

# The Challenge of Defining and Meeting the Essential Amino Acid Requirements of Fish:

Dominique P. Bureau



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# BACKGROUND

- Fish meal is increasingly being replaced by more economical protein sources with different amino acid profiles.
- Increase reliance on ingredients with poorer amino acid profiles, brings the need to pay greater attention to the EAA requirements of fish.
- Composition of aquaculture feeds has evolved rapidly and these feeds can be formulated to widely different protein, lipids and digestible energy levels.
  - Salmonid feed composition can vary from: 33-60 % CP and 12–40% lipid.
- Growth rates and feed efficiencies achieved today are much better in those in the past.
- This impose a significant challenge to our ability to interpret information on nutrient requirements in fish in the literature and then make practical recommendations.



# **Complementarity of Corn Gluten Meal and Soybean Meal as Protein Sources in the Diet of Young Atlantic Salmon**

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Department of Animal and Poultry Science  
University of Guelph, Ontario  
CANADA**

**Presented at the IX International Symposium on Nutrition and Feeding in  
Fish, Miyazaki, Japan, 2000.**

# Materials and Methods

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## **Fish:**

**Atlantic salmon (*Salmo salar* L.)  
Anadromous, LaHave River strain (wild)  
initial body weight = 5.3 g/fish**

## **Experimental Design and Conditions:**

**Six diets, 3 replicates  
Water temperature = 15°C  
Duration = 24 weeks**

## **Diets:**

**40% digestible protein (DP)  
20 MJ/kg digestible energy (DE)  
20 g/MJ DP/DE  
Nutrients in excess of NRC (1993) requirements**

# Diet Formulation

Ingredients	Diets					
	1	2	3	4	5	6
Fish meal, herring, 68% CP	20	20	20	20	56	38
Corn gluten meal, 60% CP	10	20	30	40	-	20
Soybean meal, 48% CP	35	23	12	-	-	-
Blood meal, spray-dried	5	5	5	5	5	5
Whey	8.3	9	9	9	10	10
Starch, raw	-	1.4	2.4	4.5	10	7
CaHPO <sub>4</sub>	0.4	0.4	0.4	0.4	-	-
Vitamins and minerals	2	2	2	2	2	2
Fish oil, herring	19.3	19.2	19.2	19.1	17	18
Total	100	100	100	100	100	100

# Performance of Atlantic salmon fed over 24 weeks

Parameters	Diets					
	1	2	3	4	5	6
Final body weight, g/fish	83 bc	88 a	87 a	81 c	86 ab	87 a
Feed efficiency, G:F	1.16b	1.24a	1.22a	1.16b	1.21a	1.24a
TGC	0.104bc	0.107a	0.107a	0.103c	0.106ab	0.107a
Nitrogen gain, g/fish	2.2	2.3	2.2	2.2	2.3	2.3
Energy gain, kJ/fish	689	731	751	693	698	706

**Initial body weight = 5.3 g/fish**

**Thermal-unit growth coefficient (TGC) =  $(FBW^{1/3} - IBW^{1/3}) / (\text{Day} * ^\circ\text{C})$**

# BACKGROUND

- The mode of expression of amino acid requirements of fish is a topic of disagreement between fish nutritionists.
  - I. Kim *et al.*, 1991; NRC, 1993: consider that EAA requirements are best expressed as a percentage of diet (**% diet**).
  - II. Rodehutscord *et al.*, 1997; EAA requirements should be expressed in relation to the diet energy content (e.g. **g/MJ DE**).
  - III. Cowey and Cho, 1993: EAA requirements are best expressed in relation to the dietary protein content (**% protein** or **g/16 g N**).
- Individual EAA levels deemed adequate in the diet may be different depending on: mode of expression adopted, composition of diet and amino acids profile of the ingredients.

## Different Modes of Expression = Dramatically Different and Largely Contradictory Assumptions

- **% of diet:** Assumes that the diet composition has no effect on amino acid requirement (relative to the “mass” of diet).
- **g/MJ digestible energy (DE):** Assumes that the amino acid requirement is directly to DE intake. Higher DE will need to be higher in EAA compared to lower DE feeds (since lower feed intake with high DE feeds).
- **% of protein:** Assumes when excess amino acid are catabolized for energy, first limiting amino acid is not spared compare to other, less limiting, amino acids. Assumes that if formulate to amino acid levels in excess of requirement, excess protein must be “balanced” (respect certain proportion for each amino acids) .

**One can make a case for and against each of these modes of expression**



# Arginine requirement of rainbow trout fish according to three different modes of expression.

<b>References</b>	<b>Requirement</b>
<b>NRC (1993)</b>	<b>1.5 % diet</b>
<b>Rodethutscord et al (1997)*</b>	<b>1.0 g/MJ digestible energy</b>
<b>Mambrini and Guillaume (1999)</b>	<b>4.4 % protein (g/ 16 g N)</b>

## NRC (1993) Essential Amino Acid Requirements Computed According to Different Schools of Thoughts

EAA Requirement	Lys	Met+ Cys	Arg	Thr	Trp	His	Val	Leu	Iso	Phe+ Tyr	Sum EAA
%diet	1.8	1.0	1.5	0.8	0.2	0.7	1.2	1.4	0.9	1.8	11.3
g/MJ DE	1.2	0.67	1.00	0.53	0.13	0.47	0.80	0.93	0.60	1.20	7.53
% protein	4.8	3.3	4.4	2.0	0.6	1.6	5.3	3.6	2.0	5.3	-

Encarnação and Bureau (2001)

**TABLE 6**

*Recommended dietary amino acid concentrations*

	Lysine	Methionine <sup>1</sup>	Arginine <sup>2</sup>	Threonine <sup>2</sup>	Tryptophan	Histidine	Valine	Leucine	Isoleucine
Own results <sup>3</sup>									
dry matter, g/kg	27.7	8.0	11.5	10.3	2.0	5.8	15.7	13.6	13.7
digestible energy, g/MJ	1.38	0.40	0.57	0.51	0.10	0.29	0.78	0.68	0.68
NRC (1993)									
digestible energy, <sup>4</sup> g/MJ	1.19	0.66 <sup>5</sup>	0.99	0.53	0.13	0.46	0.80	0.93	0.60

<sup>1</sup> Results from Rodehutschord et al. (1995a).

<sup>2</sup> Results from Rodehutschord et al. (1995b).

<sup>3</sup> Concentrations required to reach 95% of plateau in protein deposition.

<sup>4</sup> Recalculated values.

<sup>5</sup> Methionine + Cystine.

Rodehutschord et al. (1997)

# Digestible Lysine Content of Experimental Diets

Digestible Lysine	Diet			
	1	2	3	4
Calculated content, % DM	3.06	2.74	2.45	2.12
% above/under requirement:				
NRC (1993), % diet	70	52	36	18
NRC (1993), g/MJ DE	20	6	-6	-20
Guillaume et al. (1999), g/16 g N	51	34	17	1

# Performance of Atlantic salmon fed over 24 weeks

Parameters	Diets					
	1	2	3	4	5	6
Final body weight, g/fish	83 bc	88 a	87 a	81 c	86 ab	87 a
Feed efficiency, G:F	1.16b	1.24a	1.22a	1.16b	1.21a	1.24a
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Whey	8.3	9	9	9	10	10
Starch, raw	-	1.4	2.4	4.5	10	7
CaHPO <sub>4</sub>	0.4	0.4	0.4	0.4	-	-
Vitamins and minerals	2	2	2	2	2	2
Fish oil, herring	19.3	19.2	19.2	19.1	17	18
Total	100	100	100	100	100	100

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Solution : Compute EAA content of feeds using three modes of expression and use highest value?!

# Mode of Expression Adopted will Result in Different Formulation Targets!

Composition	Starter	Grower	High energy
Crude Protein, %	51	44	38
Lipids, %	16	28	33
Digestible energy, MJ/kg	17	20	22

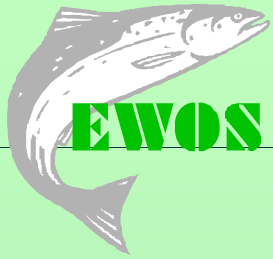
School of thought\*\*      **Lysine level deemed adequate (g/kg feed)**

1) % diet	18	18	18
2) g/MJ DE	22	24	26
3) % Protein	25	21	18

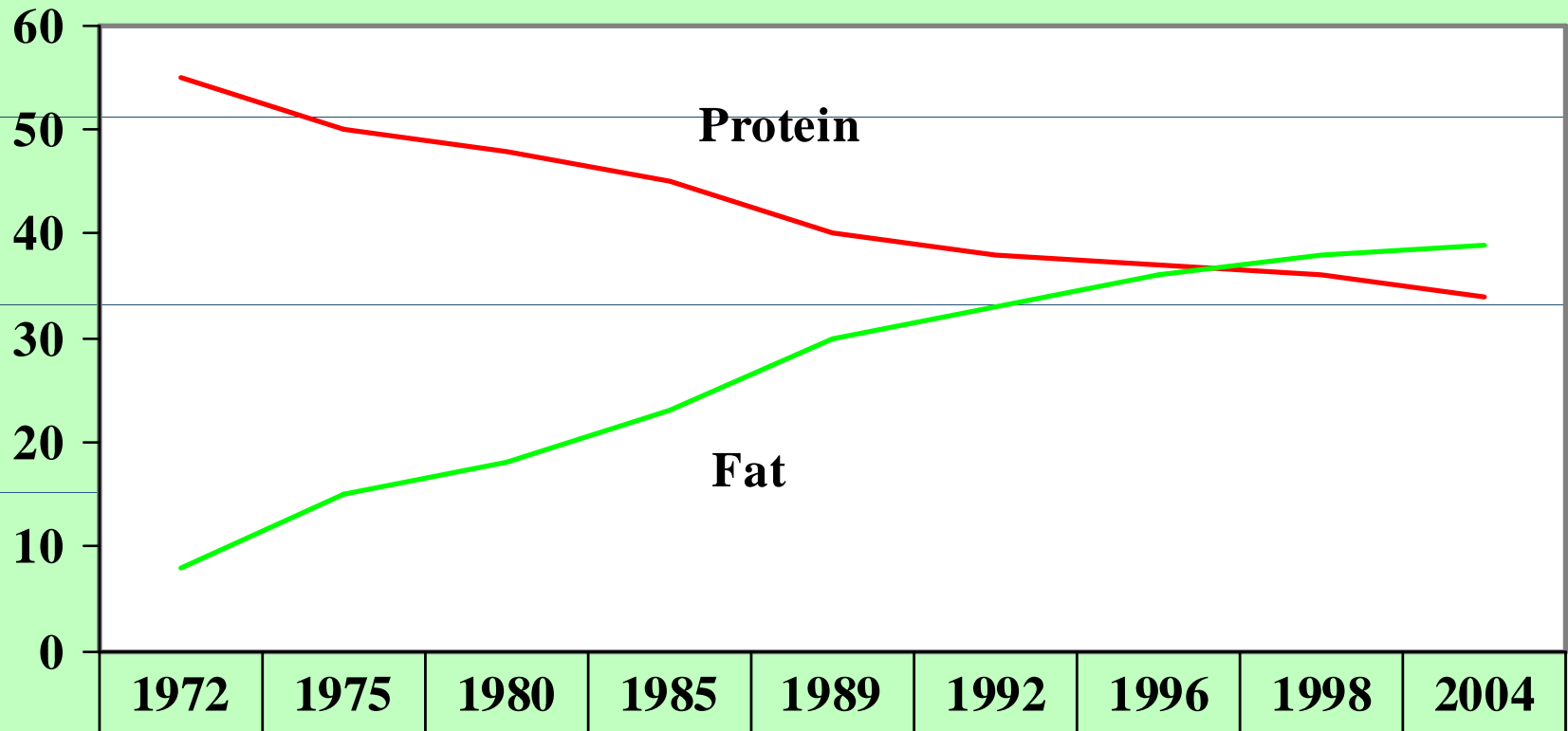
**Why are we spending so much effort on research?**

High-Low, % difference	36	33	47
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**How can we expect feed manufacturers to be able to least-cost feeds?**



# Protein/fat development in salmon feed 1972 - 2004



— Protein %	55	50	48	45	40	38	37	36	34
— Fat %	8	15	18	23	30	33	36	38	39

Grower feed Norway

Greatly reduced amount of feed needed for one kg biomass gain



# Central Questions

**How reliable are estimates of amino acid requirements found in the reference literature (e.g. NRC, 1993)?**

**What the best mode of expression of essential amino acid requirements?**

**How does composition of the diet affect essential amino acid utilization and requirements?**

**How does fish species, life stage, growth rate, feed efficiency, etc. affect utilization and requirement of essential amino acids?**

## Meeting NRC (1993) Lysine Requirement (1.8% diet)

Ingredients	Diet			
	1	2	3	
Fish meal	40	18	18	
Corn gluten meal	11	49	49	
Fish oil	14	14	14	
L-Lysine	-	-	0.5	
<b>Composition</b>				
Digestible protein, %	43	43	43	
Digestible energy, MJ/kg	19	19	19	
Digestible Lysine				<b>NRC</b>
% diet	3.2	1.8	2.3	<b>1.8</b>
% protein	7.4	4.0	5.2	<b>4.8</b>

## Performance of Rainbow Trout Fed Diets Meeting NRC (1993) Lysine Requirement (1.8% Diet) vs. Diet with >2.2% Lysine

Digestible Lysine	Diet			NRC
	1	2	3	
% diet	3.2	1.8	2.3	1.8
% protein	7.4	4.0	5.2	4.8

### Part 1 (Week 1-12)

Growth rate, TGC  
Feed eff., gain:feed

0.26a	0.21b
1.19a	0.94b

### Part 2 (Week 13-16)

Growth rate, TGC  
Feed eff., gain:feed

0.26a	0.28a
1.07a	1.11a

\*TGC = 100 (FBW<sup>1/3</sup> - IBW<sup>1/3</sup>) / (Temp. (°C) \* days)

## Lysine Requirement of Rainbow Trout – Summary of Published Studies

Reference	CP	Lipid	No. of Levels	Lysine Conc.	TGC	Response variable	Model	Est. Lysine Requirement
	%	%	n	%	%			
Ketola (1983)	47	12	5	0.5-2.9	0.12	Weight gain	ANOVA	2.9% diet
Walton et al.(1984)	45	17	7	1.0-2.6	0.17	Weight gain	Broken Line	1.9% diet
Lanari et al.(1991)	40	n/a	n/a	n/a	n/a	Weight gain	Broken Line	2.2% diet
Kim et al.(1992)	35	10	8	0.7-1.6	0.20	Weight gain	Broken Line	1.3% diet
Pfeffer et al.(1992)	47	15	8	1.5-3.0	0.13	Protein gain	Polynomial	1.8% diet
Rodehutschord et al. (1997)	32	28	21	0.5-5.8	0.23	Weight gain	Exponential	2.3% diet
Encarnação et al.( 2004)	40	24	6	1.2-2.4	0.22	Weight gain	Exponential	2.3% diet

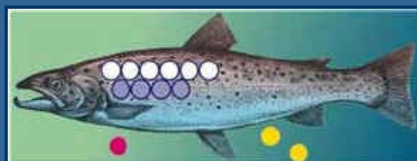
NRC (1993) “established” lysine requirement at 1.8% diet

# Effect of Diet Composition on Lysine Utilisation and Requirement in Rainbow Trout (*Oncorhynchus mykiss*)

PhD Thesis

Pedro Encarnação

Fish Nutrition Research Laboratory  
Department of Animal and Poultry Science  
University of Guelph



# OBJECTIVES

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- Generate information required to improve our understanding of the factors affecting EAA utilization and requirements of fish.
- Examining the effects of diet composition (DE and different energy-yielding nutrients) on lysine utilisation and requirements of rainbow trout.

# Study 1

Effect of dietary DE level on lysine requirements and utilization by rainbow trout

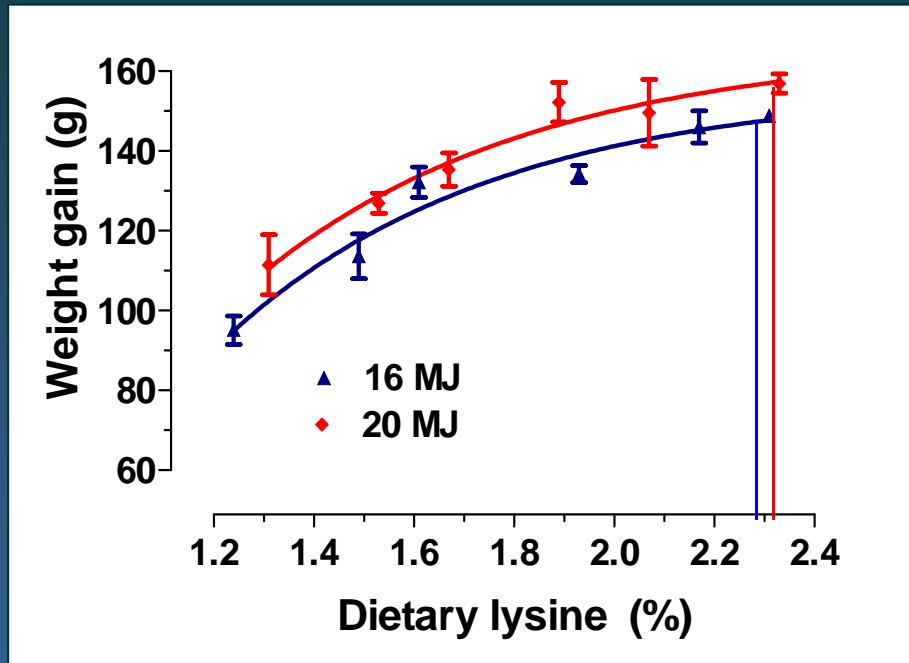
## OBJECTIVES

To assess the effect of DE level/intake in the diet on lysine requirements :

- How does DE intake or dietary level affect the lysine intake and dietary requirement ?
- How DE level affects lysine utilization?

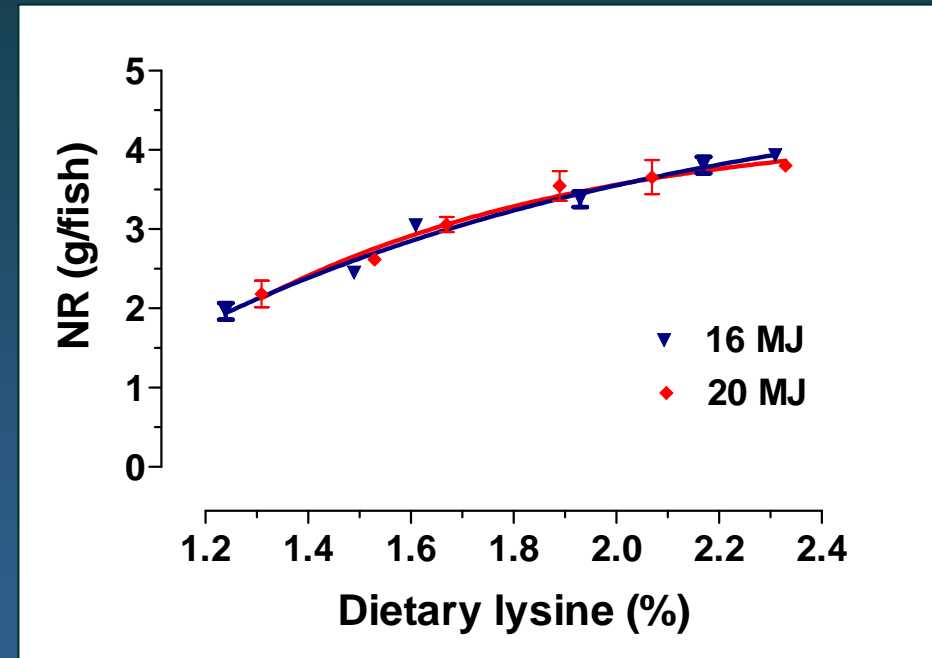
# RESULTS

Figure 1 - Live weight gain in response to lysine intake at two DE levels.



- ✓ Significant linear and quadratic effects to lysine and DE levels, no effect of DE level on lysine requirements (2.28 vs 2.33 %)

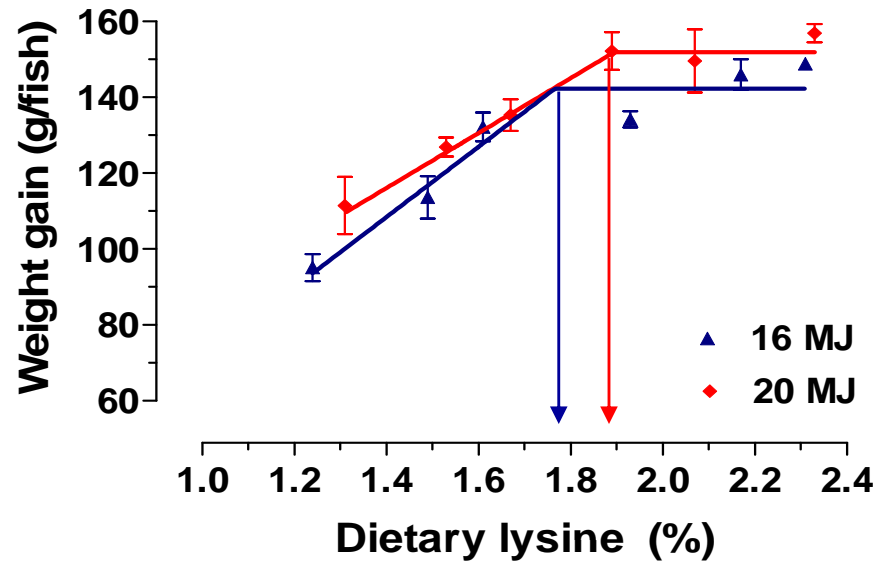
Figure 2 – N retention in response to dietary lysine concentration at two DE levels.



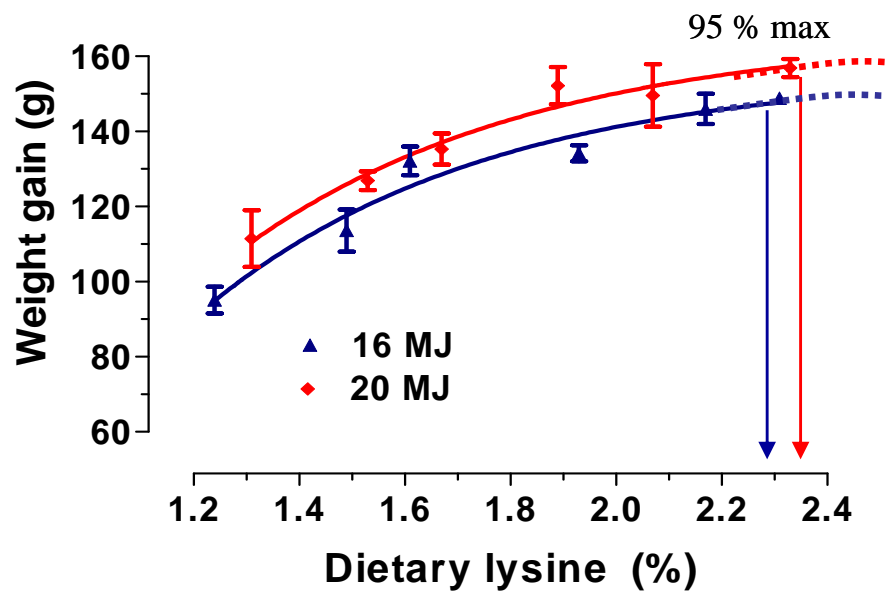
- ✓ No effect of dietary DE level on nitrogen retention.



# Model Adopted Can Significantly Affect Estimate of Requirement

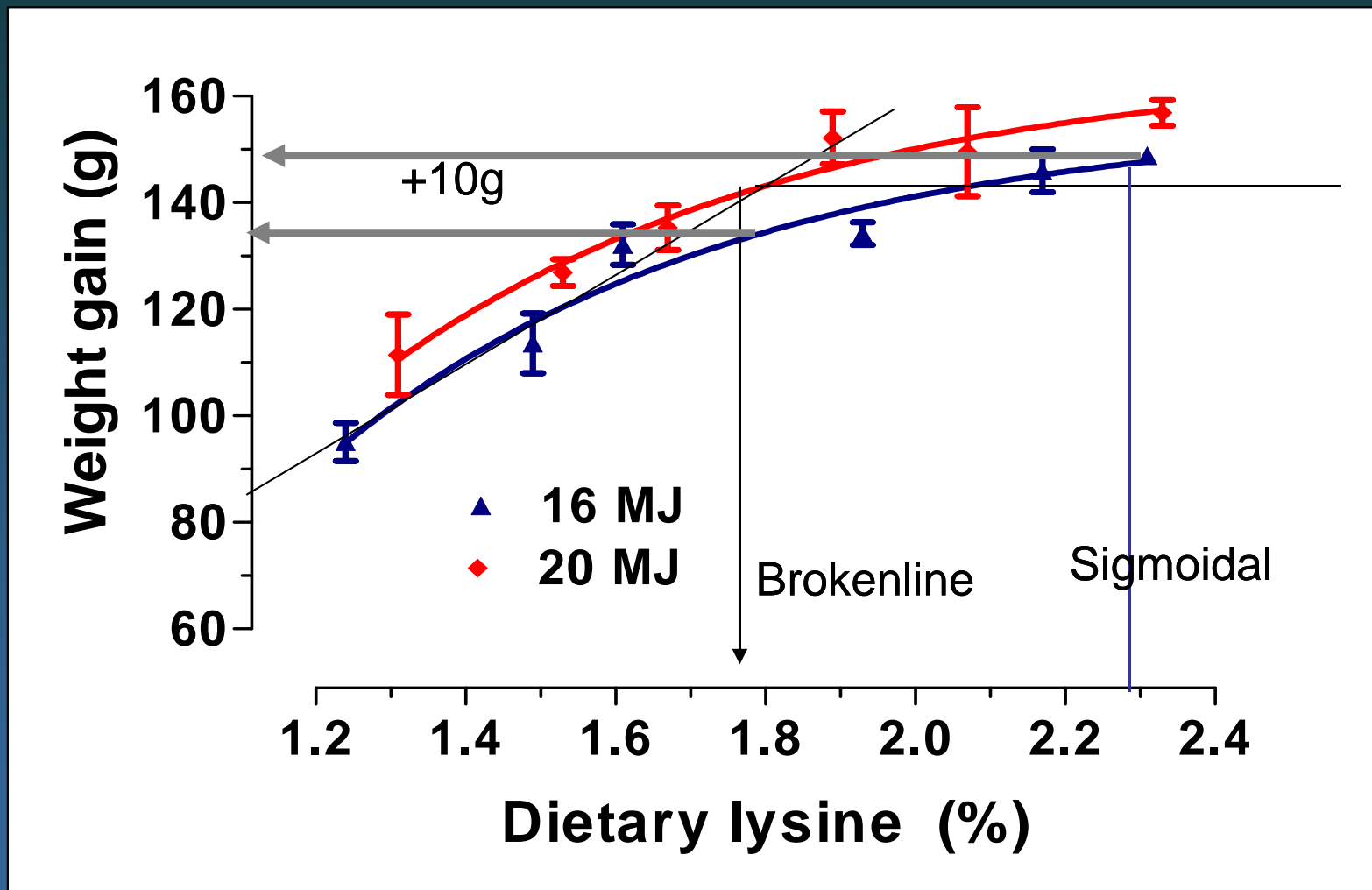


Broken line model  
= 1.8% diet

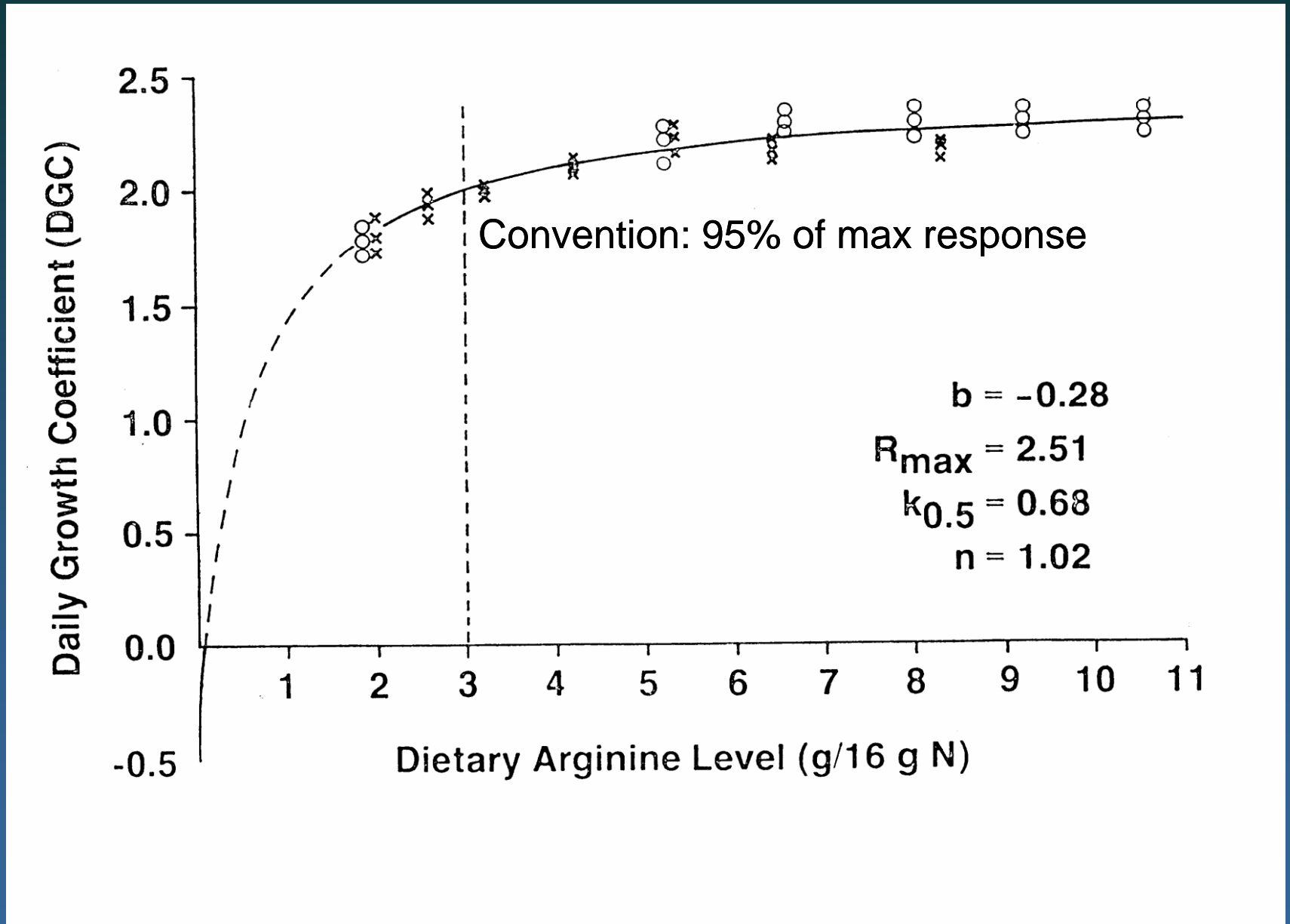


Nutritional kinetic model  
= 2.3% diet

# Model Adopted Can Very Significantly Affect Estimate of Requirement



# Response of Rainbow trout to Increasing Arginine Levels



# Expressing Lysine Requirement as % of the Protein Content of the Diet is not Entirely Appropriate

**Encarnacao et al. (2004):**

**Estimate of requirement :2.3% of diet DM  
Diet: 40% crude protein**

**Estimated requirement = 5.75 g/ 100 g Protein**

**Rodehutscord (1997)**

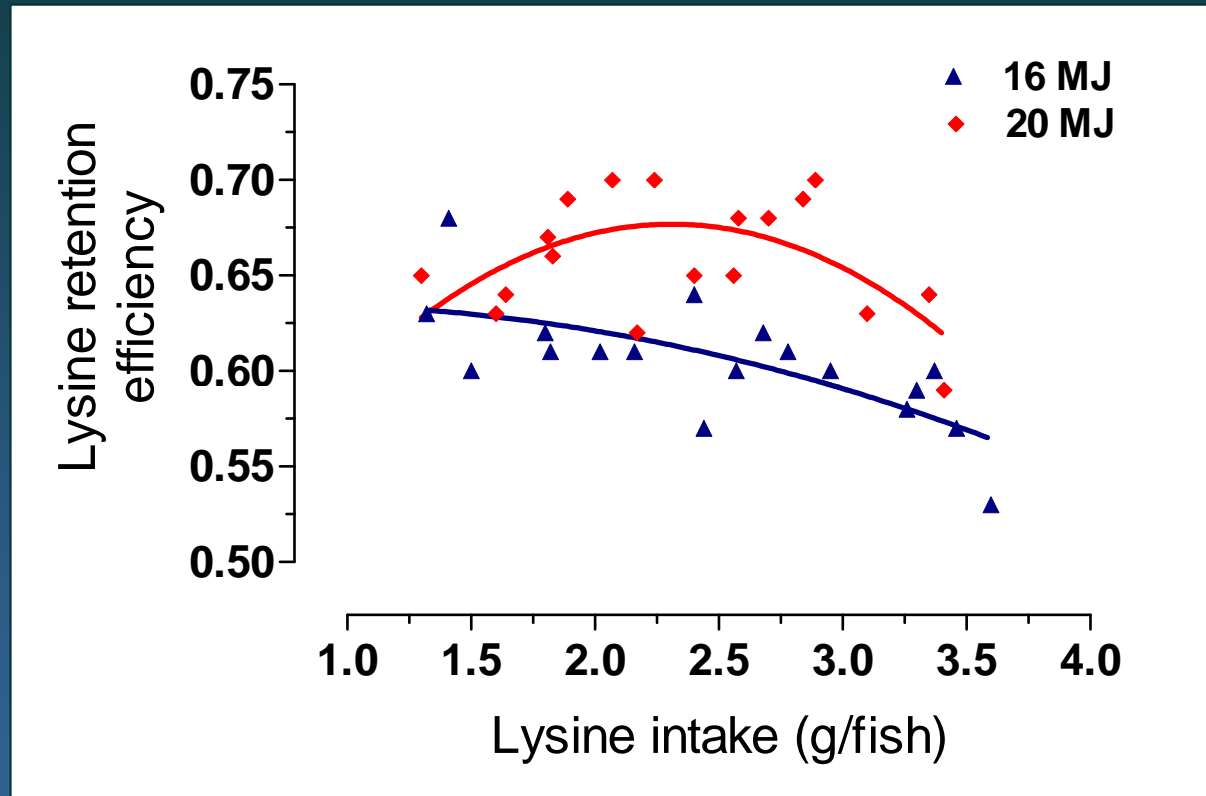
**Estimate of requirement :2.3% of diet DM  
Diet: 32% crude protein**

**Estimated requirement = 7.25 g/ 100 g Protein**

**Increasing number of other studies suggest that expressing amino acid requirement as % of the protein of the diet is not entirely appropriate, unless formulating to very low protein level where all amino acids are equally limiting.**

# RESULTS

Fig. 3 – Lysine efficiency in response to the lysine intake of fish.



- ✓ Higher efficiency of lysine utilization at higher dietary DE levels.

# CONCLUSIONS

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- Expressing EAA requirements in relation to DE content of the diet is not appropriate.
- Diet digestible energy (DE) content affects marginal efficiency of lysine utilization for protein deposition.
- When lysine was limiting, additional energy supplied by fish oil allowed lysine to be spared for protein deposition.
- Regulation of EAA utilization in fish could be different from other monogastric animals, at least pigs.

## Mode of Expression Adopted will Result in Different Formulation Targets!

Composition	Starter	Grower	High energy
Crude Protein, %	51	44	38
Lipids, %	16	28	33
Digestible energy, MJ/kg	17	20	22
School of thought**	Lysine level deemed adequate (g/kg feed)		
1) % diet	18	18	18
2) g/MJ DE	22	24	26
3) % Protein	25	21	18
High-Low, % difference	36	33	47

# Estimating Dietary Lysine Requirements for Live Weight Gain and Protein Deposition in Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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1 Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-Sen University, Guangzhou, P. R. China

2 Department of Animal and Poultry Science, University of Guelph, Ontario, Canada

3 Evonik-Degussa Corporation, Kennesaw, GA, 30144, USA

**Presented at the XIV International Symposium on Nutrition and Feeding in Fish, Qingdao, China, 2010.**



# Introduction

Most of the estimates of essential amino acid (EAA) requirements have been determined based on live body weight gain as the response criteria.

Results from a number of studies have suggested that lysine requirement for maximum protein gain of rainbow trout is significantly higher than that for maximizing weight gain.

Reference	Estimates of Lysine Requirement	
	Live weight gain	Protein gain
	% diet DM	
Pfeffer et al. (1992)	1.8	2.2
Rodehutscord et al. (1997)	2.3	2.7
Encarnaç�o et al. (2004)	2.3	~2.7

The experimental design (# of treatments, range of dietary lysine levels, # of replicates) of these studies was not sufficiently powerful to confidently determine if requirement for body protein gain is greater than that for live body weight gain.

There is insufficient information on effect of EAA on body composition as well as on efficiency of EAA utilization (useful information for nutritional models).

# Objectives

- 1) To compare estimates of lysine requirement of rainbow trout using live weight gain and body protein deposition as the response criteria and different response fitting models.
- 2) To determine the efficiency of lysine utilization by rainbow trout

# Materials and Methods

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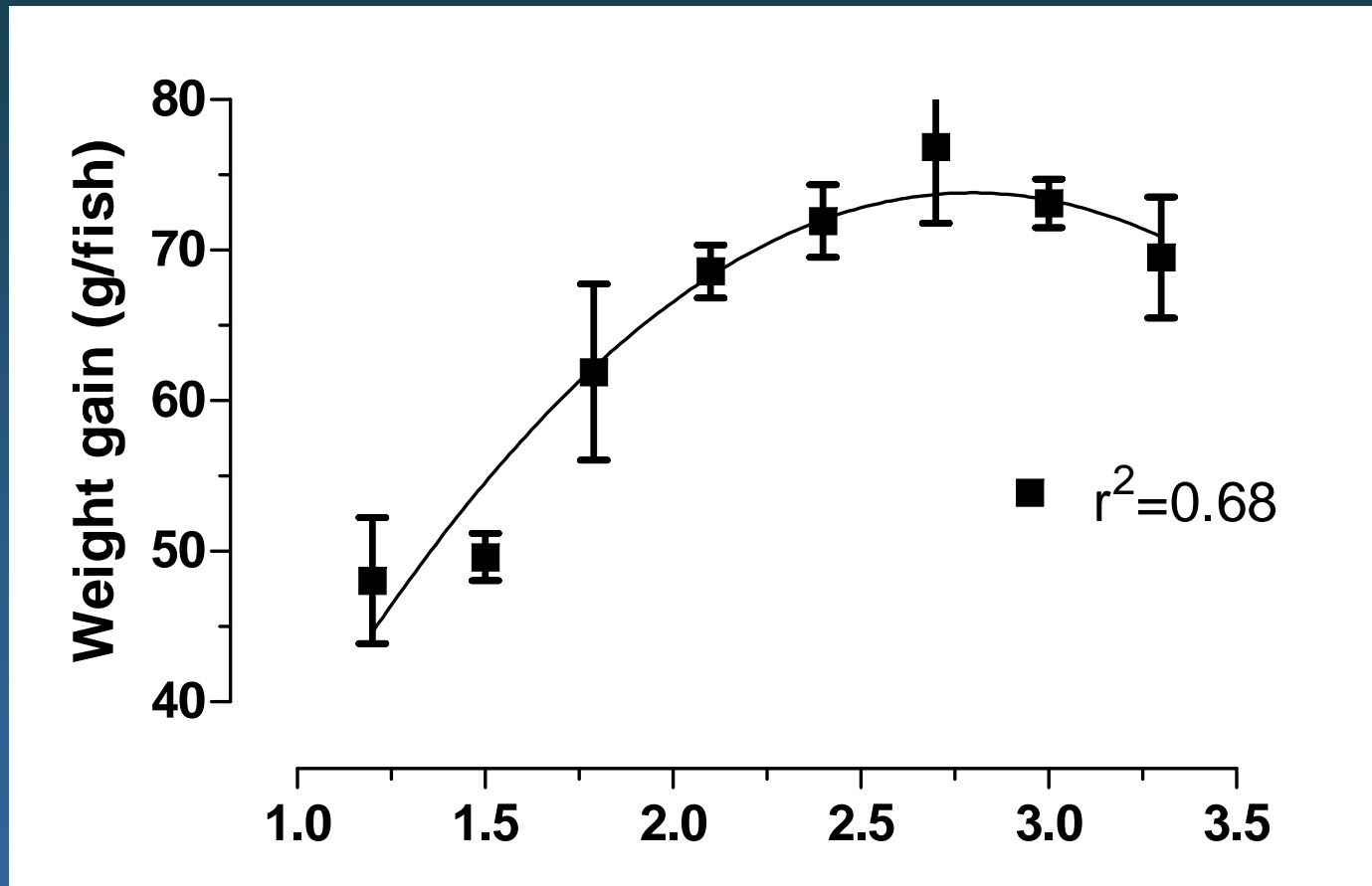
**Fish:** Rainbow trout (*Oncorhynchus mykiss*)  
initial body weight = 5 g/fish

**Design:** 9 diets, 4 replicates  
Complete Randomized Block Design  
Water temperature =15°C  
Duration= 12 weeks

**Diets:** >42% digestible protein (DP)  
19 MJ/kg digestible energy (DE)  
Nutrients >> in excess of NRC (1993) req.  
EAA levels >110% of Rodehutscord (1997)  
except lysine

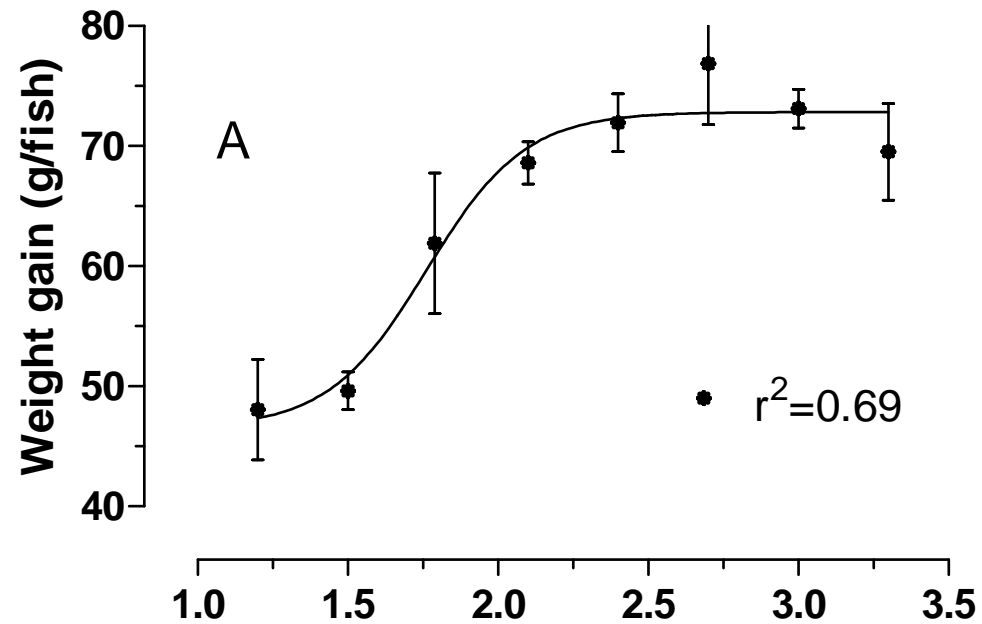
# Results

**Criteria:** Live weight gain **Model:** Quadratic



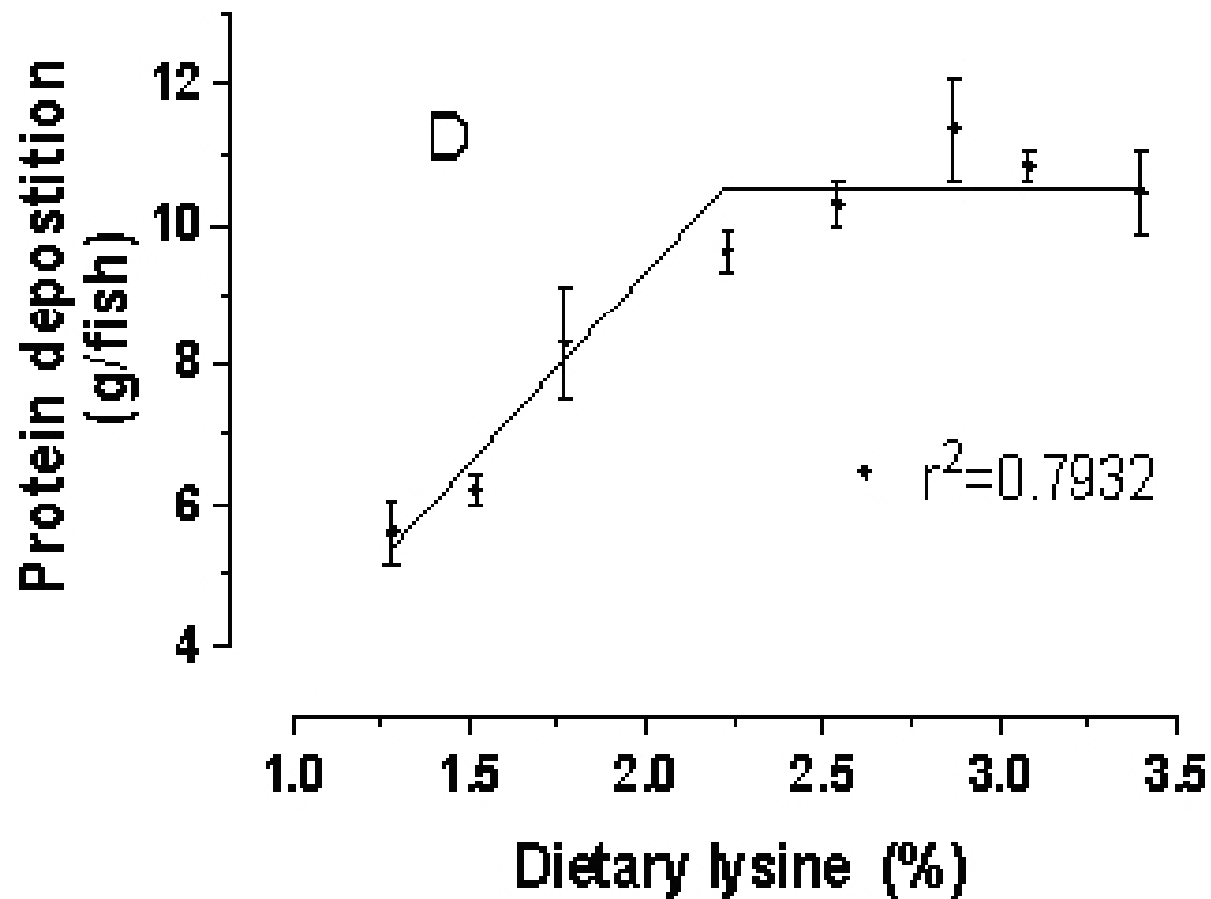
# Results

**Criteria:** Live weight gain **Model:** Four parameter logistic



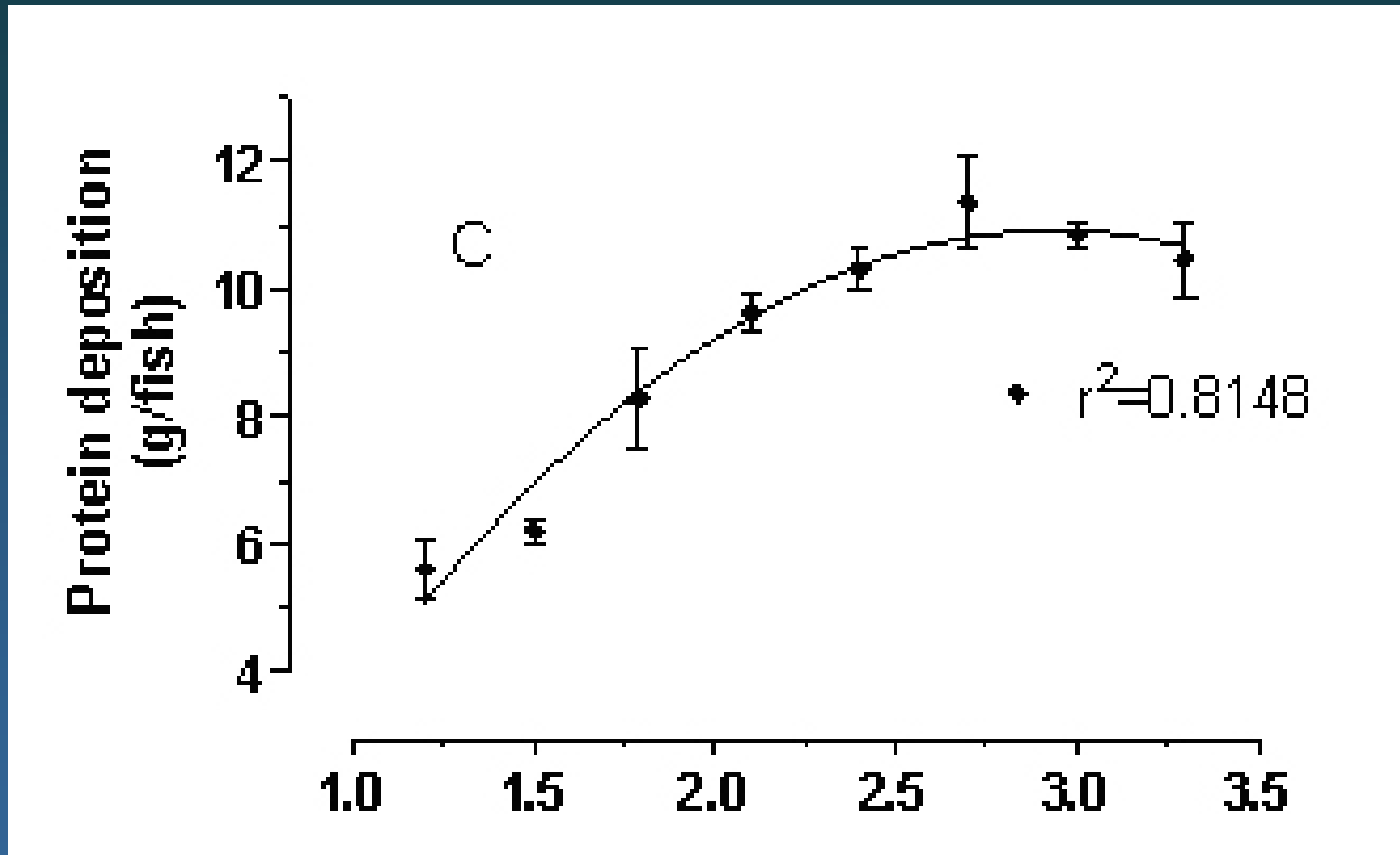
# Results

**Criteria:** Protein Deposition **Model:** Broken-line



# Results

**Criteria:** Protein Deposition **Model:** Quadratic



# Results

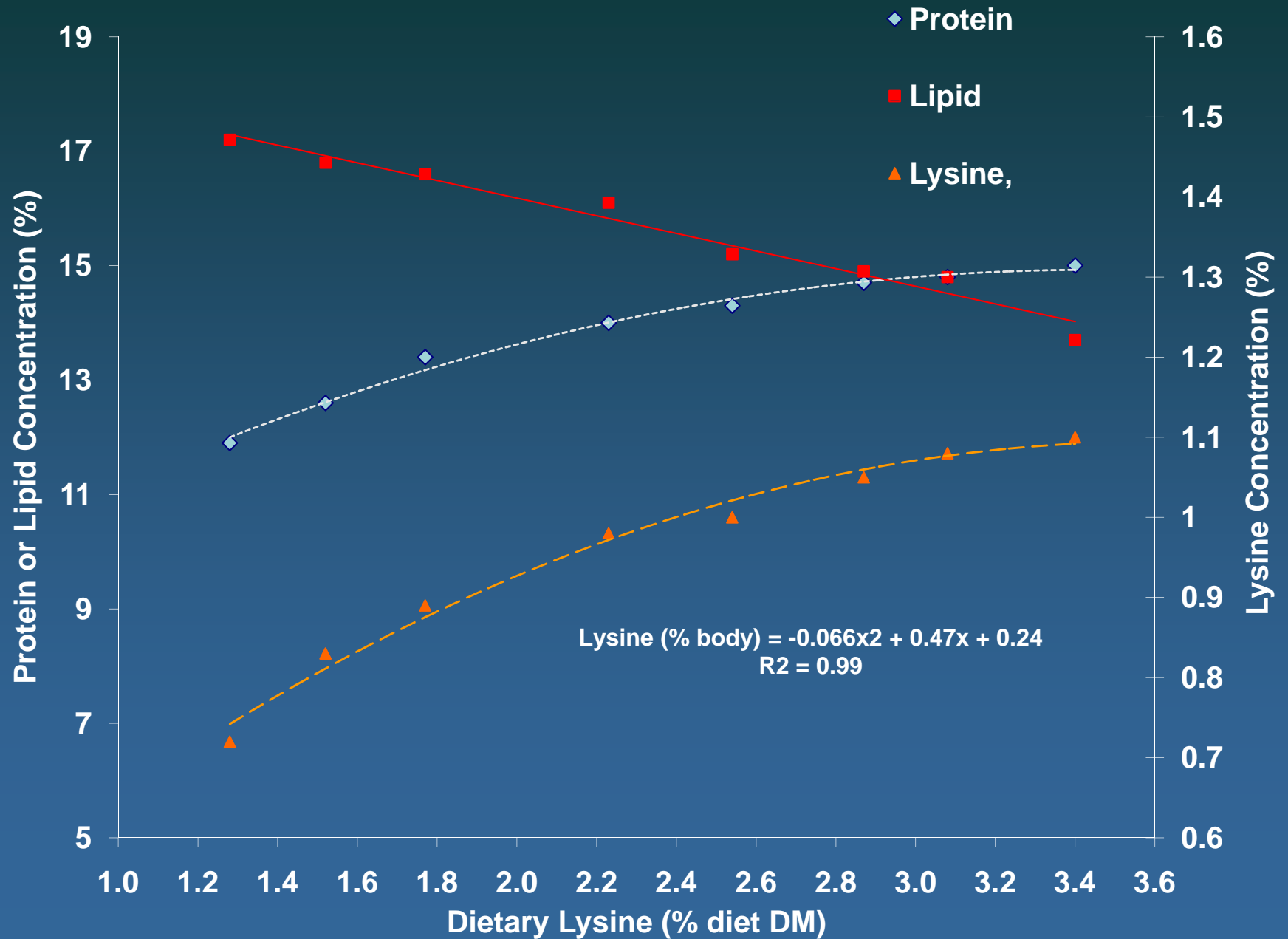
Criteria	Model			
	Four parameter logistic	Exponential	Polynomial	Broken line
Weight gain	2.11	2.68	2.23	2.19
Protein deposition	2.44	3.15	2.41	2.22

Use of different response fitting models resulted in very different estimates of lysine requirements

With the exception of broken line model, estimates of lysine requirement for protein gain appear to be 5-15% higher than those for live weight gain



# Results



# Conclusions

Results from this study suggests that lysine requirement for maximum protein gain of rainbow trout is slightly higher than that for maximizing weight gain.

However, model used for fitting the data has a greater impact on estimate of lysine requirement than the criteria selected.

Increasing dietary lysine levels appear to increase whole body protein and lysine concentrations. This could impact flesh quality and fillet yield.

# Central Questions

**How reliable are estimates of amino acid requirements found in the reference literature (e.g. NRC, 1993)?**

**What the best mode of expression of essential amino acid requirements?**

**How does composition of the diet affect essential amino acid utilization and requirements?**

**How does fish species, life stage, growth rate, feed efficiency, etc. affect utilization and requirement of essential amino acids?**

Too Many Questions, Too Little Time!

Perhaps We Need a Better Approach!

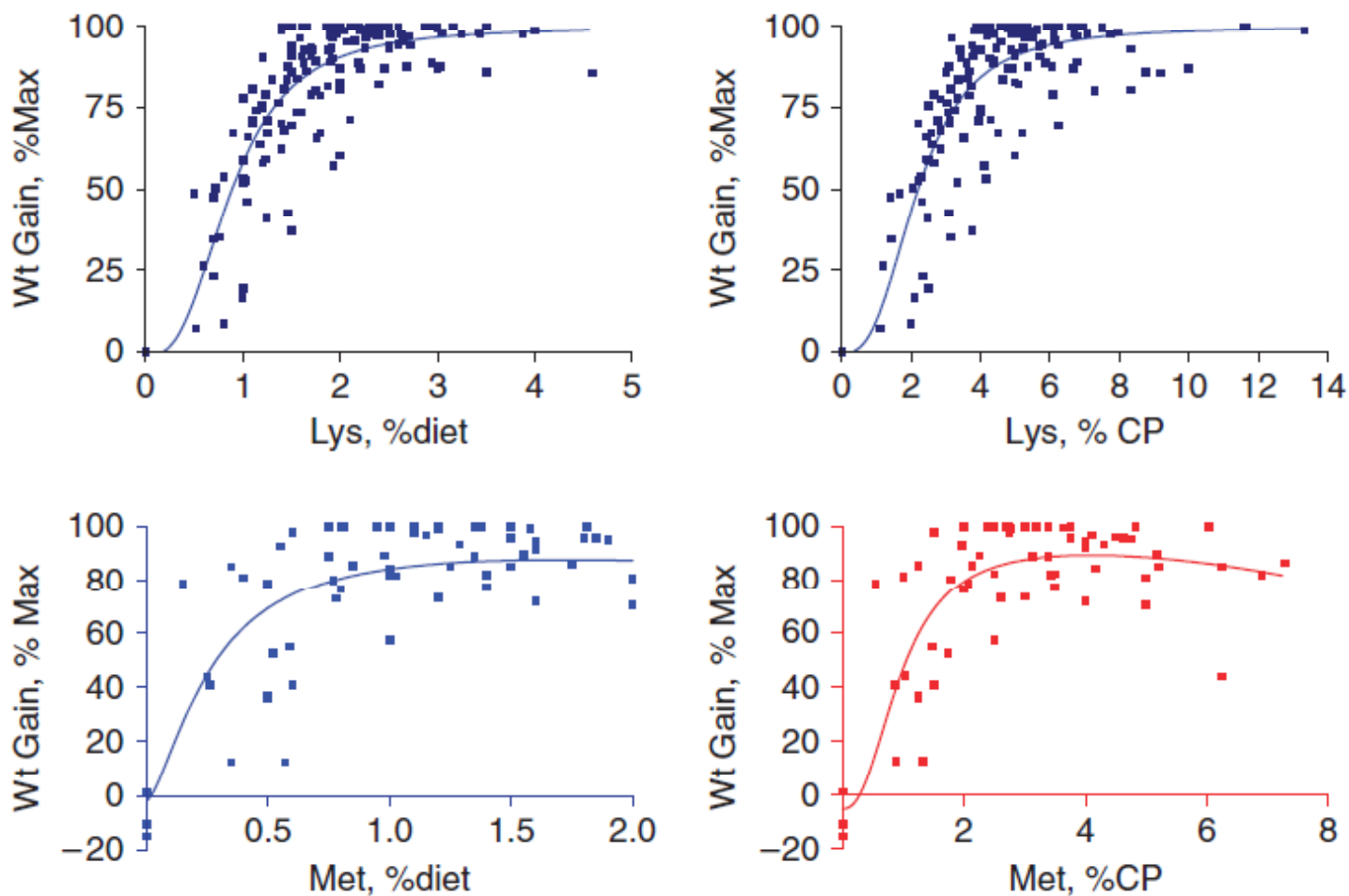
Close to 300 studies published on the essential amino acid requirement of fish have already been published

Probably 3 times more have been carried out but not published

Why reinvent the wheel?

May be we simply need to re-analyze existing data?

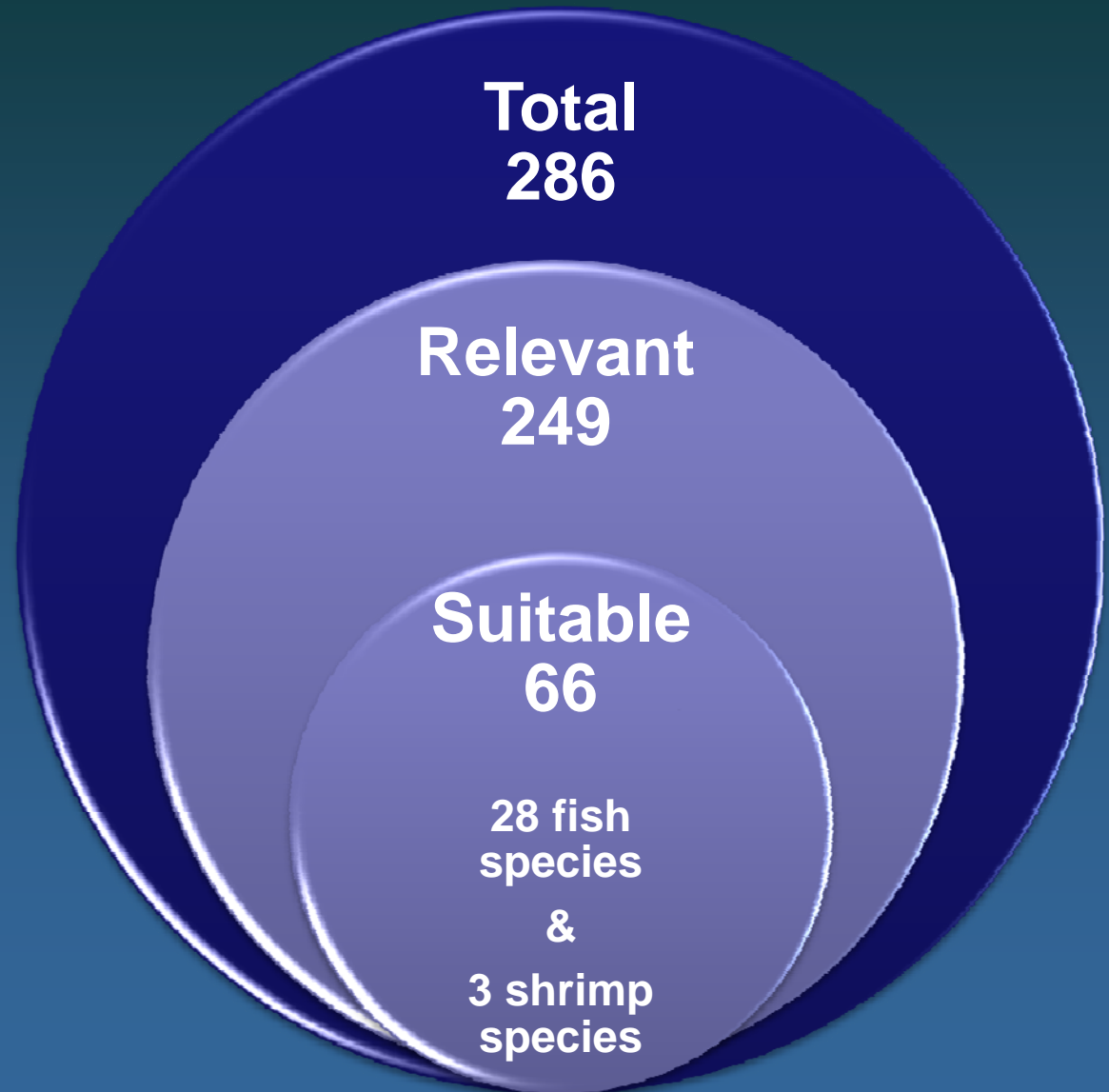
Why not carry out meta-analysis and look at the effect of fish species, diet composition, growth rates, achieved feed efficiency, etc.



**Figure 1** Analysis of literature data on lysine and methionine requirements of different species of fish and shrimp.

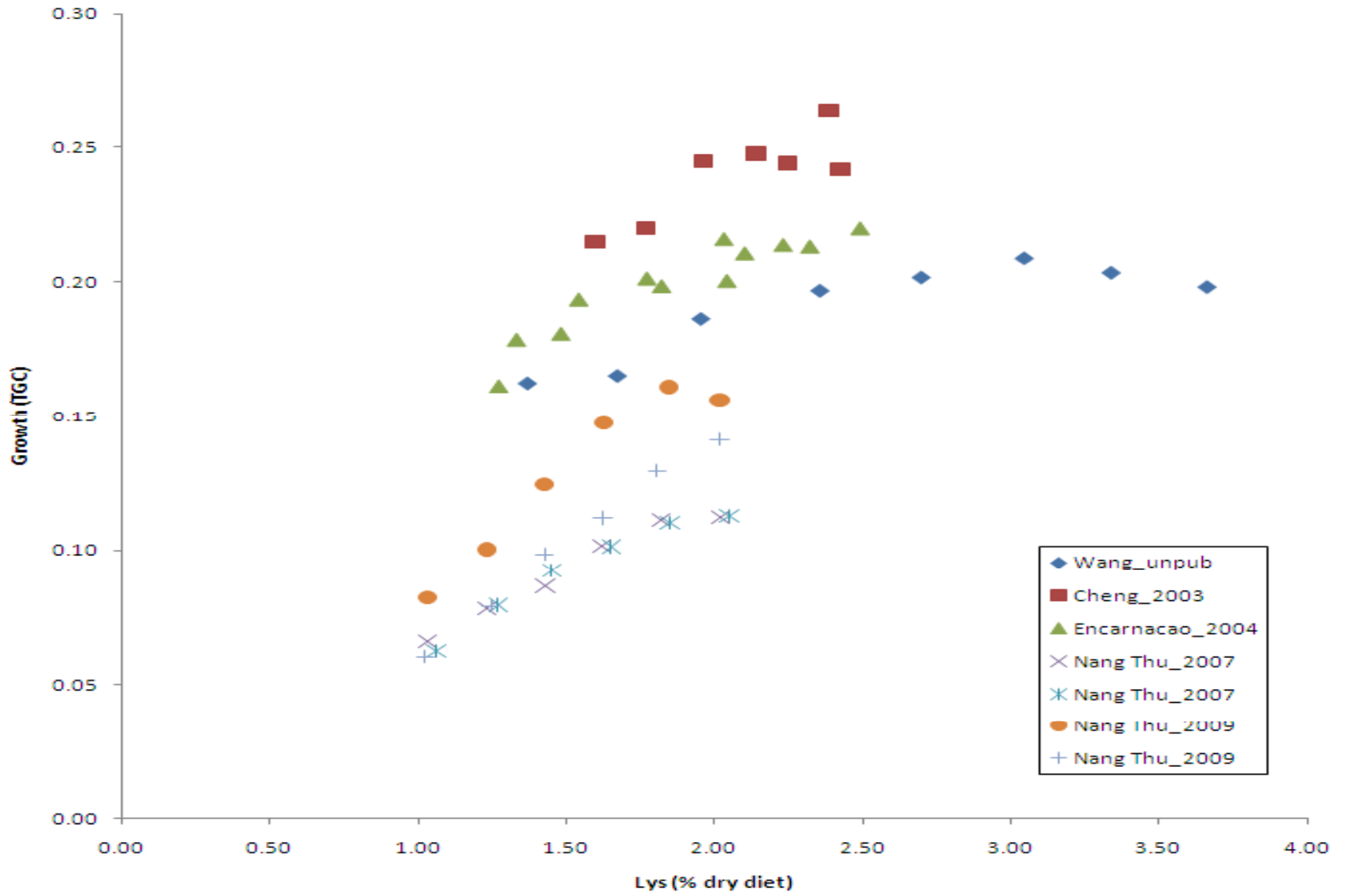
# Meta-Analysis of Essential Amino Acid Requirements of Fish

- <25% studies met criteria!
- Main reasons for rejection
  - Experimental design
    - Too few graded EAA levels
  - Poor growth
  - Low treatment differences
  - Missing information



Salze, Hua, Quinton, Bureau (in progress)

### O. mykiss - Lys vs. growth





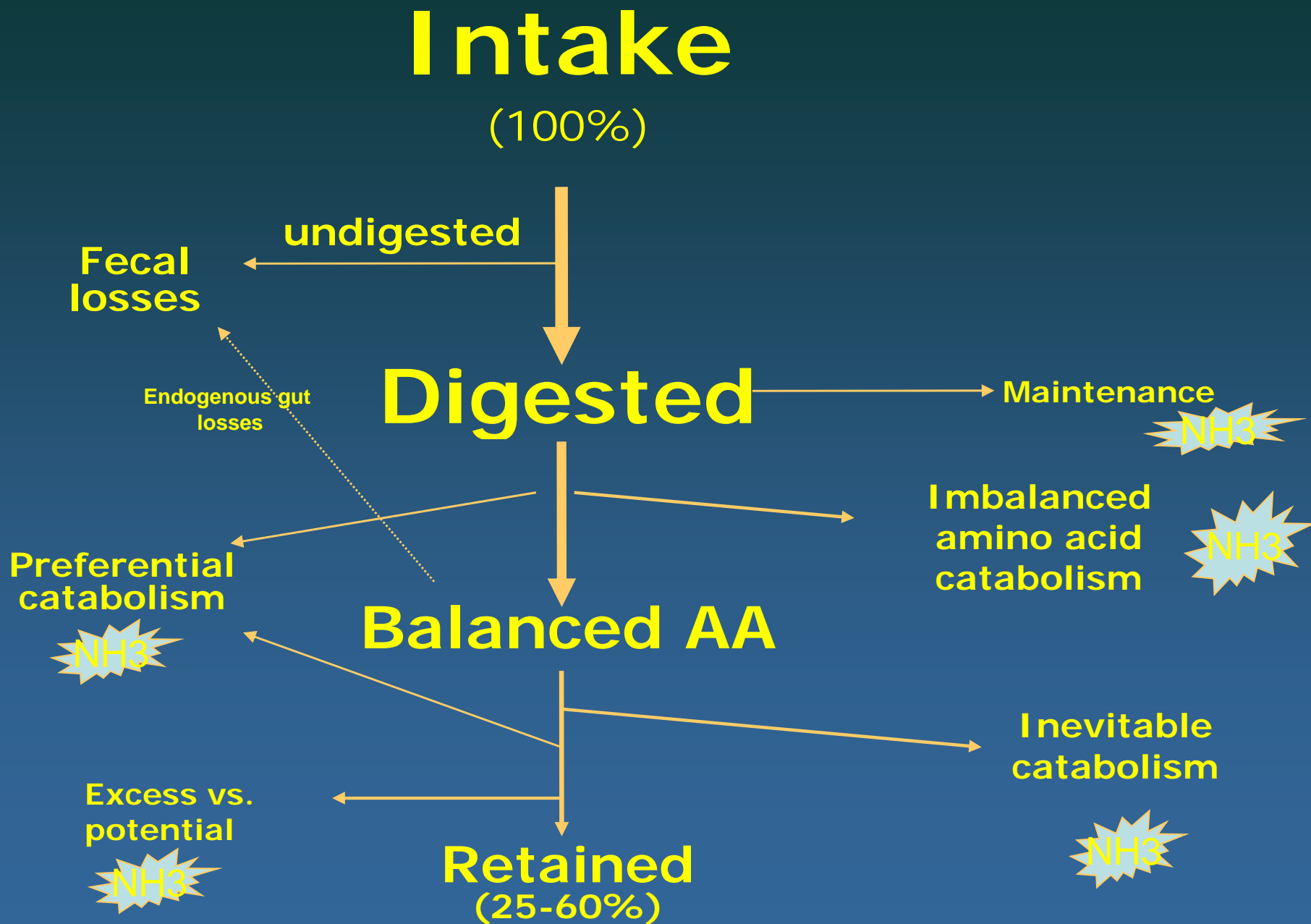
# Dynamic Computation of Essential Amino Acid Requirements through the Use of Factorial Requirement Models



# Factorial Essential Amino Acid Requirement Models

- Based on factorial partitioning scheme for essential amino acids
  - Example: Moughan (2002)
- Compute requirement as the sum of amount of essential amino acid (e.g. lysine) deposited and lost through maintenance, inevitable catabolism and digestion
- Generates absolute estimates of essential amino acid requirements (e.g. mg per fish per day)
- This absolute amount is converted into a dietary concentration (a relative amount) on the basis of the expected feed efficiency / feed intake of the animal

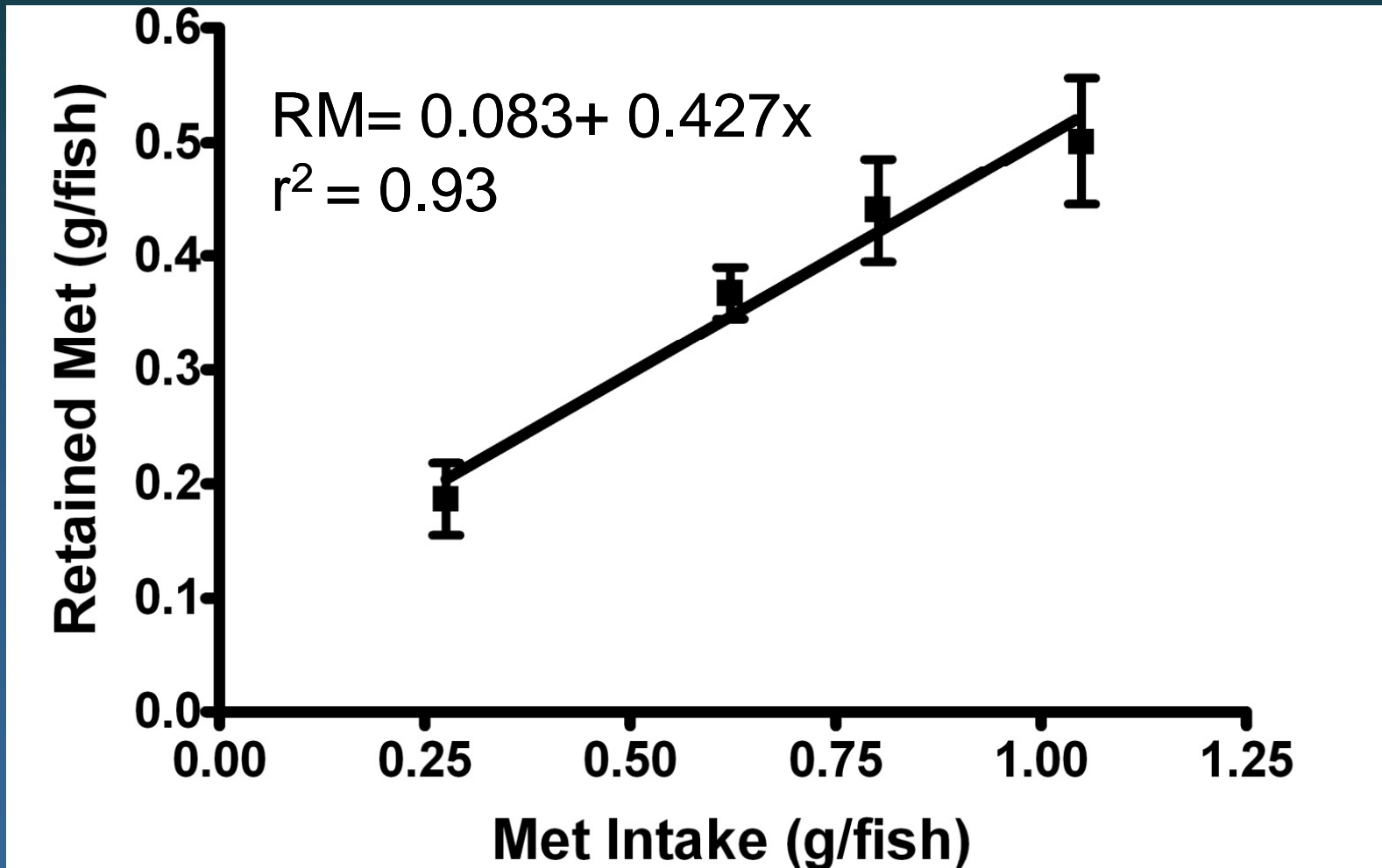
# Factorial Model of Amino Acid Utilization



## Amino Acid Composition (g/16 g N) of Various Fish and Shrimp Species

	Rainbow Trout	Atlantic Salmon	Channel Catfish	Largemouth bass	European Sea Bass	Gilthead Seabream	Turbot	Penaeid Shrimp
Alanine	6.6	6.5	6.3	6.0	6.8	6.8	7.3	5.6
Arginine	6.4	6.6	6.7	8.5	7.5	8.8	7.7	7.4
Asparate	9.9	9.9	9.7	11.8	9.5	9.4	10.3	8.8
Cysteine	0.8	1.0	0.9	0.8	1.0	1.0	1.1	0.8
Glutamate	14.2	14.3	14.4	13.3	15.5	15.1	16.5	16.2
Glycine	7.8	7.4	8.1	7.8	8.1	7.9	9.7	9.0
Histidine	3.0	3.0	2.2	2.1	2.6	2.7	2.5	2.5
Isoleucine	4.3	4.4	4.3	4.0	4.3	4.3	4.3	3.6
Leucine	7.6	7.7	7.4	8.0	7.1	7.3	7.5	6.5
Lysine	8.5	9.3	8.5	8.1	7.9	8.1	8.1	7.8
Methionine	2.9	1.8	2.9	2.6	2.7	3.0	3.4	2.3
Phenylalanine	4.4	4.4	4.1	4.0	4.3	4.7	4.5	3.6
Proline	4.9	4.6	6.0	6.0	5.3	5.3	5.5	8.0
Serine	4.7	4.6	4.9	4.2	4.5	4.5	5.2	3.6
Threonine	4.8	5.0	4.4	4.4	4.4	4.6	4.6	3.8
Tryptophan	1.0	0.9	0.8	0.9	N/A	N/A	N/A	N/A
Tyrosine	3.4	3.5	3.3	2.8	3.9	4.0	4.1	7.5
Valine	5.1	5.1	5.2	4.6	4.7	4.8	4.7	5.1

# Efficiency of Methionine Utilization

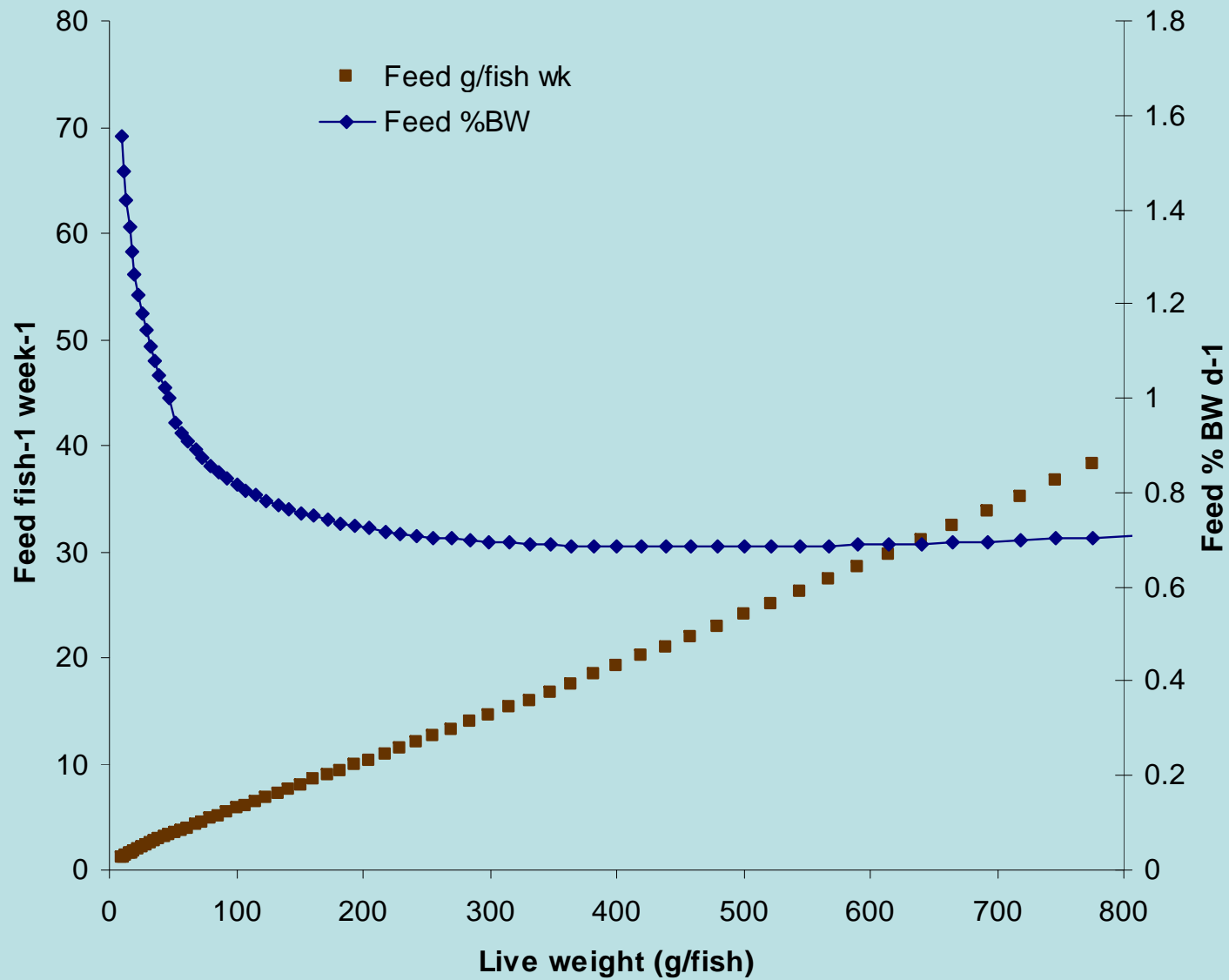


Retained methionine (g/fish) vs. methionine intake (g/fish)

# Factorial Essential Amino Acid Requirement Models

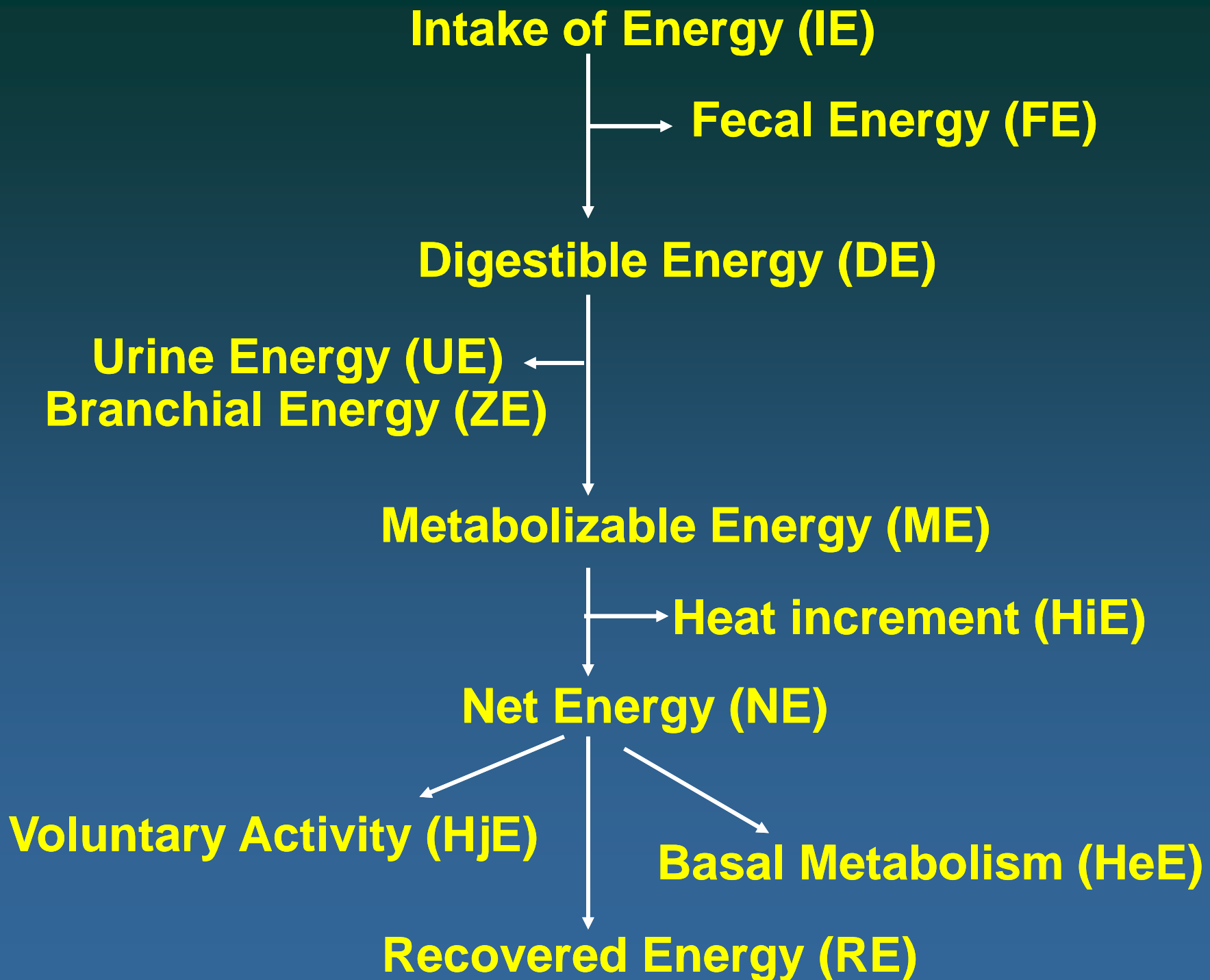
- Based on factorial partitioning scheme for essential amino acids
  - Example: Moughan (2002)
- Compute requirement as the sum of amount of essential amino acid (e.g. lysine) deposited and lost through maintenance, inevitable catabolism and digestion
- Generates absolute estimates of essential amino acid requirements (e.g. mg per fish per day)
- This absolute amount is converted into a dietary concentration (a relative amount) on the basis of the expected feed efficiency / feed intake of the animal

# Estimated Feed Intake of Rainbow Trout

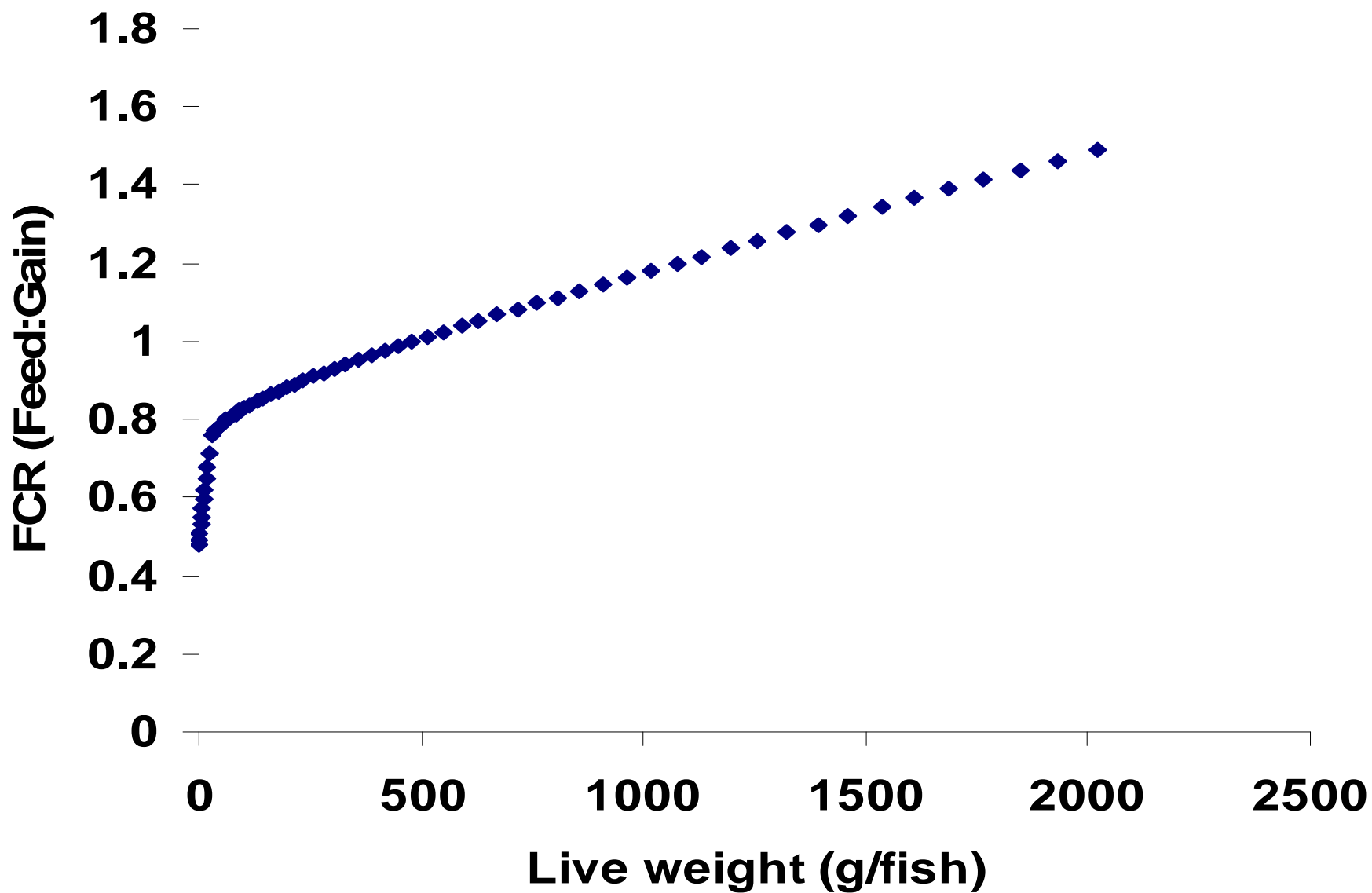


(TGC= 0.180, Temperature = 9°C, Diet 18 MJ DE, 22 g DP/MJ DE)

Bureau et al. (2002)



# Simulation of FCR of rainbow trout at different weights



Bureau et al. (2008)



Digestible EAA Requirements (% Diet Dry Matter) Estimated Using a Factorial Model for Rainbow Trout of Different Weights Fed Diets with 4.78 Mcal DE (20 MJ DE)

Essential Amino Acids	Weight Class		
	0.2–20 g	20–500 g	500–1,500 g
	% diet DM		
Arg	1.91	1.77	1.62
His	0.83	0.77	0.69
Ile	1.27	1.19	0.98
Leu	2.26	2.11	1.78
Lys	2.47	2.31	1.92
Met + Cys	1.32	1.23	1.10
Phe + Tyr	2.49	2.33	1.82
Thr	1.77	1.63	1.60
Trp	0.43	0.40	0.42
Val	1.90	1.76	1.64

# Observations

**Current models compute independent estimates of EAA requirements and assume no effect of composition of the diet and life stage of the animal and that feed intake and feed efficiency are determinant factors unrelated to efficiency of EAA utilization**

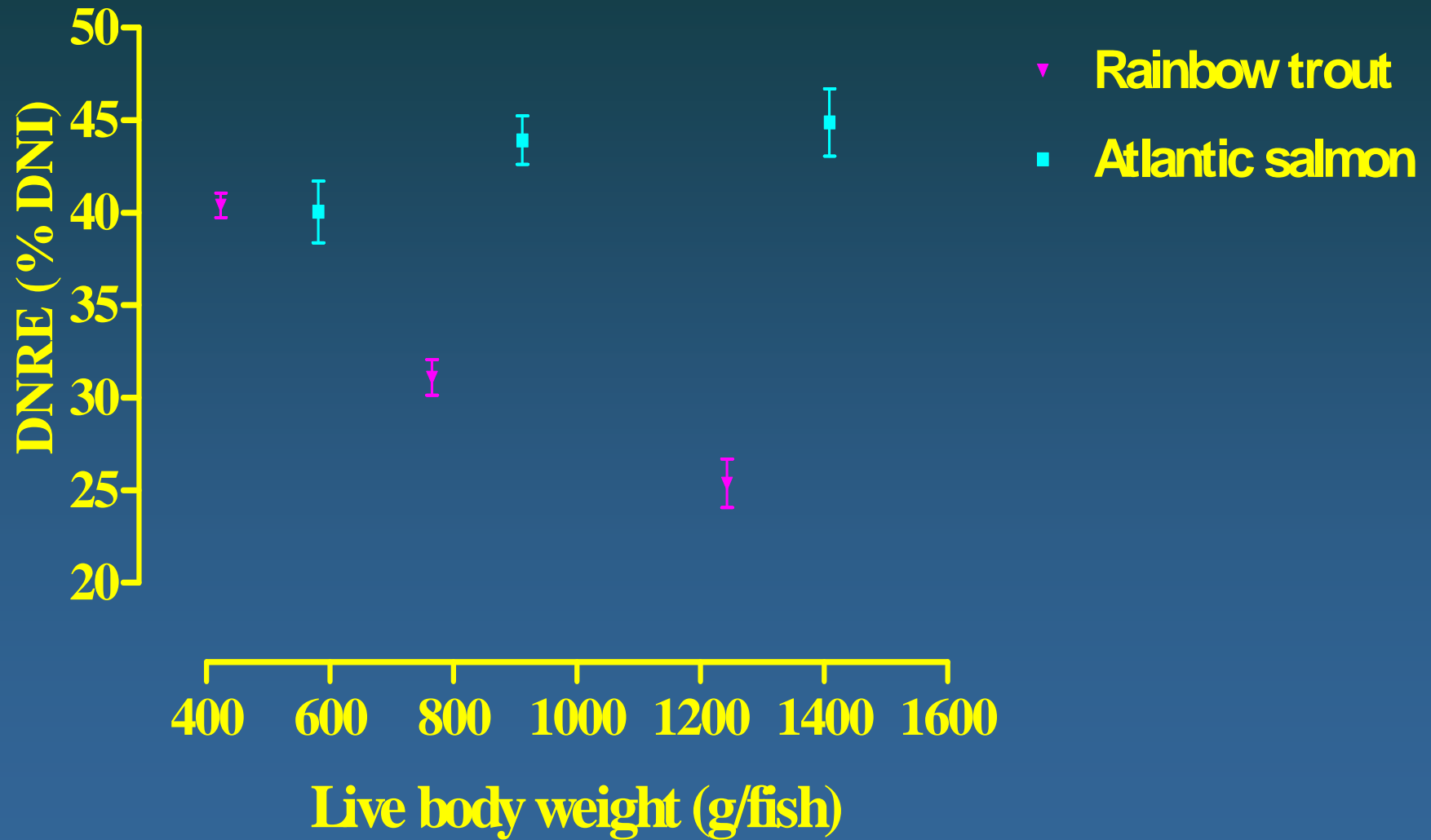
**The simple model derived from monogastric animals (poultry and swine) are too simplistic and do not adapt perfectly to fish**

**Endogenous factors (species and life stage) appear to have as great impact on efficiency of amino acid utilization than dietary manipulations.**

**Different energy-yielding nutrients (digestible energy sources) have different effect on efficiency of protein and lysine utilization**

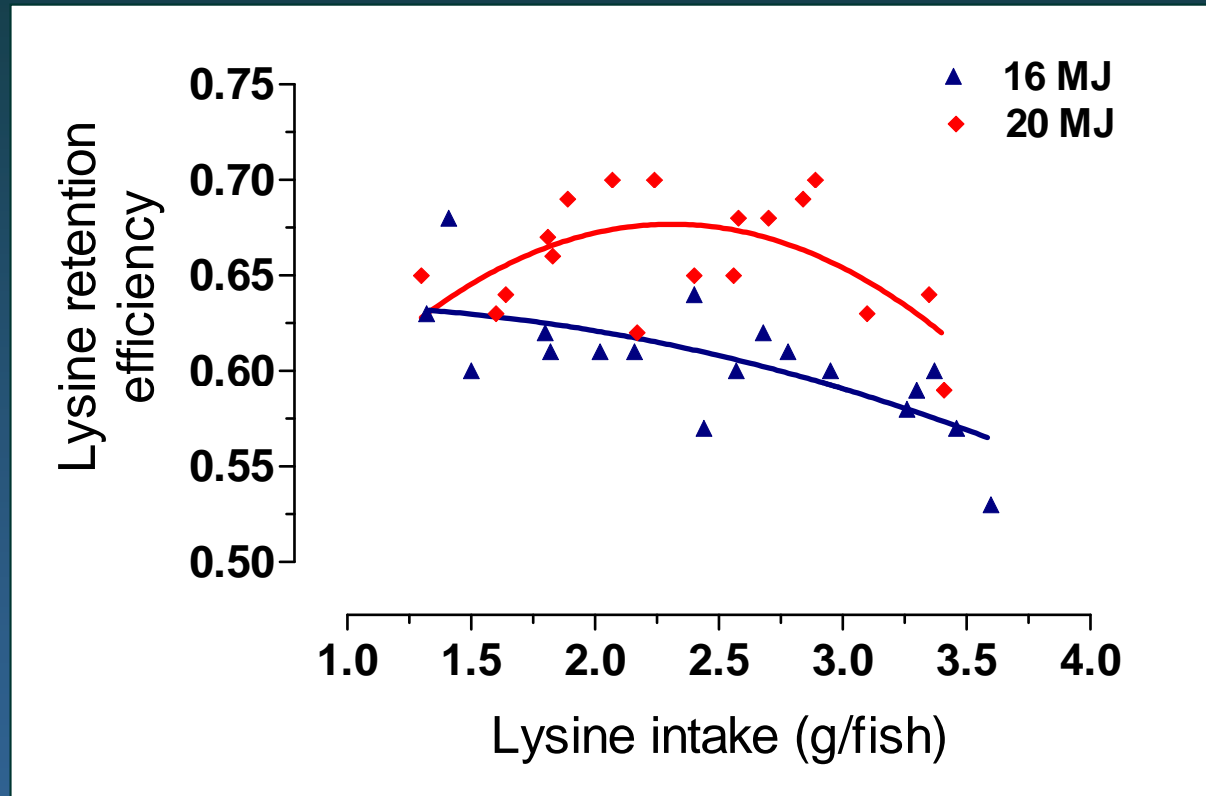
**Fish have an endogenously determined target for protein and lipid deposition and they will eat / metabolize feed nutrients to achieve this target. Efficiency of protein utilization is largely determined by the animal itself, not the human feeding this animal**

## Efficiency of N retention is affected by size in rainbow trout



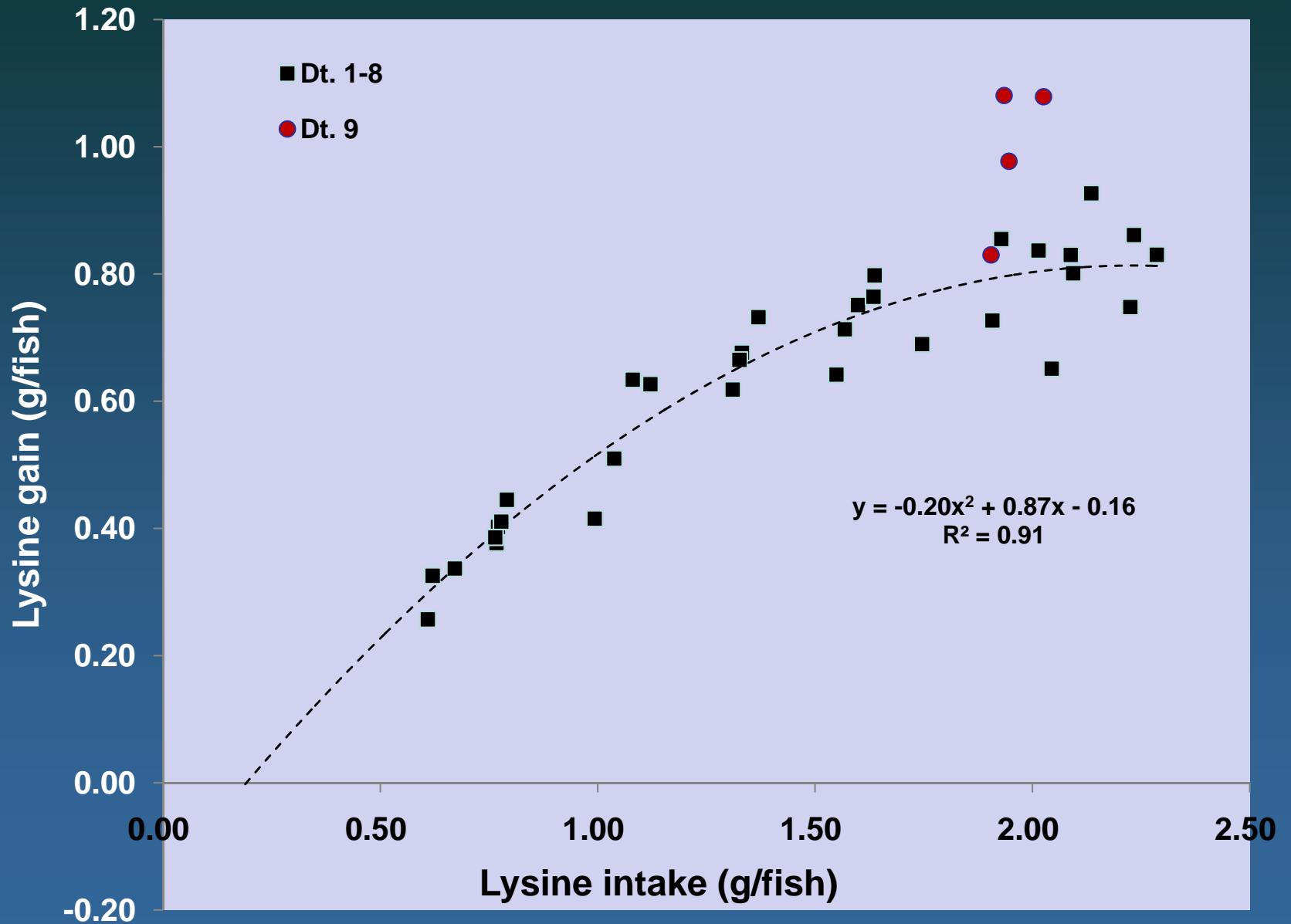
# RESULTS

Fig. 3 – Lysine efficiency in response to the lysine intake of fish.

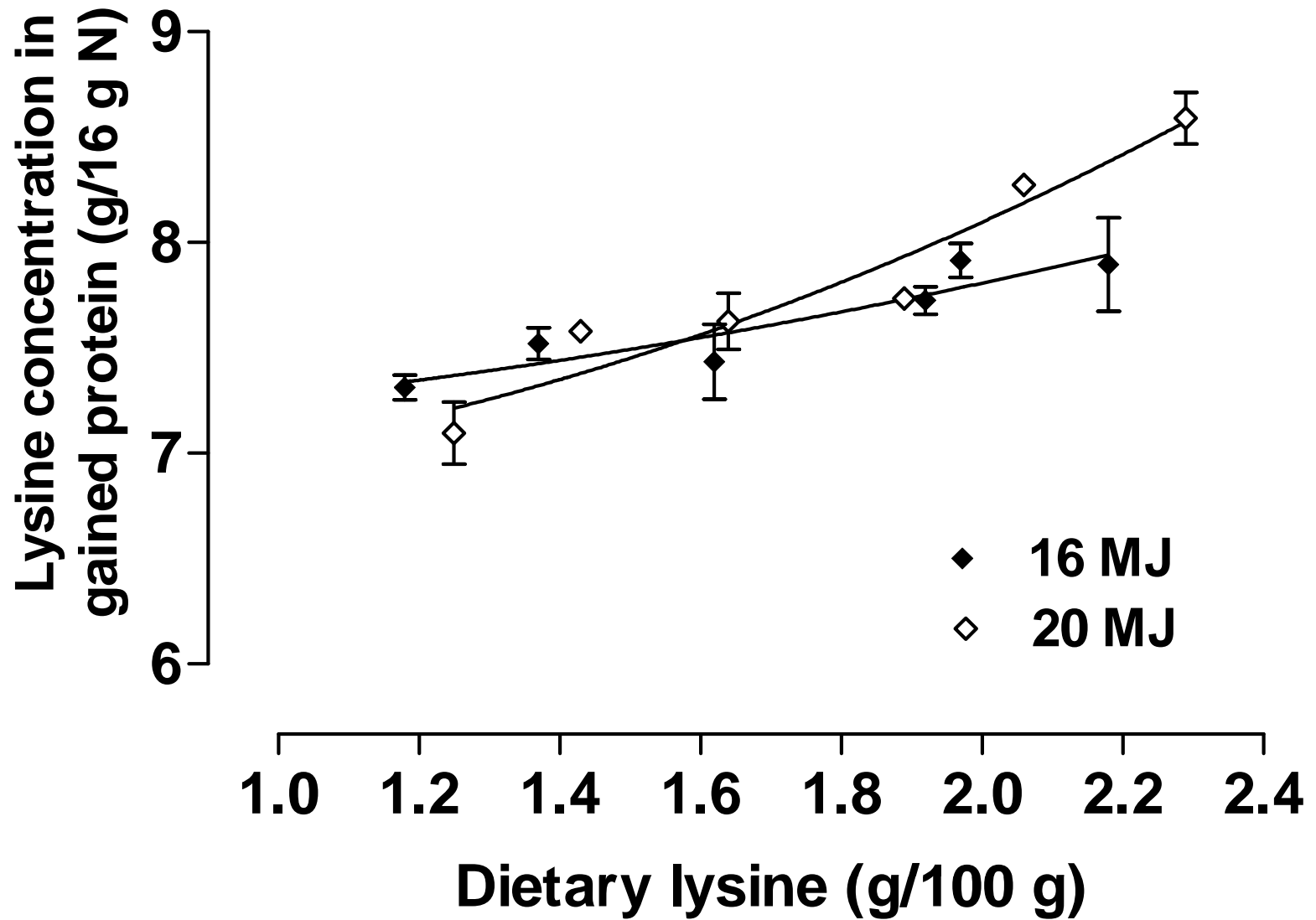


- ✓ Higher efficiency of lysine utilization at higher dietary DE levels.

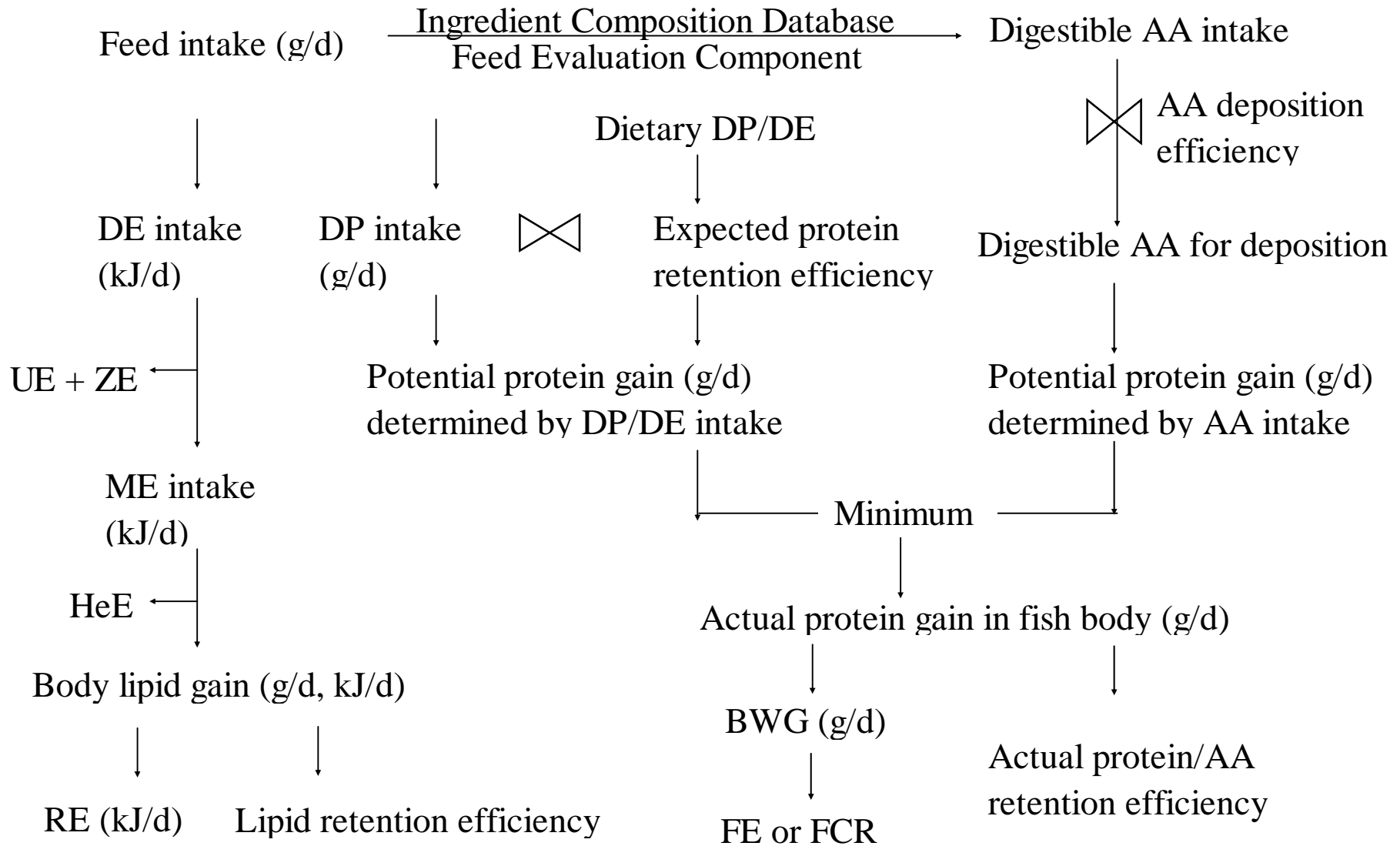
# Lysine Deposition as a Function of Lysine Intake



# Results



# A Novel Hybrid Nutrient-Flow Bioenergetics Growth Model for Fish



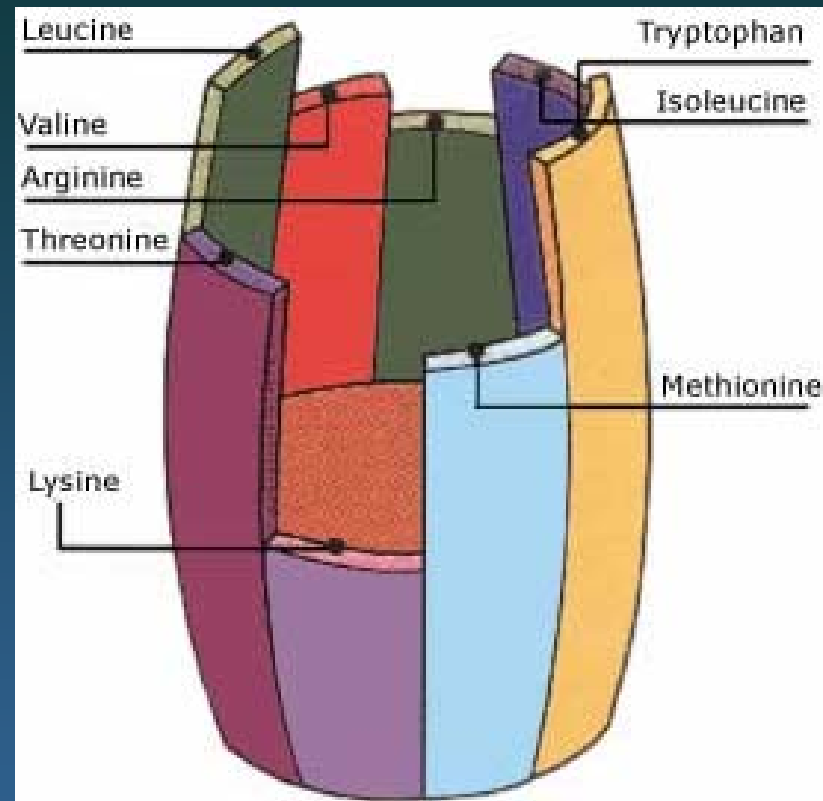
Hua and Bureau (in progress)



# Ideal Protein Concept



# Liebig's Law of the Minimum



**Efficiency of Amino Acid Utilization will not be higher than lowest stage**

**At origin of the concept of “Ideal Protein Pattern”**

**Profile exactly meeting all essential amino acid requirements  
No EAA in excess in comparison to one another**

# Rainbow trout feeds (35% CP, 15% lipid) formulated to have different ideal protein patterns

Table 5. Study 2: Essential amino acid concentrations (g kg<sup>-1</sup> dry feed) of experimental diets

Amino acid	Diet			
	1) RT	2) NRC	3) Nonlinear model	4) EAA deletion
Arginine	16.0	17.3	11.5	16.8
Histidine	7.4	8.1	5.7	6.4
Isoleucine	10.8	10.4	13.5	9.9
Leucine	19.0	16.1	13.4	17.5
Lysine	21.2	20.7	27.4	19.3
Methionine	7.2	9.0	7.9	8.1
Cystine	2.0	2.5	3.0	2.2
Phenylalanine	10.9	11.7	11.2	13.7
Tyrosine	8.4	9.0	8.6	10.5
Threonine	11.9	9.2	10.3	12.3
Tryptophan	2.3	2.3	2.0	2.1
Valine	12.7	13.8	15.5	11.2
Total	130.0	130.0	130.0	130.0

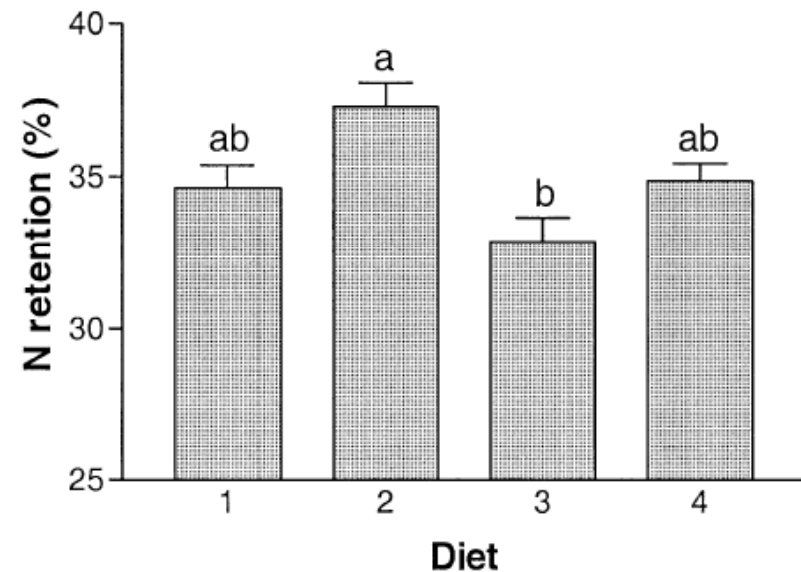


Figure 3. Study 2: Mean N retention (% of N intake) of rainbow trout fed four experimental diets. Error bars indicate SE (n=4). Values not sharing the same letter are significantly different (P<0.05, Tukey test).

**“Ideal amino acid pattern” derived from NRC (1993) gave best nitrogen retention efficiency.**

**Green and Hardy (2002)**

# However, results are interesting but not conclusive...

Table 7. Study 2: Mean weight gain, thermal-unit growth coefficient (TGC) and feed efficiency ratio (FER) of rainbow trout fed four experimental diets (mean  $\pm$  SE, n=4). Values in the same column not sharing the same superscript are significantly different ( $P < 0.05$ , Tukey test)

Dietary treatment	Gain (g) <sup>1</sup>	TGC	FER
1) RT	23.3 $\pm$ 0.4 <sup>ab</sup>	0.102 $\pm$ 0.001 <sup>ab</sup>	0.735 $\pm$ 0.014 <sup>ab</sup>
2) NRC	24.6 $\pm$ 0.4 <sup>a</sup>	0.106 $\pm$ 0.001 <sup>a</sup>	0.763 $\pm$ 0.006 <sup>a</sup>
3) Nonlinear model	21.9 $\pm$ 0.6 <sup>b</sup>	0.098 $\pm$ 0.002 <sup>b</sup>	0.690 $\pm$ 0.011 <sup>b</sup>
4) EAA deletion	23.2 $\pm$ 0.4 <sup>ab</sup>	0.101 $\pm$ 0.001 <sup>ab</sup>	0.722 $\pm$ 0.011 <sup>ab</sup>

<sup>1</sup>Mean initial weight per fish  $\pm$  SE for all treatments = 15.5  $\pm$  0.6 g.

**Growth and feed efficiency values obtained were relatively low**

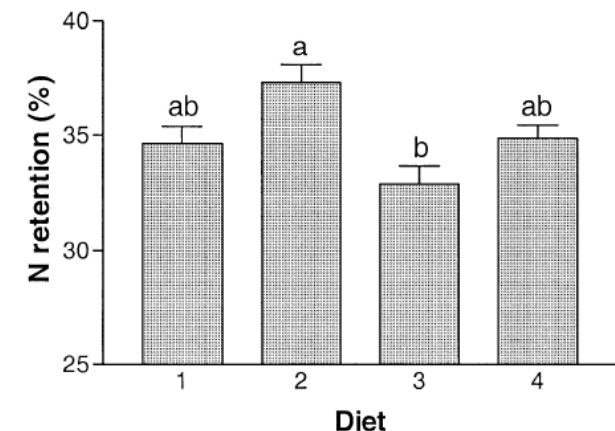


Figure 3. Study 2: Mean N retention (% of N intake) of rainbow trout fed four experimental diets. Error bars indicate SE (n=4). Values not sharing the same letter are significantly different ( $P < 0.05$ , Tukey test).

**Nitrogen retention efficiency was relatively low**

# Deficiency in amino acids may explain the results

**Diets used were likely deficient**

Table 5. Study 2: Essential amino acid concentrations ( $\text{g kg}^{-1}$  dry feed) of experimental diets

Amino acid	Diet			
	1) RT	2) NRC	3) Nonlinear model	4) EAA deletion
Arginine	16.0	17.3	11.5	16.8
Histidine	7.4	8.1	5.7	6.4
Isoleucine	10.8	10.4	13.5	9.9
Leucine	19.0	16.1	13.4	17.5
Lysine	21.2	20.7	27.4	19.3
Methionine	7.2	9.0	7.9	8.1
Cystine	2.0	2.5	3.0	2.2
Phenylalanine	10.9	11.7	11.2	13.7
Tyrosine	8.4	9.0	8.6	10.5
Threonine	11.9	9.2	10.3	12.3
Tryptophan	2.3	2.3	2.0	2.1
Valine	12.7	13.8	15.5	11.2
Total	130.0	130.0	130.0	130.0

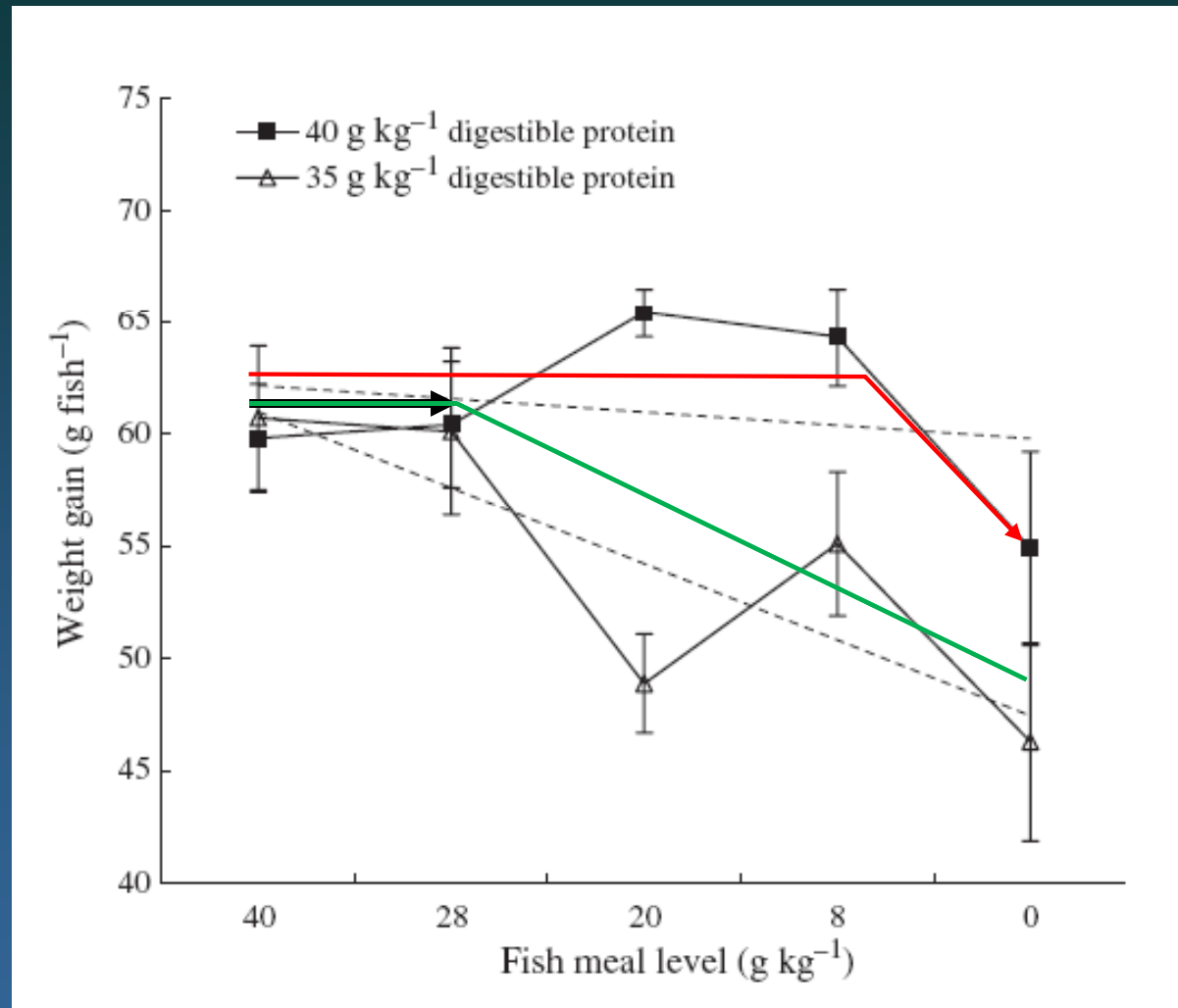
**Marginally  
Deficient  
in  
Iso, Cys**

**Marginally  
Deficient  
in  
Lys**

**Deficient  
in  
Arg, His**

**Marginally  
Deficient  
in  
Lys, His, Iso**

## Effect of replacement of high quality protein by a lower quality one at two different protein levels



At higher protein levels, essential amino acid deficiencies occur at lower fish meal (higher alternative ingredient) levels. It is the essential amino acid intakes that matter, not the fish meal level or “relative level” of essential amino acids of the diet

# What is more important?

Meeting absolute amino acid requirement (mg/fish per day, % diet)?

or

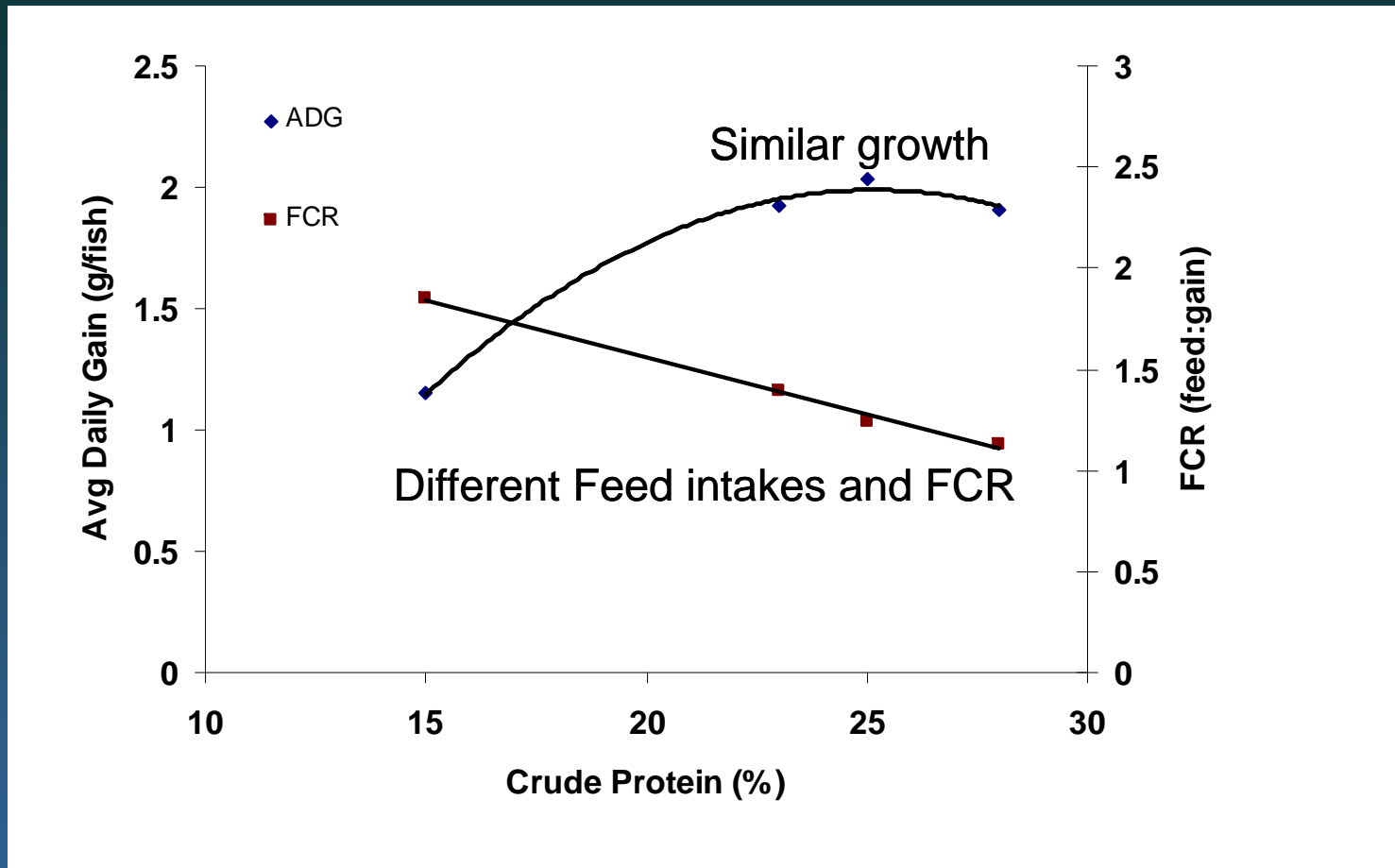
Meeting amino acid requirements as function of all other amino acid supplied?

## Usefulness of Ideal Protein Concept

Predicting requirements for 10 EAA when only information on requirements for one or two EAA is available

Formulating low protein diets (diets meeting exactly EAA requirement of fish and have no EAA in excess)

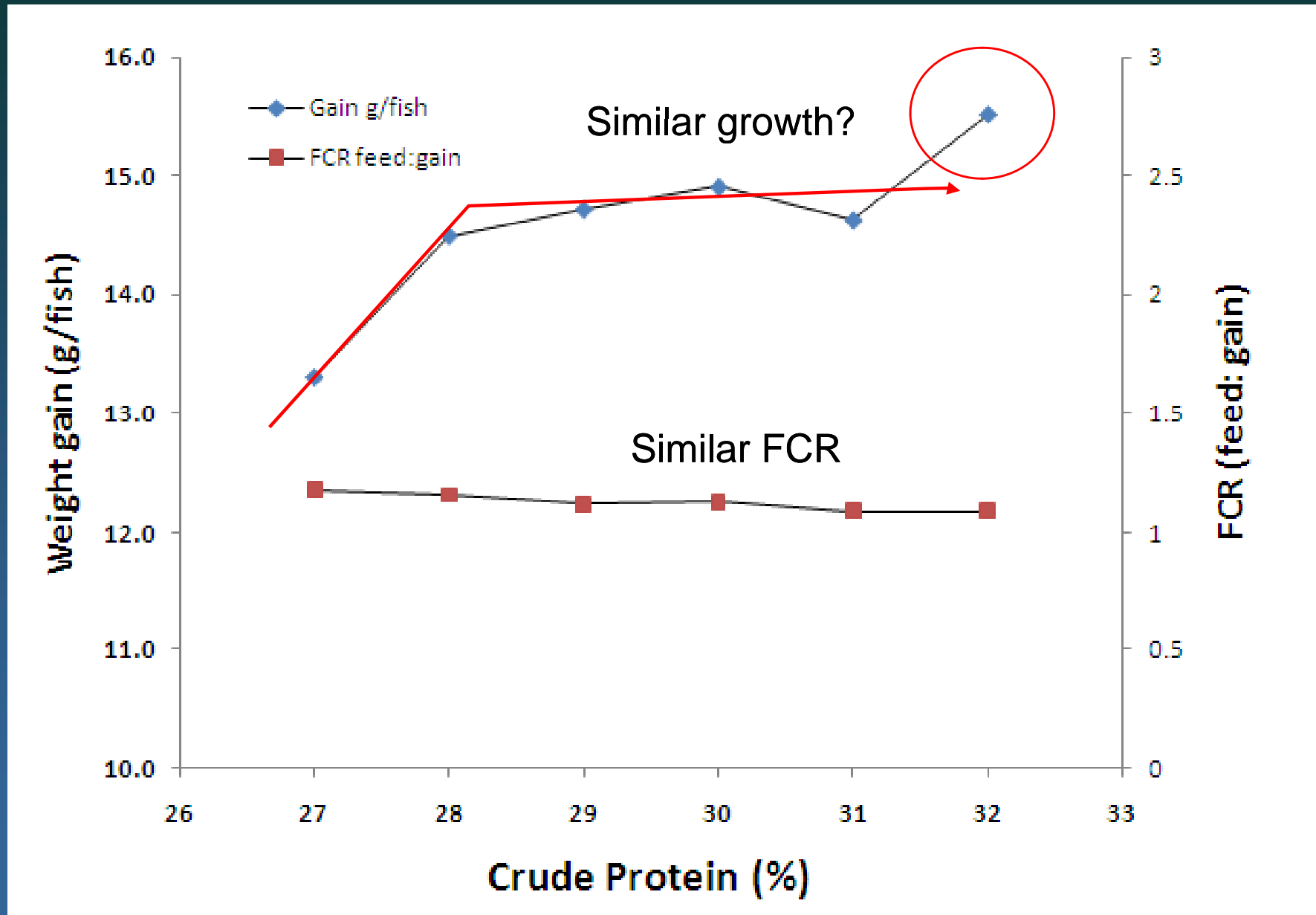
# Daily Weight Gain and Feed Conversion Ratio of Nile Tilapia Fed Commercial Feeds with Different Nutrient Densities



Fish are capable of eating more of the more “diluted” lower protein feed in order to obtain enough essential amino acids.

What if we supplemented the low protein feeds with enough essential amino acids to meet the requirement (% diet) of the fish?

# Protein Requirement of Tilapia fed Diet with Different Protein Levels but Formulated to Ideal Protein Concept





# Acknowledgments

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