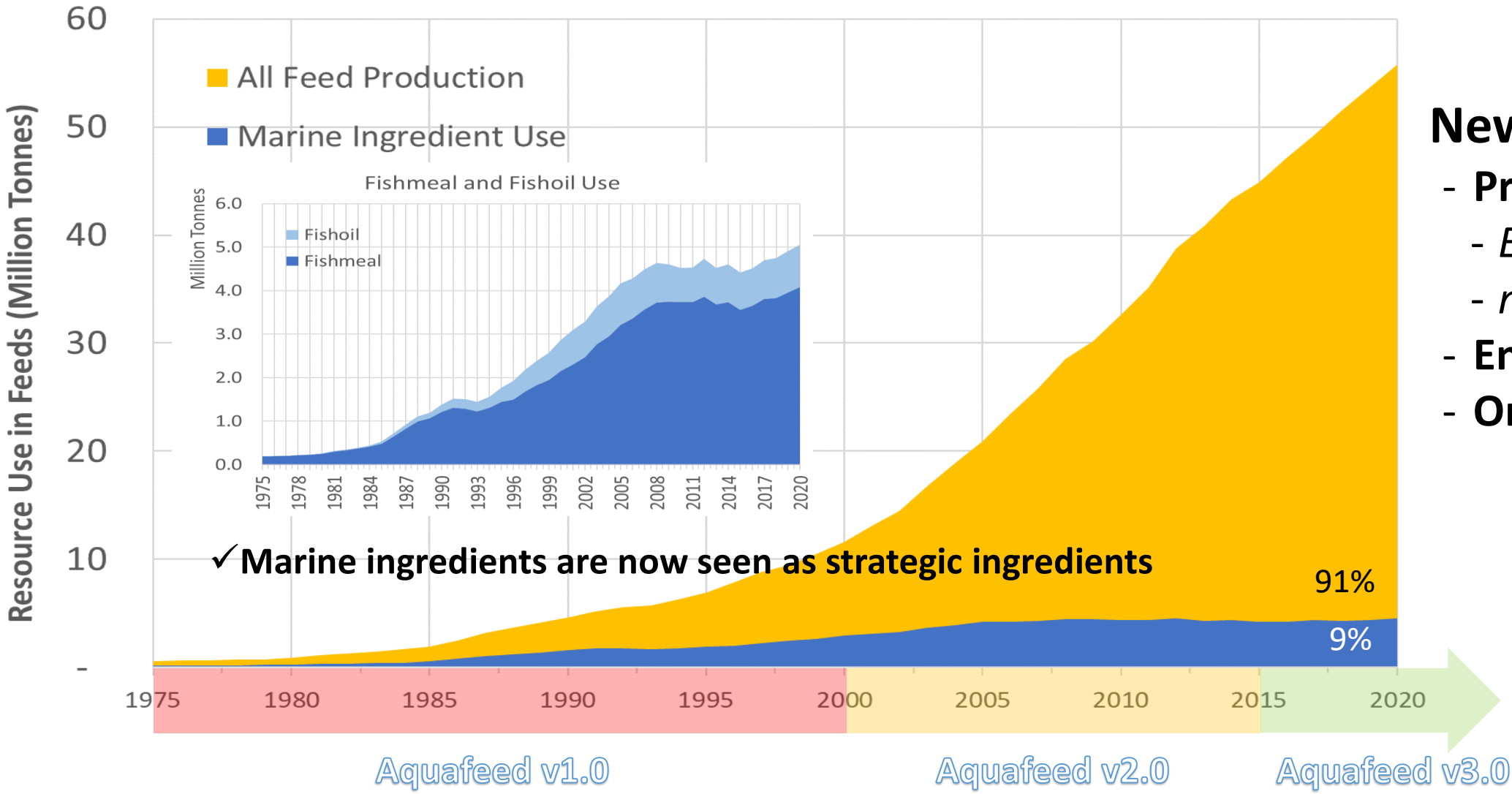
An Ideal Ingredient?



What Does Fish Meal Bring That Plant Feed Ingredients Don't?

Components/Parameters	Fish meal	Plant Proteins
Essential amino acid profile	Excellent	Excellent/Poor
Digestible amino acids	Excellent/Good	Excellent/Good
LC n-3 HUFA (EPA+DHA)	**Excellent**	**None**
LC n-6 HUFA (ARA)	**Good/Moderate**	**None**
Available phosphorus	Excellent	Moderate/Poor
Digestible energy	Good	Good/Moderate
Micro-minerals	***Excellent***	***Variable/Poor***
Phospholipids	***Excellent***	***Moderate/Poor***
Cholesterol	***Excellent***	***None***
Hormones/ Bio-active compounds	***Moderate/Low***	***Low/Moderate***
Taurine	***Excellent***	***None***
Nucleotides	***Excellent***	***Moderate/None***
Soluble fibers / Oligosaccharides	*Absent*	*Moderate/High*
Insoluble fibers (cellulose, lignin)	*Absent*	*Moderate/High*
Misc. anti-nutritional factors	***Low/absent***	***Moderate/High***
Contaminants	Moderate	Low/Moderate
Phytates	*None*	*High/Moderate*
Attractants	***High***	***Low/Moderate***

The Future WILL Need Additional Ingredients



New Sources

- **Protein**
  - *EAA*
  - *nEAA*
- **Energy**
- **Omega-3**

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Animal Nutrition = Balanced Understanding of
Nutritional Requirements and Ingredient Quality

You can’t disconnect nutritive value of ingredients and nutritional requirements of the animal

How Diet Formulation Works

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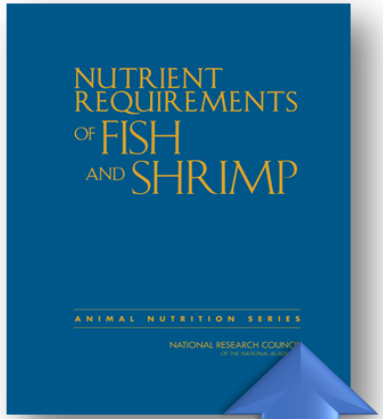
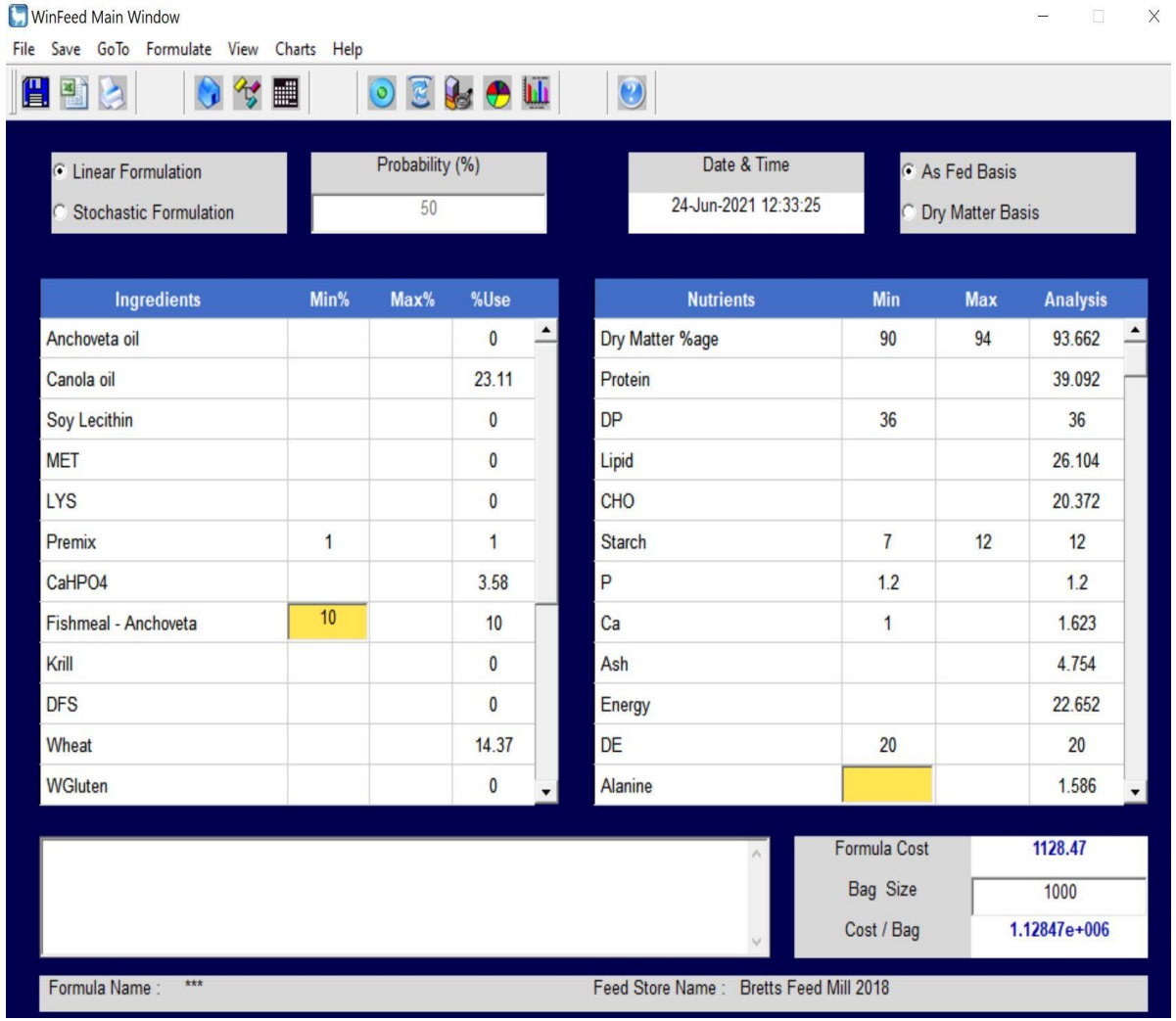
Requirement Specifications



Constraints

- Nutrient requirements
- Raw material tolerances
- Antinutrient thresholds
- Contaminant thresholds
- Processing parameters
- Legal constraints
- Social attitudes
- Quality expectations
- Price

Ingredient Database



Assessing Nutritional Effects of Ingredients

Characterisation

Functionality

Gene / Protein Expression

Immune Function

Blood Chemistry

Feed Intake

Digestibility

Utilisation

(Growth/Retention/Composition)

Aquaculture Nutrition 2007 13; 17–34

A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds

B.D. GLENCROSS[1], M. BOOTH[2] & G.L. ALLAN[2]

[1] Department of Fisheries – Western Australia, Research Division, North Beach, WA, Australia; [2] New South Wales Department of Primary Industries, Port Stephens Fisheries Centre, Nelson Bay, NSW, Australia

Abstract

The evaluation of feed ingredients ... research and feed development for ... evaluating ingredients for use in aqua... several important knowledge comp... understood to enable the judicious ... ingredient in feed formulation. This ... (1) ingredient digestibilities, (2) ingred...

Received: 28 February 2020 | Revised: 29 May 2020 | Accepted: 15 June 2020

DOI: 10.1111/anu.13138

Check for updates

REVIEW

Aquaculture Nutrition WILEY

A feed is *still* only as good as its ingredients: An update on the nutritional research strategies for the optimal evaluation of ingredients for aquaculture feeds

Brett D. Glencross

As Much a Study of the Ingredient…

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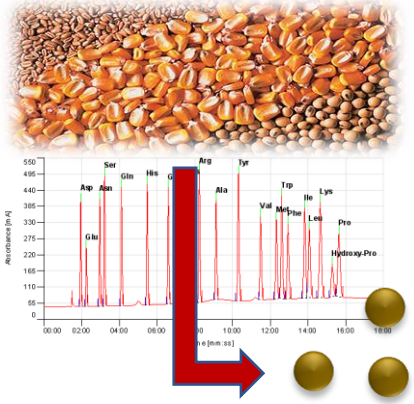
1. Characterisation

What nutrients and how much does it have?



5. Functionality

Does it impact the pelleting process?

2. Feed Intake

How palatable is it to this species?

Aquaculture Nutrition 2007 **13**; 17–34

A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds

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REVIEW

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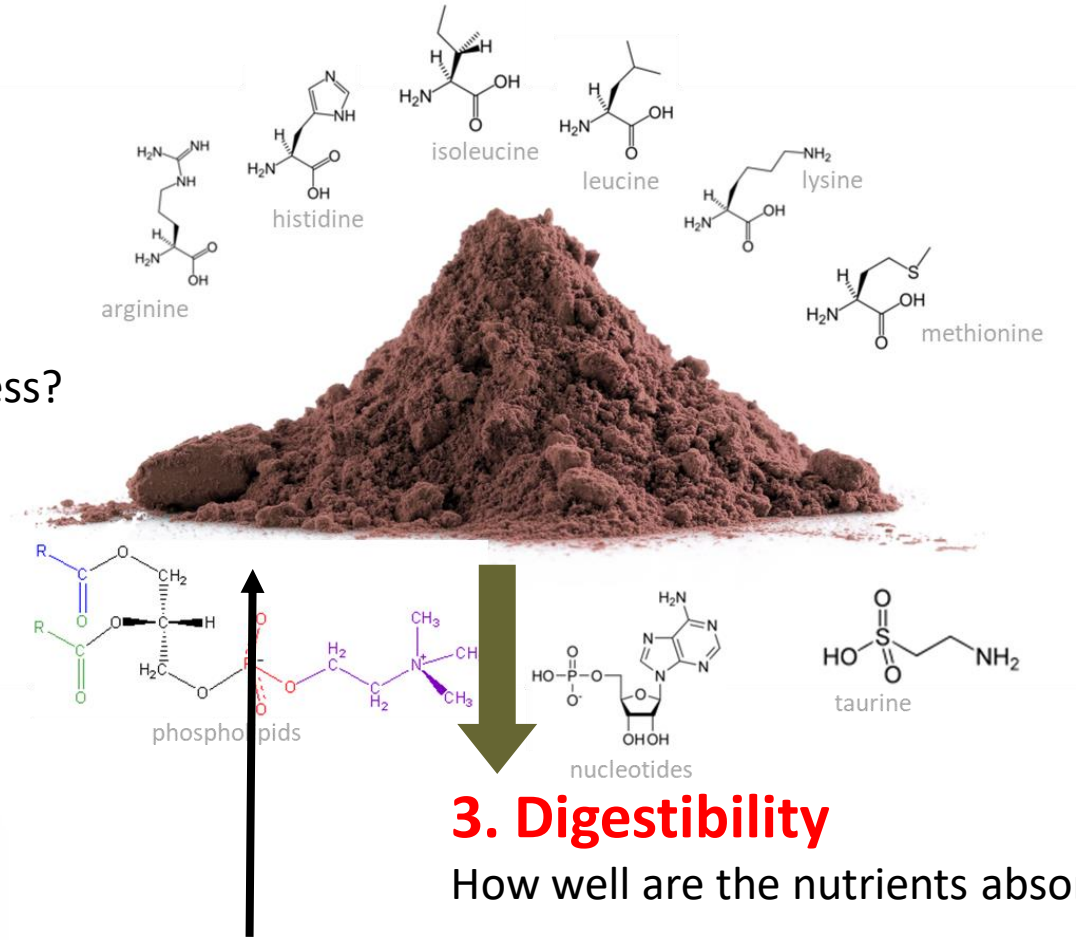


3. Digestibility

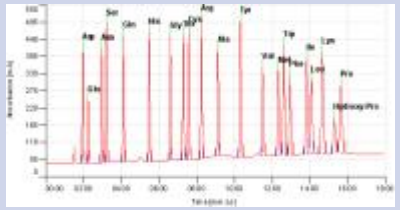
How well are the nutrients absorbed?

4. Utilisation

Is there any antinutrient (toxic) impact on utilisation?

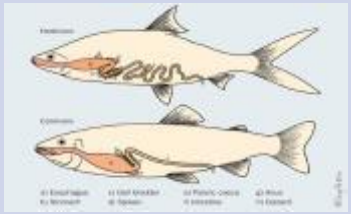
Assessing Nutritional Effects of Ingredients

STEP 1



Characterisation

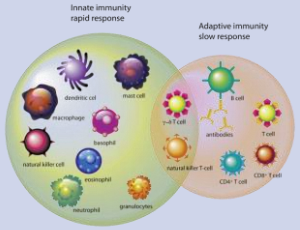
STEP 2

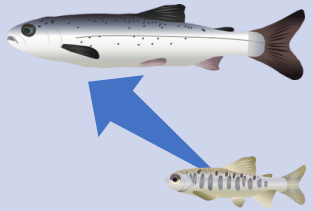


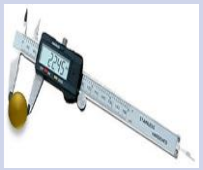
Digestibility

Palatability

STEP 3







Immune Response

Utilisation

Functionality

STEP 4

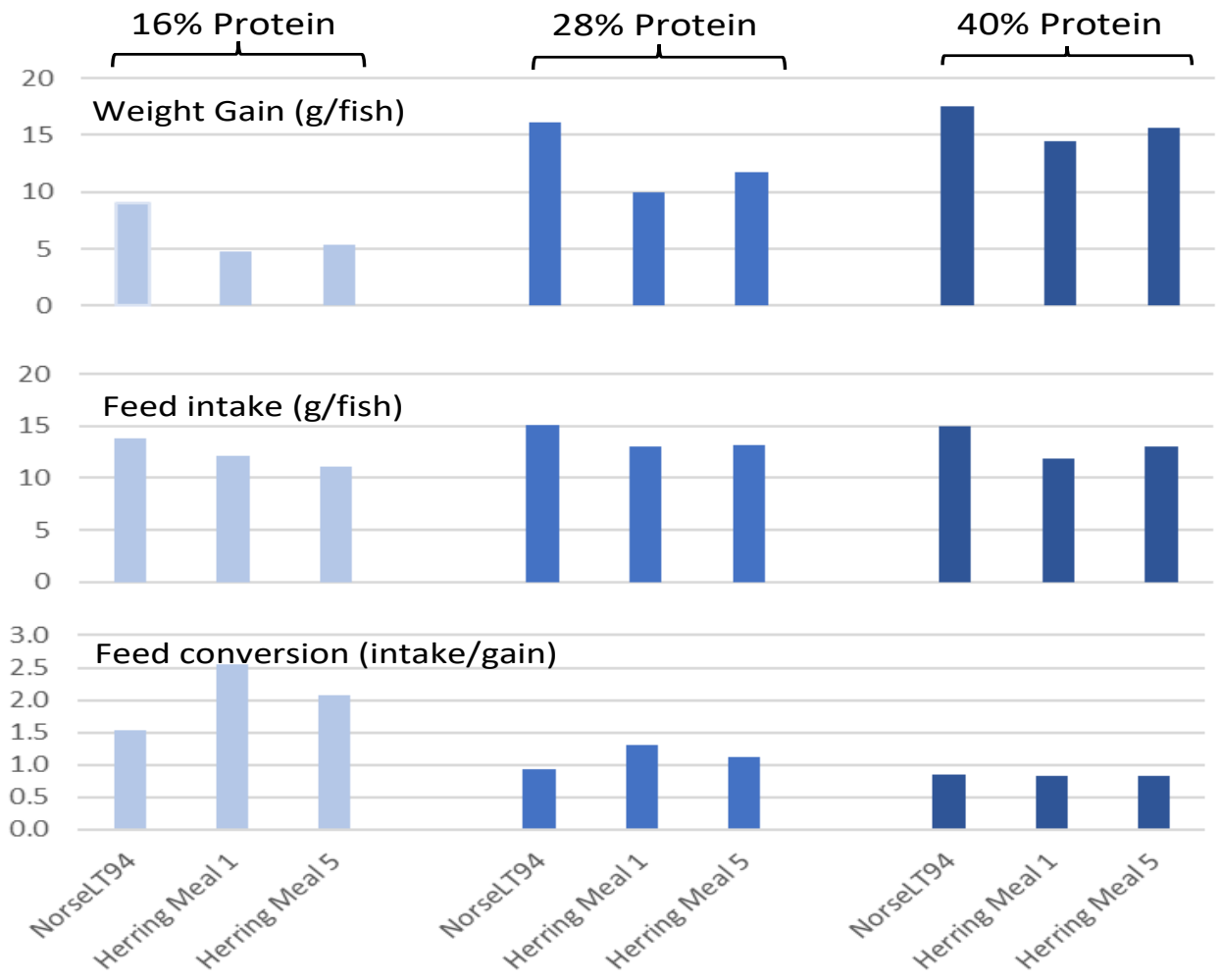
Omics Responses

Meat Quality

LCA Assessment

Assessing Nutritional Quality Through Performance Trials

From: Anderson et al 1993. Aquaculture 115: 305-325.



Pepsin Digestibility

NorseLT94: ADC_{Pro} = 0.97

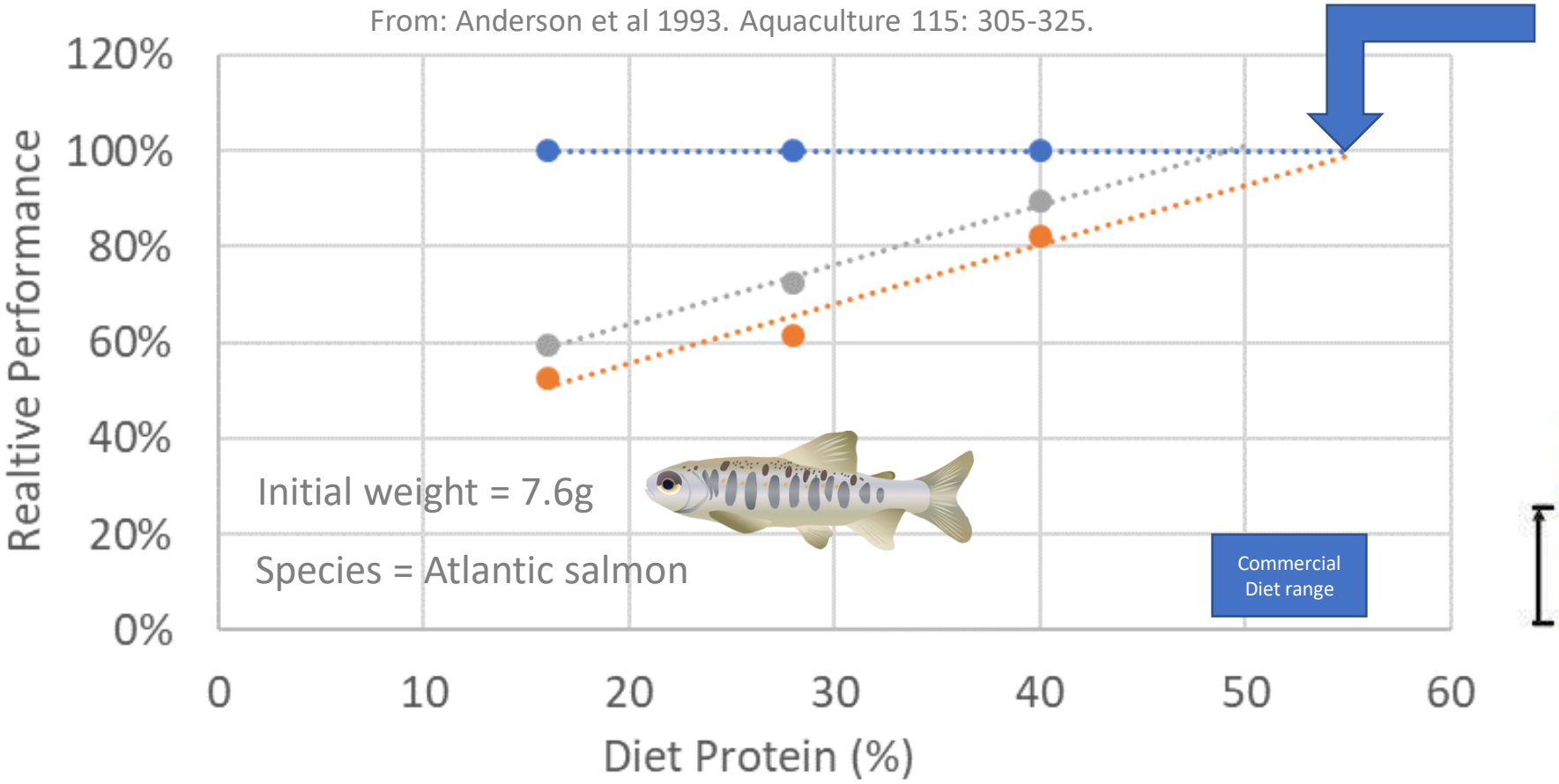
Herring Meal 1: ADC_{Pro} = 0.91

Herring meal 5: ADC_{Pro} = 0.95

Ability to use growth studies to define quality is highly dependant on diet design (e.g. protein levels)

Assessing Nutritional Quality Through Performance Trials

From: Anderson et al 1993. Aquaculture 115: 305-325.

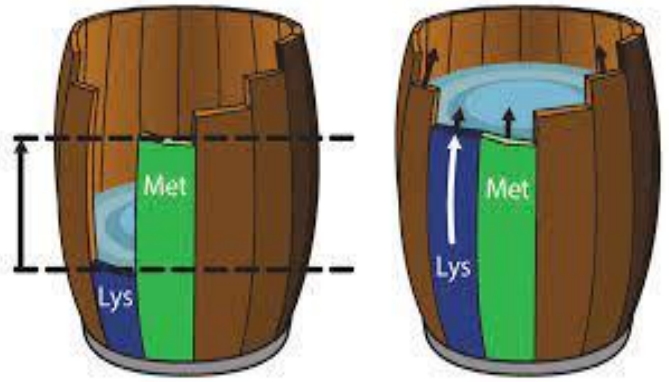


NorseLT94 — Pepsin Digestibility ADC_{Pro} = 0.97

Herring Meal 1 — ADC_{Pro} = 0.91

Herring Meal 5 — ADC_{Pro} = 0.95

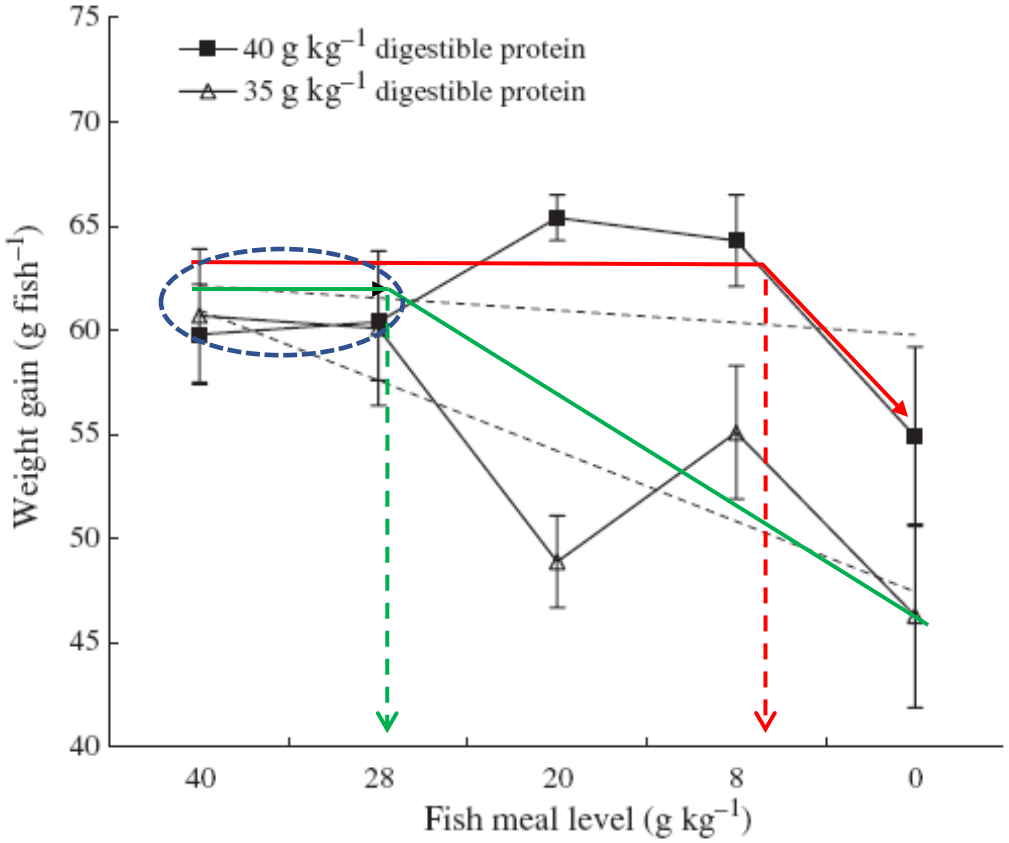
At this protein level it is no longer possible to tell a good ingredient from a bad one!



Leibig's Law:
Growth is only responsive to the first limiting nutrient

Marine Fish Cage Farm on Nanao Island, Guangdong, China

Effect of Replacement of Fish Meal by a Mixture of Animal Proteins in Marine Fish Feeds Formulated to Two Digestible Protein Levels

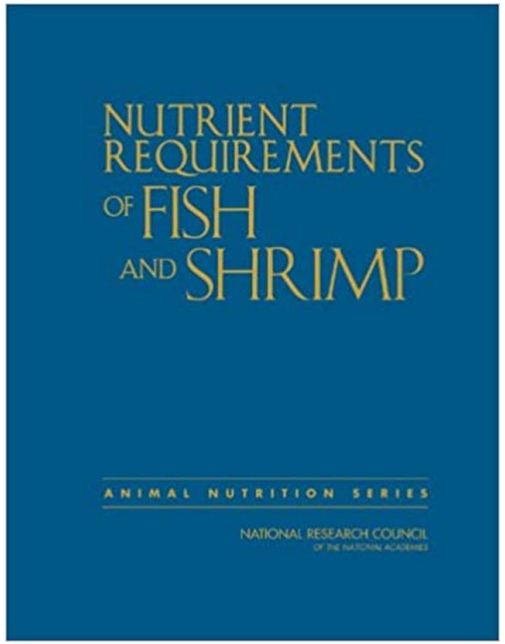


Wang et al. (2010)

At higher protein levels, essential amino acids (EAA) deficiencies occur at much lower fish meal and higher alternative ingredient levels. It is the essential nutrient levels of the diet that matter, not the amino acid balance/profile.

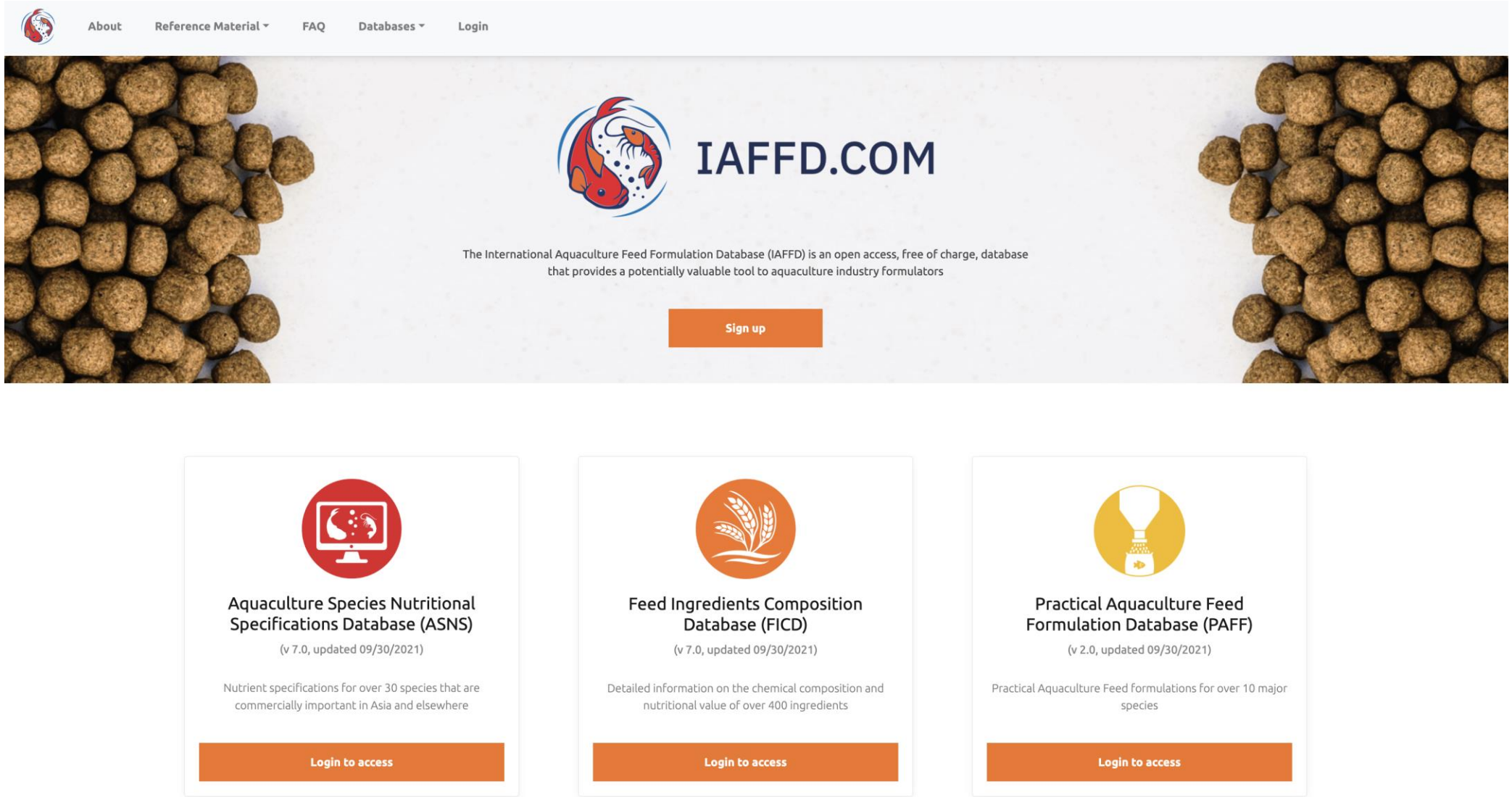
Essential Amino Acid Requirements of Different Fish Species

Source: NRC (2011)



Amino Acids	Atlantic Salmon	Common Carp	Nile Tilapia	Channel catfish	Rainbow Trout	Asian Seabass	European Seabass	Japanese Flounder	Red Drum	Yellowtail
Arginine	1.8	**1.7**	1.2	1.2	1.5	1.8	1.8	2.0	1.8	1.6
Histidine	0.8	0.5	**1.0**	0.6	**0.8**	NT	NT	NT	NT	NT
Isoleucine	1.1	**1.0**	**1.0**	0.8	**1.1**	NT	NT	NT	NT	NT
Leucine	1.5	1.4	1.9	1.3	1.5	NT	NT	NT	NT	NT
Lysine	2.4	**2.2**	**1.6**	1.6	**2.4**	2.1	2.2	2.6	1.7	1.9
Methionine	0.7	0.7	0.7	0.6	0.7	0.8	NT	0.9	0.8	0.8
Met+Cys	1.1	1.0	1.0	1.0	1.1	1.2	1.1	NT	1.2	1.2
Phenylalanine	0.9	1.3	1.1	0.7	0.9	NT	NT	NT	NT	NT
Phe+Tyr	1.8	2.0	1.6	1.6	1.8	NT	NT	NT	NT	NT
Threonine	1.1	**1.5**	1.1	0.7	**1.1**	NT	1.2	NT	0.8	NT
Tryptophan	0.3	0.3	0.3	0.2	0.3	NT	0.3	NT	NT	NT
Valine	1.2	**1.4**	**1.5**	0.8	1.2	NT	NT	NT	NT	NT
Taurine	NR	NR	NT	NR	NR	R	0.2	R	R	R

www.iaffd.com



IAFFD Essential Amino Acid Specifications (v 6.0)

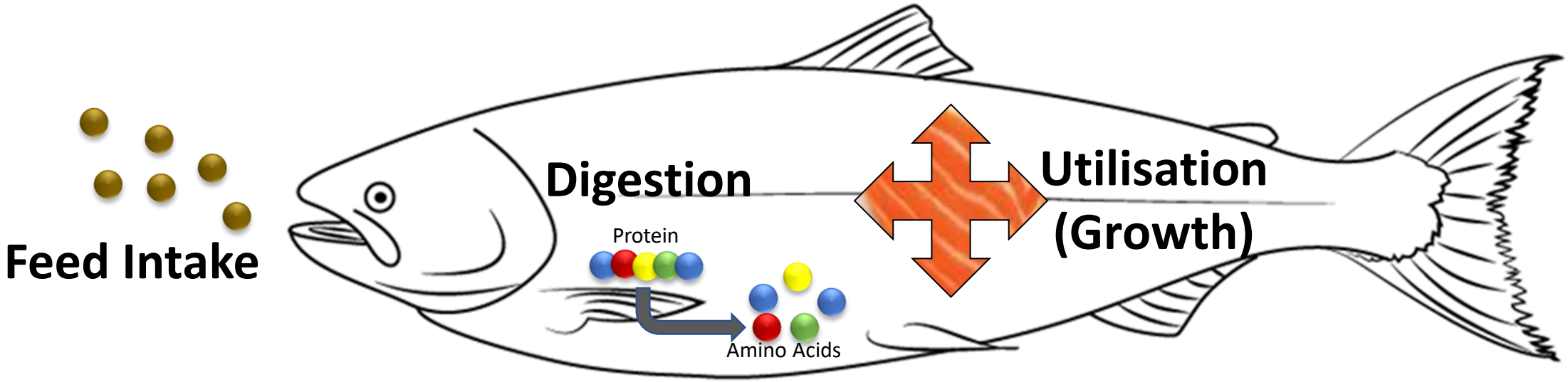
Asian sea bass – Intensive culture

Code	Nutrient				104I_1 Starter	104I_2 Fry	104I_3 Pre-grower	104I_4 Grower1	104I_5 Finisher	104I_6 Brood
					< 5 g	*5-50 g*	*50-200 g*	*200-500 g*	*500-1500 g*	*>1500 g*
107 AA01	Arginine	ARG	%	Minimum	2.64	2.49	2.39	2.33	2.26	2.26
107 AA02	Histidine	HIS	%	Minimum	0.50	0.47	0.46	0.45	0.43	0.43
107 AA03	Isoleucine	ILE	%	Minimum	1.66	1.55	1.48	1.44	1.38	1.38
107 AA04	Leucine	LEU	%	Minimum	2.90	2.72	2.61	2.54	2.45	2.45
107 AA05	Lysine	LYS	%	Minimum	2.80	2.62	2.50	2.43	2.34	2.34
107 AA06	Methionine	MET	%	Minimum	1.05	0.99	0.96	0.93	0.90	0.90
107 AA07	Phenylalanine	PHE	%	Minimum	1.41	1.31	1.26	1.22	1.18	1.18
107 AA08	Threonine	THR	%	Minimum	1.62	1.53	1.48	1.45	1.41	1.41
107 AA09	Tryptophan	TRP	%	Minimum	0.46	0.44	0.43	0.42	0.41	0.41
107 AA10	Valine	VAL	%	Minimum	1.97	1.85	1.79	1.74	1.68	1.68
107 AA11	Cystine	CYS	%	Minimum	0.52	0.49	0.47	0.46	0.45	0.45
107 AA12	TSAA (Met+Cys)	SAA	%	Minimum	1.57	1.48	1.43	1.39	1.35	1.35
107 AA13	Tyrosine	TYR	%	Minimum	0.97	0.90	0.86	0.83	0.79	0.79
107 AA14	Phe+Tyr	Phe+Tyr	%	Minimum	2.38	2.21	2.11	2.05	1.97	1.97

Animal Nutrition = Balanced Understanding of
Nutritional Requirements and Ingredient Quality

You can’t disconnect nutritive value of ingredients and nutritional requirements of the animal

Hierarchy of Impacts



- Three primary points of influence
- Sequential influence (intake → digestion → utilisation)
- Declining impact on performance through the sequential influence

  …but effects often accumulate

Performance and Fishmeal Replacement

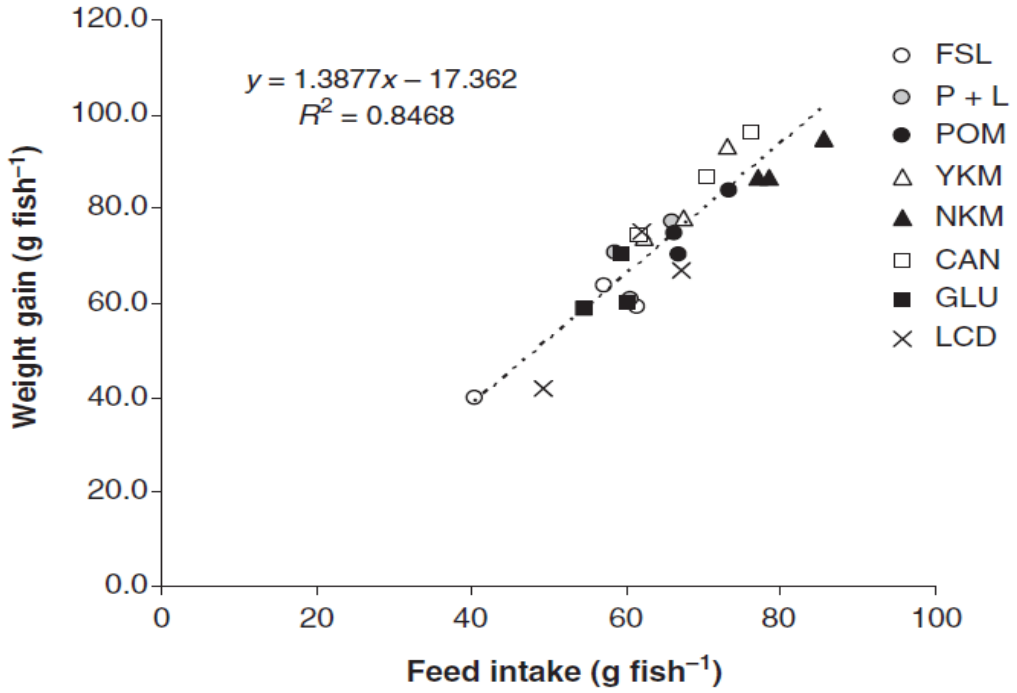


Figure 2 Fish weight gain as a function of feed intake among the different treatments. Diet acronyms are given in Table 1.

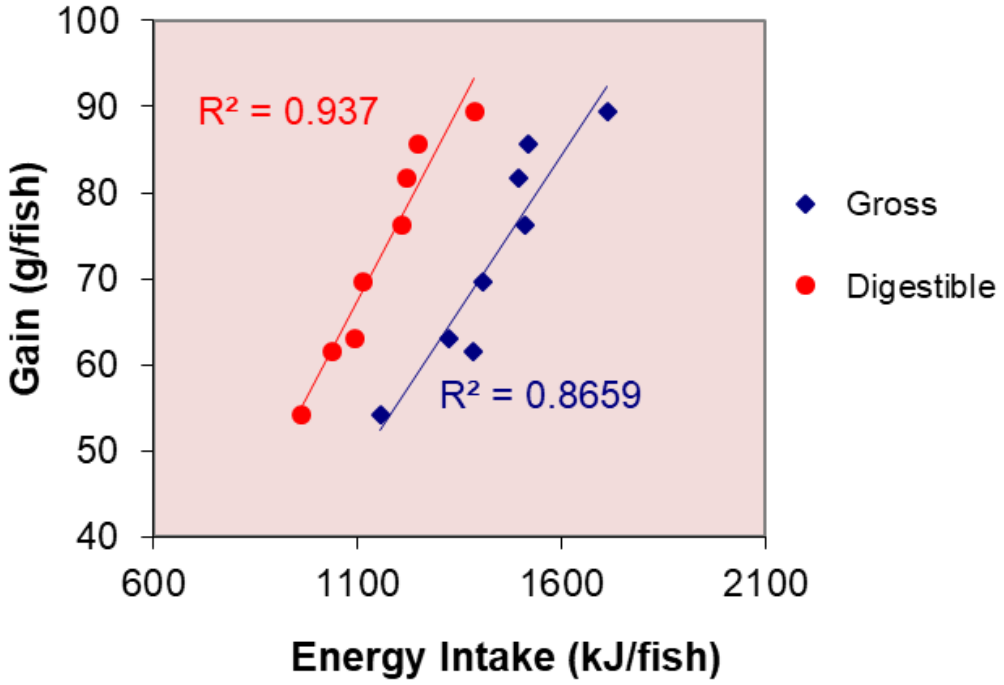

Aquaculture Nutrition
doi: 10.1111/j.1365-2095.2010.00834.x
Aquaculture Nutrition 2011 17; e722–e732

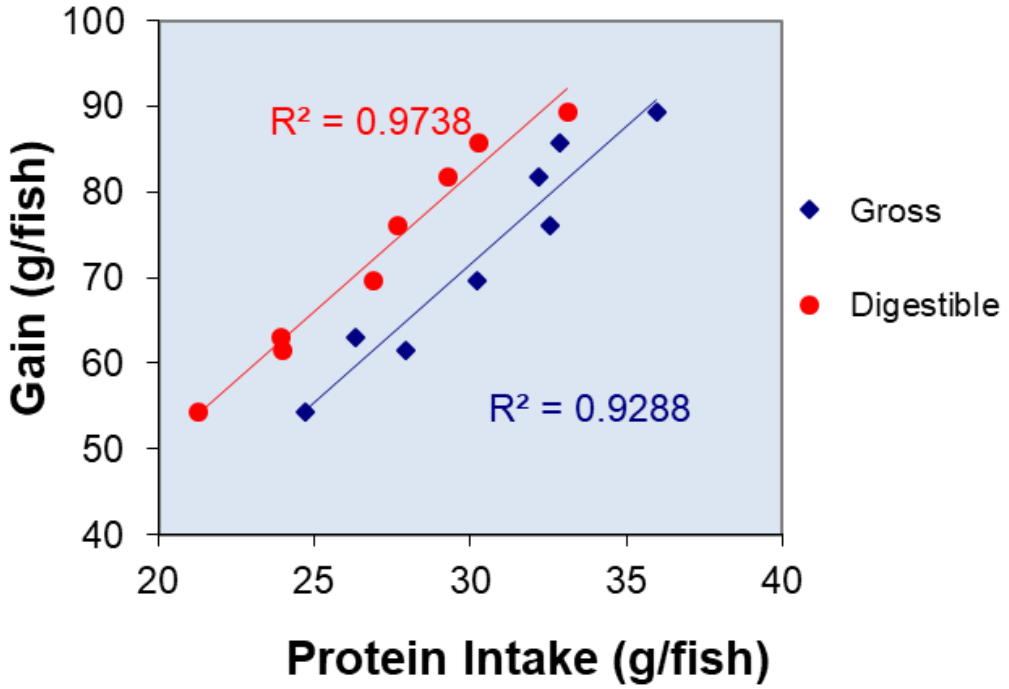
Evaluating options for fishmeal replacement in diets for juvenile barramundi (*Lates calcarifer*)

B. GLENCROSS, N. RUTHERFORD & B. JONES
Department of Fisheries – Research Division, North Beach, WA, Australia

- All diets formulated to equal DPro and DE
- More than 80% of the variation in growth could be explained by feed intake.

Intake vs Digestion vs Growth





- Examining that same data further on a digestible energy and digestible protein basis increased the R^2 value.
- Although feed intake defines most of the effect on growth, adding in factors for digestible nutrient (rather than gross nutrient) intake explains additional variability in the growth response.

Driving Palatability Responses?

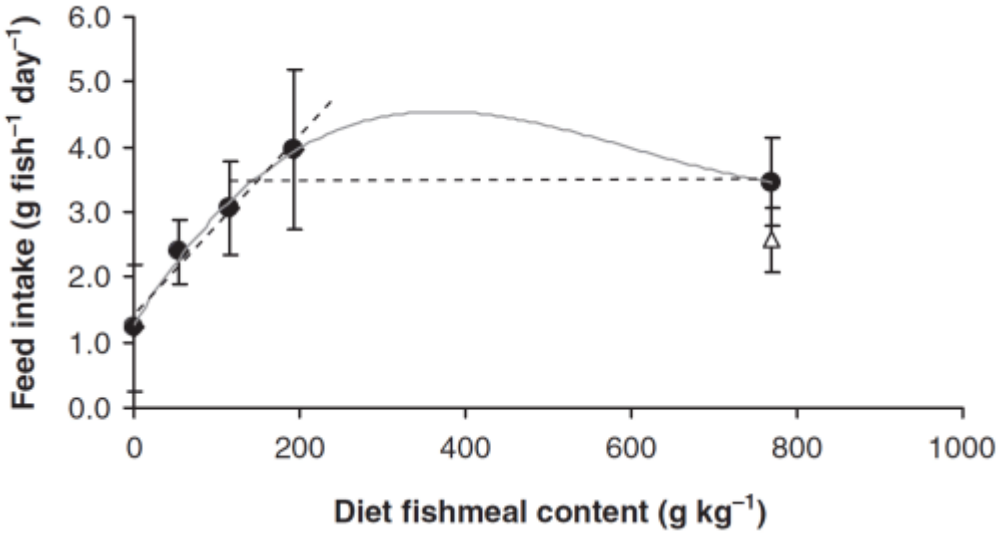
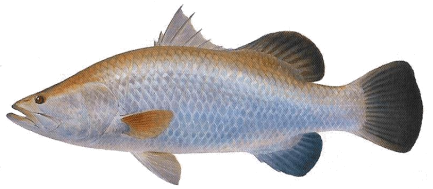


Figure 1 Feed intake (g fish^{-1} day^{-1}) of juvenile barramundi fed diets with varying fishmeal content (●), when the diets are prepared on an equivalent digestible protein and energy basis blended with lupin protein concentrate. Indicated also is the diet with 10 g kg^{-1} of sodium sulfamerazine (△) as a feed intake deterrent.

- All diets formulated to equal DPro and DE
- Critical threshold to feed intake estimate at ~15% FM inclusion.

Moving to Complete Replacement?



- Complete replacement of FM had clear impacts on intake, growth and FCR.
  - Effects were clearly intake linked.
  - Threshold refined to <10%.
- Replacement of the FO (with ricebran oil) had a no significant impacts on performance.

Aquaculture 451 (2016) 298–309

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journal homepage: www.elsevier.com/locate/aqua-online

ELSEVIER

An evaluation of the complete replacement of both fishmeal and fish oil in diets for juvenile Asian seabass, *Lates calcarifer*

Brett Glencross [a,*], David Blyth [b], Simon Irvin [b], Nicholas Bourne [a], Marcel Campet [c,1], Pascal Boisot [d], Nicholas M. Wade [a]

[a] CSIRO Agriculture Flagship, PO Box 2583, Brisbane, QLD 4001, Australia
[b] CSIRO Agriculture Flagship, 141 North St, Woorim, QLD 4706, Australia
[c] InVivo NSA, An Phu, Binh Duong Province, Viet Nam
[d] InVivo NSA, St Nolff 56250, France

A. Weight Gain

	30%FM	20%FM	10%FM	0%FM	mean
FO 100%	189.8	196.4	175.6	167.5	182.3
FO 30%	198.2	199.0	189.5	172.5	189.8
FO 15%	221.0	190.0	199.0	167.6	194.4
FO 0%	201.9	167.1	197.1	171.9	184.5
mean	202.7	188.1	190.3	169.9	

Summary Statistics	*F*	*p value*
Fishmeal (FM)	4.839	**0.007**
Fishoil (FO)	0.714	0.551
FM x FO	0.857	0.572

B. Intake

	30%FM	20%FM	10%FM	0%FM	mean
FO 100%	195.2	194.5	184.6	181.1	188.9
FO 30%	204.7	201.0	192.7	182.0	195.1
FO 15%	230.8	187.3	201.4	176.2	198.9
FO 0%	215.6	174.1	198.4	193.9	195.5
mean	211.6	189.2	194.3	183.3	

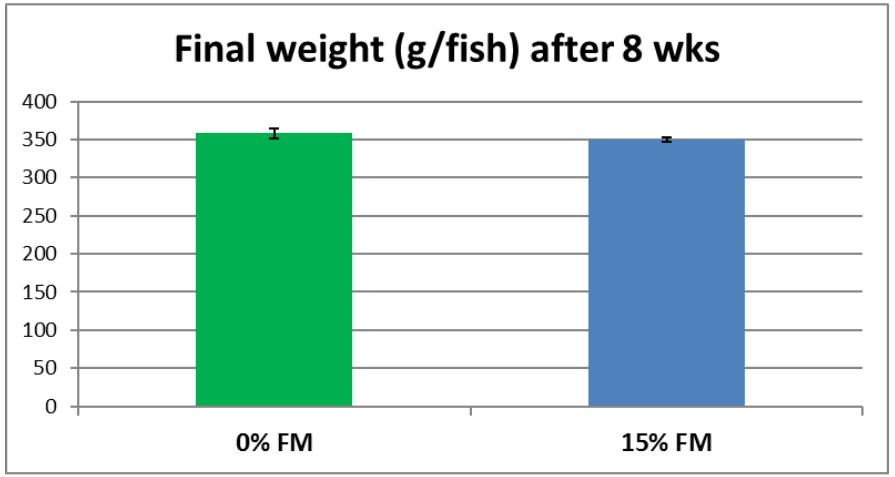
Summary Statistics	*F*	*p value*
Fishmeal (FM)	5.623	**0.003**
Fishoil (FO)	0.667	0.578
FM x FO	1.285	0.283

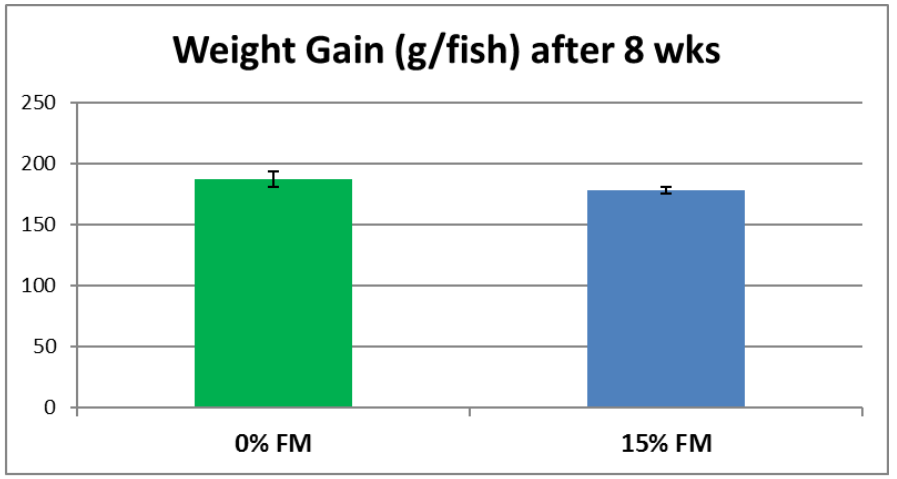
C. Feed Conversion

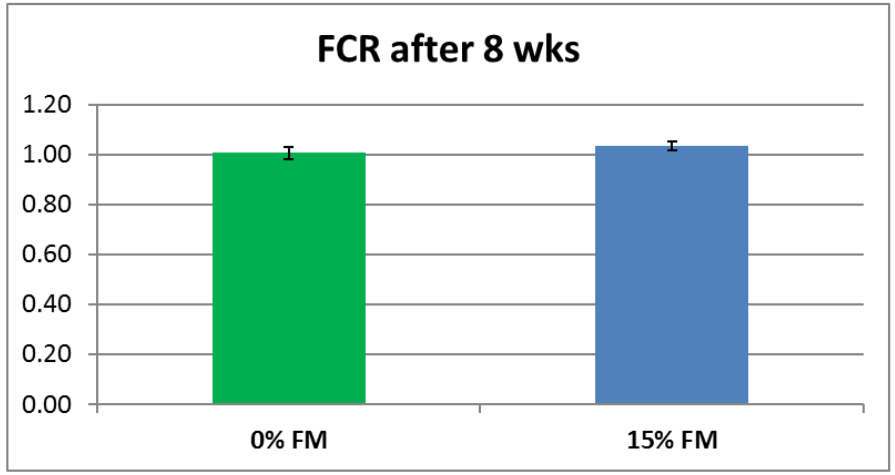
	30%FM	20%FM	10%FM	0%FM	mean
FO 100%	1.03	0.99	1.05	1.08	1.04
FO 30%	1.03	1.01	1.02	1.06	1.03
FO 15%	1.04	0.99	1.01	1.05	1.02
FO 0%	1.07	1.04	1.01	1.13	1.06
mean	1.04	1.01	1.02	1.08	

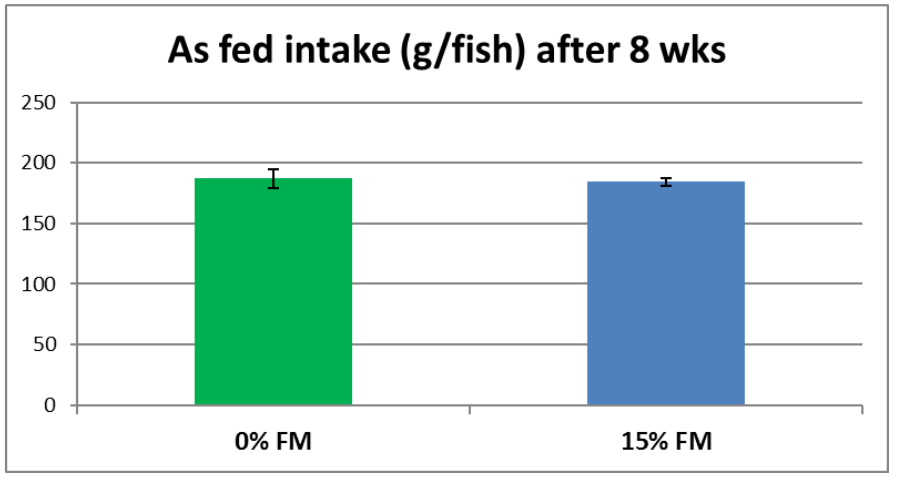
Summary Statistics	*F*	*p value*
Fishmeal (FM)	3.114	**0.040**
Fishoil (FO)	0.809	0.498
FM x FO	0.478	0.879

Zero Fishmeal…









- Attractants used to avert palatability issues
- Initial weight = 171 g/fish, Temp = 27 - 29ºC, Salinity = 32 g/L
- 6 reps per treatment, Fed twice daily

Suri Tani Pemula (Japfa Comfeed Group) - Ciranjang Research Station (West Java, Indonesia)

Ingredients	Commercial	No Fish Meal
Soybean meal, 48%	24.9	41.2
Fish meal, SE Asia, 57-59% CP	10.1	-
Corn, yellow	7.7	2.1
Wheat flour	18.0	18.0
Meat and bone meal, 53% CP	7.0	7.0
Poultry by-products meal, 65% CP	5.5	5.5
Feather meal, 80% CP	2.6	0.7
Fish oil, SE Asia	1.1	1.7
Soy lecithin	1.0	1.7
Palm olein	0.9	0.3
L-Lysine HCl	0.11	0.09
DL-Methionine (99%)	0.06	0.12
Common ingredients, vitamin, and minerals	21.0	21.7
Chemical Composition (analyzed, as is)		
Dry matter, %	87.6	89.0
Crude protein, %	32.1	32.6
Lipids, %	6.3	6.2

Performance of African catfish (*Clarias gariepinus*) fed commercial feeds with or without fish meal

Treatment	Biomass Initial	Biomass Final	FCR Biomass	IBW	FBW	TGC	FCR Individual	Mort.
	kg/tank	kg/tank	Feed:Gain	g/fish	g/fish	%	Feed:Gain	%
Commercial Type	0.442	15.1	1.30	5.5	282	0.144	1.01	33
No Fish meal	0.440	11.9	1.33	5.5	222	.129	1.04	33

Shrimp Performance and Fishmeal

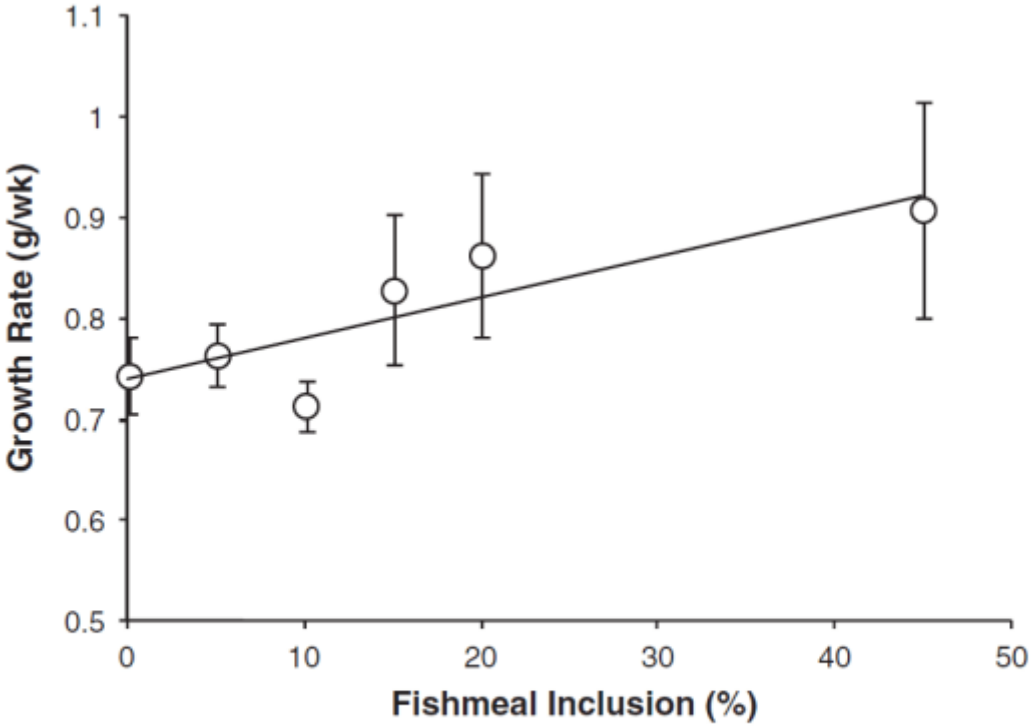


Fig. 1. Effect of fishmeal reduction in formulation on growth rate of shrimp in Experiment 1. Shown are the linear regressions through the means ± SEM of the data.

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Effective use of microbial biomass products to facilitate the complete replacement of fishery resources in diets for the black tiger shrimp, *Penaeus monodon*

Brett Glencross [a,b,*], Simon Irvin [a,c], Stuart Arnold [a,c], David Blyth [a,c], Nicholas Bourne [a,b], Nigel Preston [a,b]

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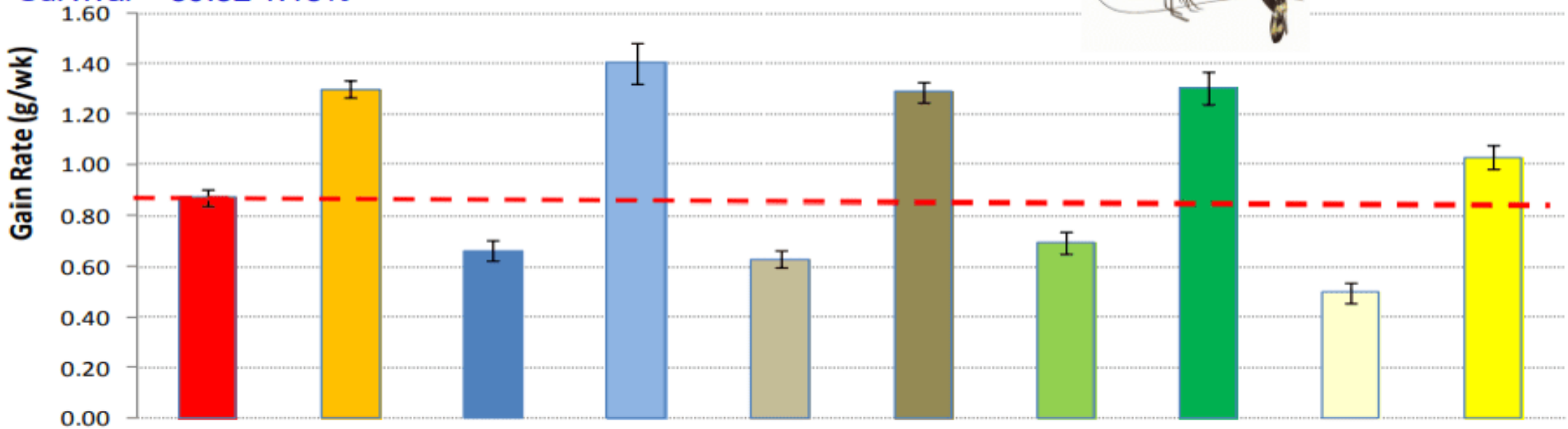
[c] CSIRO Marine and Atmospheric Research, PO Box 2066, Woorim, QLD 4507, Australia

- All diets formulated to equal CPro and GE, and balanced for EAA demands.
- More difficult to reconcile effects with feed intake due to issues of measuring this accurately in shrimp.

Performance and Fishmeal Replacement

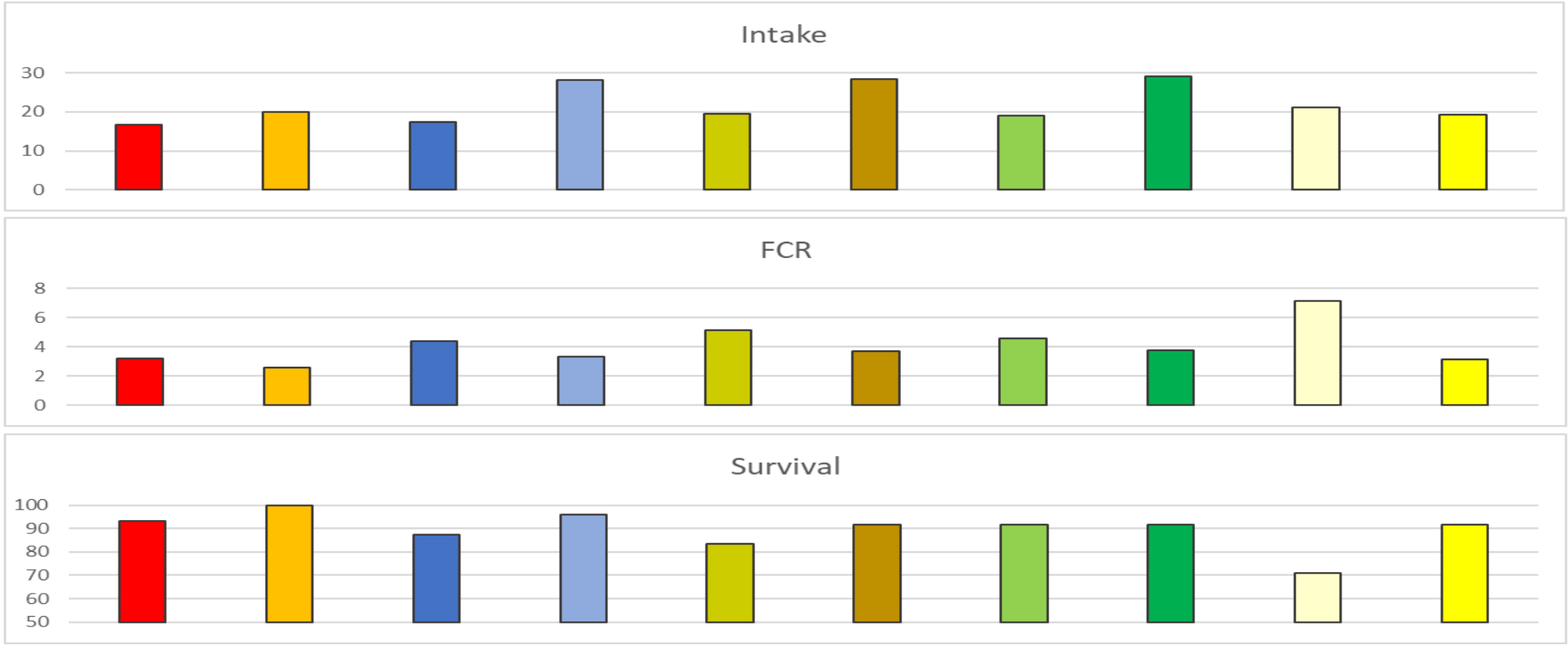
- **Indoor Tank Trial – 2**
- All diets formulated to 42%Protein, 7%Lipid
- Lupin kernel meal and Poultry Offal Meal used as alternative protein sources, Linseed oil as alternative oil
- Each treatment replicated n=5
- Trial run for 42days
- Survival = 89.8± 1.13%

P. monodon



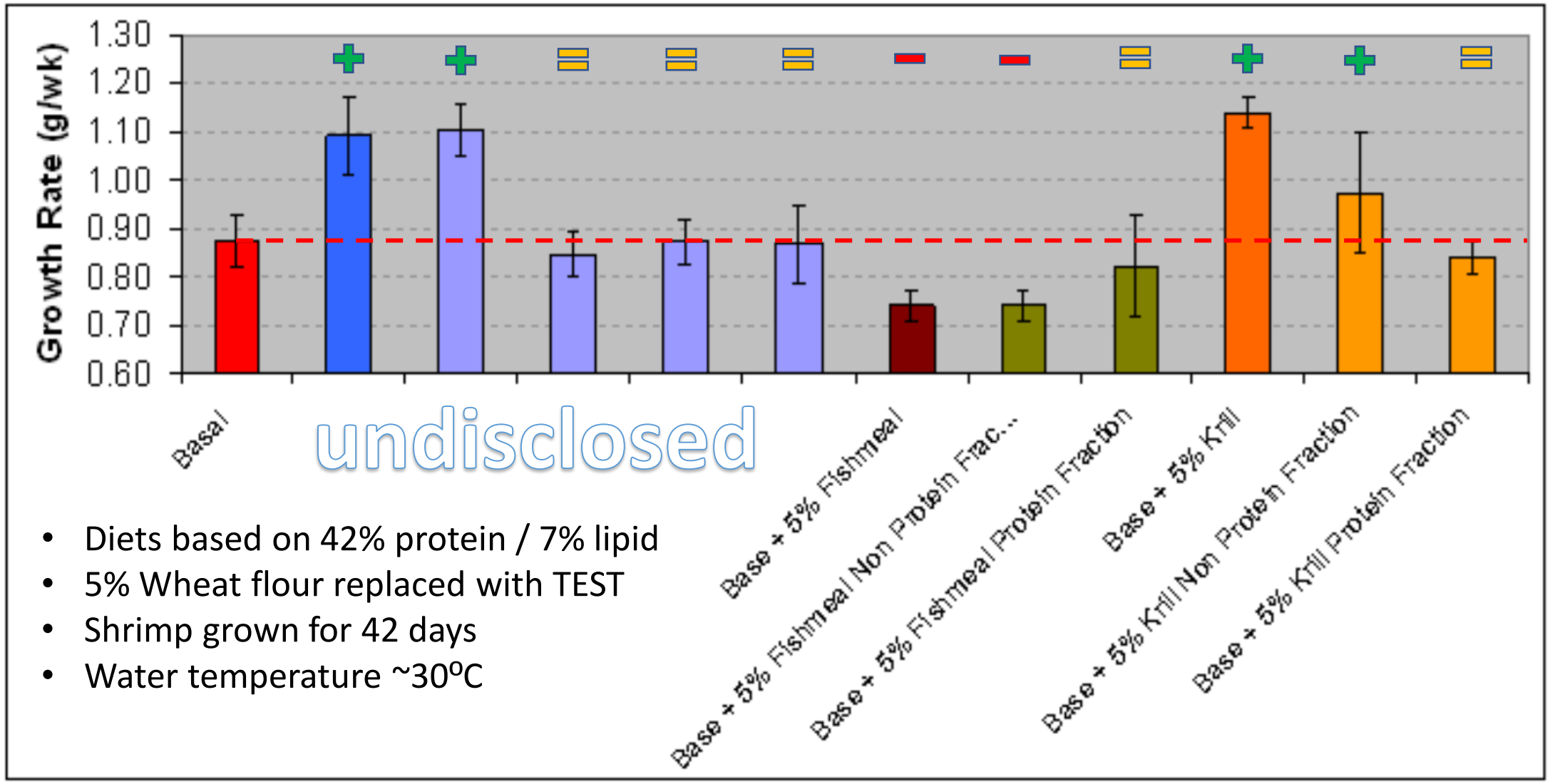
Fish meal	50%	50%	10%	10%	N	N	10%	10%	N	N
Fish oil	2%	2%	2%	2%	2%	2%	N	N	N	N
Bioactive	N	10%	N	10%	N	10%	N	10%	N	10%

Performance and Fishmeal Replacement



Fish meal	50%	50%	10%	10%	N	N	10%	10%	N	N
Fish oil	2%	2%	2%	2%	2%	2%	N	N	N	N
Bioactive	N	10%	N	10%	N	10%	N	10%	N	10%

Isolating Bioactives in Ingredients



- Diets based on 42% protein / 7% lipid
- 5% Wheat flour replaced with TEST
- Shrimp grown for 42 days
- Water temperature ~30^{o}C

Bioactives in Marine Ingredients

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Evidence of a growth factor in some crustacean-based feed ingredients in diets for the giant tiger shrimp *Penaeus monodon*

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Our studies, while providing evidence for the presence of a shrimp growth factor in crustacean meals, do not identify the nature of the factor nor specifically its mode of action. However, we are able to conclude that the growth factor is almost certainly of insoluble protein origin. It is possibly the residue of one or more of the bioactive neurosecretory hormones of the X-organ-sinus gland complex and which in the

Fishmeal Replacement

- Cost-effectively meeting nutritional requirements of animals
- Production risk management
  - e.g. Disease and stress resistance of animals
- Feed characteristics
  - From the fish perspective (palatability)
  - From the farmers' perspective (smell, colour)
- Final product quality / composition