USSEC 4th IAFFD Feed Formulation Workshop
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DIGESTIBILITY: MAKING SENSE OF, AND ADEQUATELY USING, PUBLISHED OR R&D DATA

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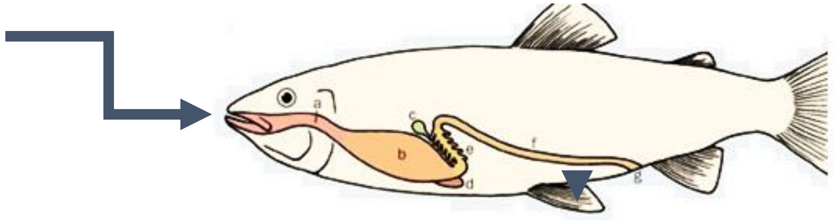
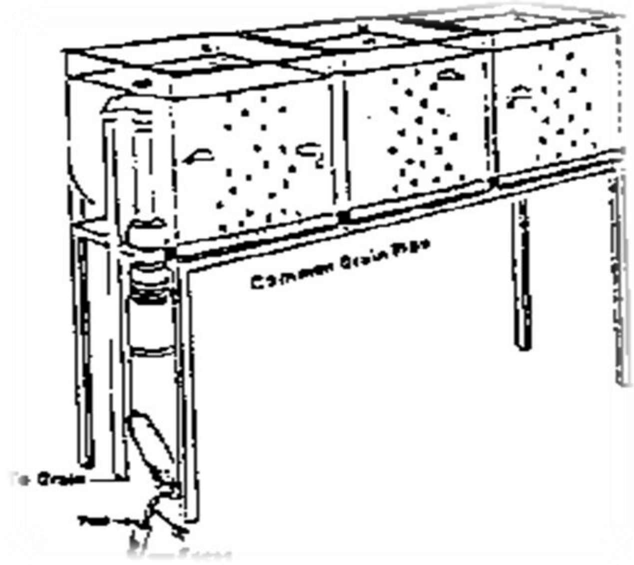
Digestibility = First rational step to assess potential nutritive value of ingredients

Intake



Faeces

Guelph System (Developed in Early 1970's)



Introduction

- Increasing amount of information of the apparent digestibility coefficient (ADC) of nutrients of different ingredients
- Digestibility of nutrients is an important aspect to consider in commercial feed formulation. If not digestible, it is not available to the animal!
- Feed manufacturers are progressively moving from formulating on a ‘total nutrient’ basis to formulating on “digestible nutrient” basis
- Very tedious and costly to maintain R&D program on digestibility of feed ingredients so manufacturers have to rely on published data or 3rd party estimates
- Critical to ensure that the information available is reliable and limitations of this information are well-understood by nutritionists/feed formulators

Outline

1) Understanding digestibility

2) Methodological approaches used to estimate digestibility of nutrients of complete feeds and feed ingredients

3) Potential limitations and pitfalls associated with digestibility measurements

4) Determinants of the digestibility of nutrients: It's a matter of chemistry

5) Strategies to properly do your job (or putting in practice what you have learned – Focus of Day 2)

1. Understanding Digestibility

Concepts – It's a mess out there…

Dietary habits

vs.

Digestive Anatomy / Physiology / Biochemistry

vs.

Digestibility

vs.

Absorption

vs.

Assimilation/ Utilization

vs.

Deposition/Accretion

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	$\frac{100-25}{100}$	75%
Protein	40	4	$\frac{40-4}{40}$	90%
Lipid	20	1	$\frac{20-1}{20}$	95%

Issues:

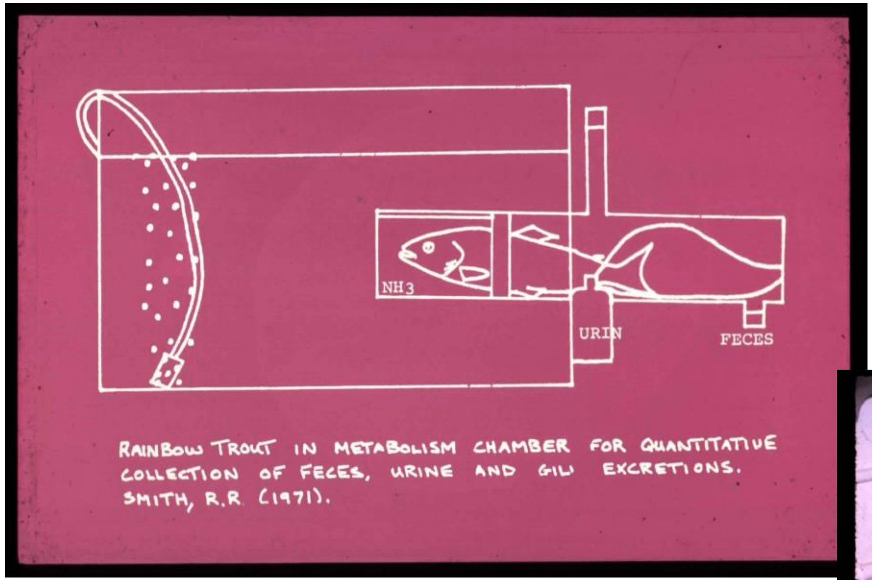
Collection
Total collection in water feasible?

Time
How long should we collect?
24H?

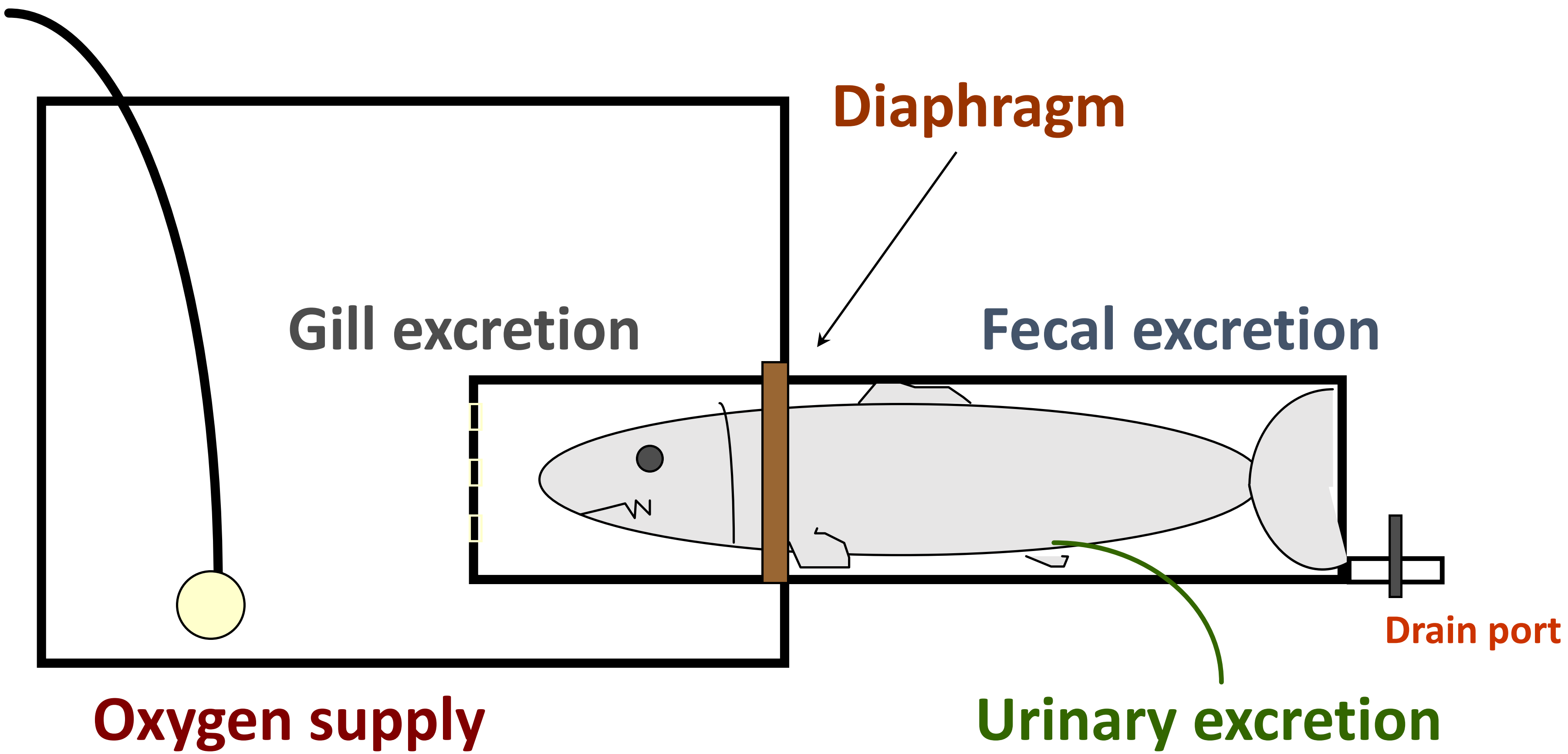
One meal?
Representative of normal state?

R. Smith Metabolic Chamber (Cornell University, New York)

Used to estimate faecal (FE) and non-faecal losses (UE+ZE)



Smith's Metabolic Chamber



"ICU" fish. Not a happy camper!

Digestibility – Indirect Method

Requires:

- Use of digestion indicator (marker) = 100% indigestible, non-toxic, pass at same rate as all dietary components
- Collection of representative samples fecal material produced

Apparent Digestibility Coefficient (ADC) = 1- (F/D x Di/Fi)

	Feed %	Feces %	Digestibility	%
Dry matter	95	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**

Collection of fecal sample:

- That is representative
- Free of uneaten feed
- No or minimal leaching

2. Methodological Issues

Feces Collection Equipment and Protocol

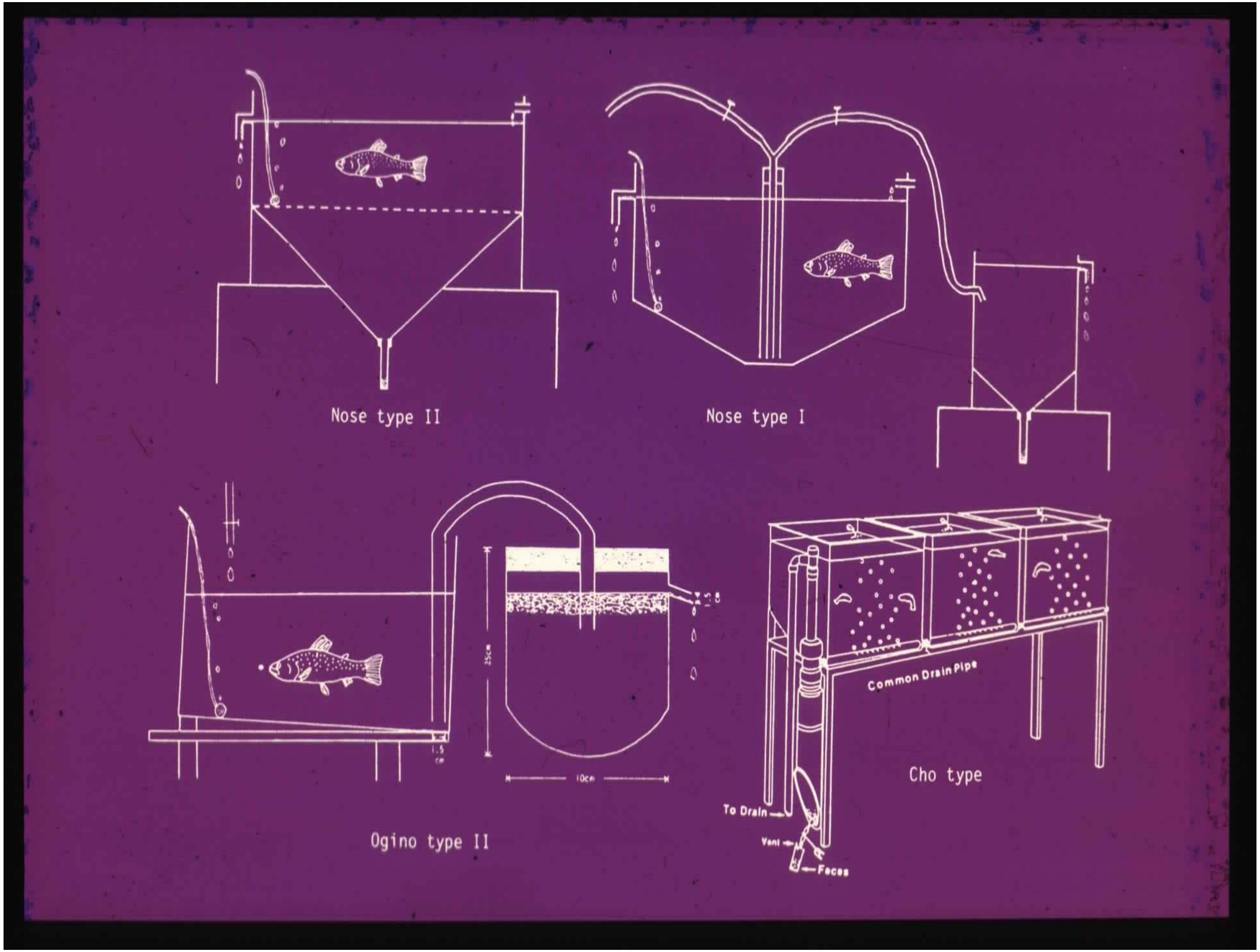
Measuring Digestibility in Fish

Several Methods:

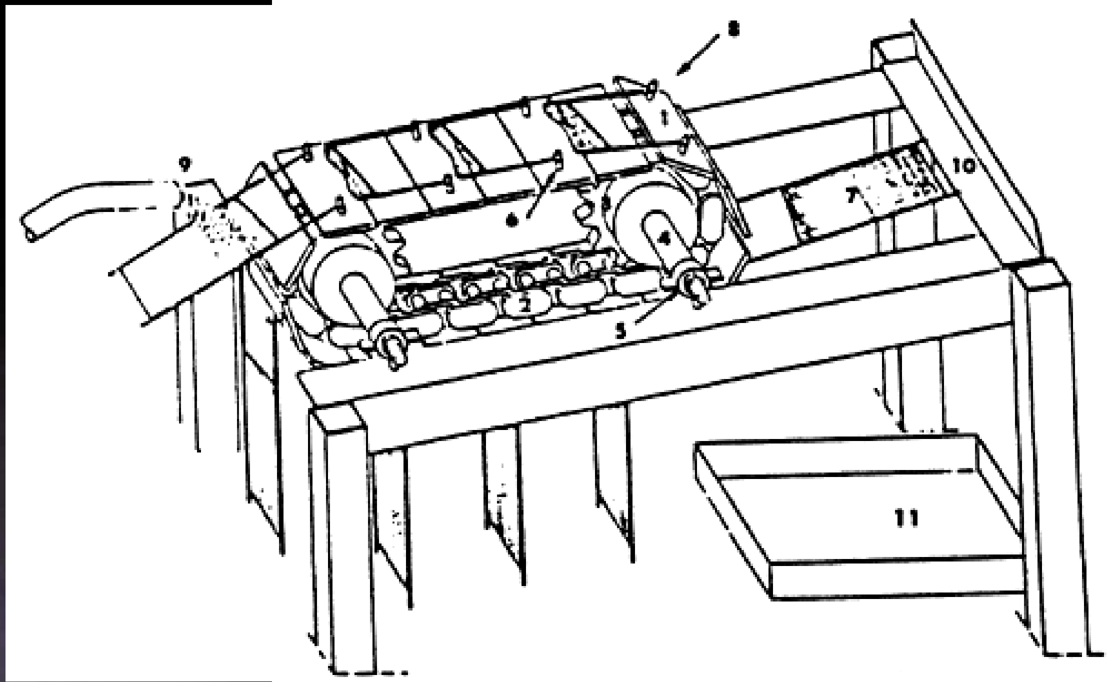
Stripping, dissection, siphoning

Three passive collection methods believed to be more reliable:

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

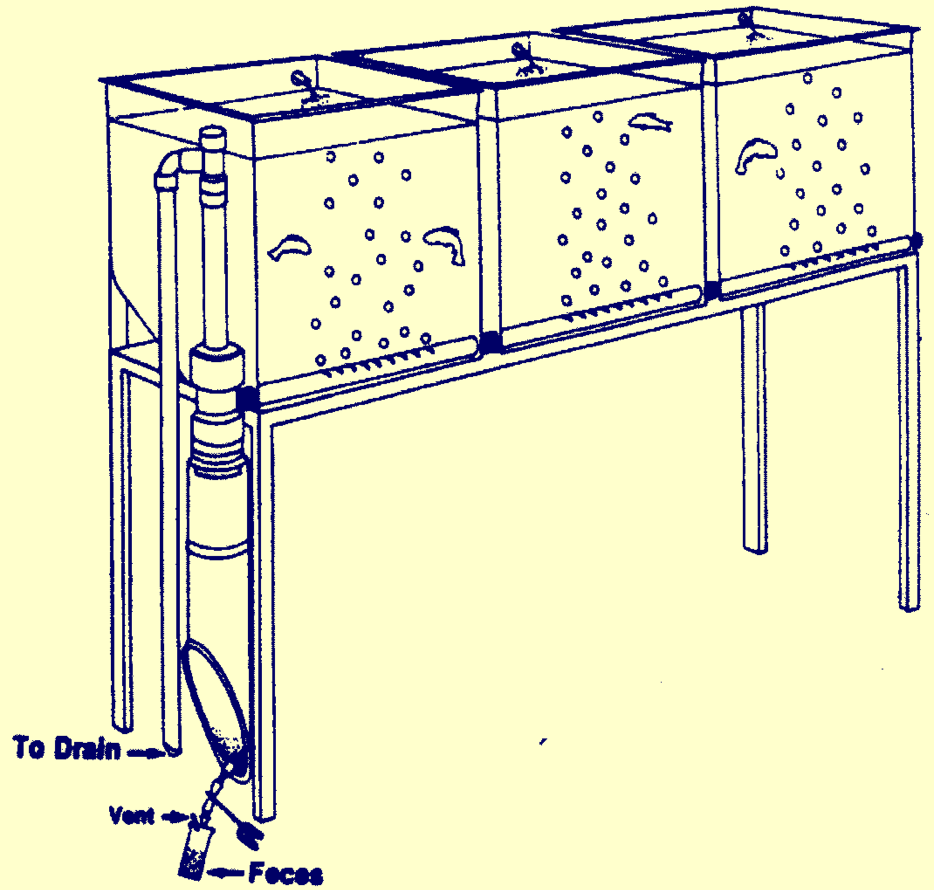

Nose type II
Nose type I
Ogino type II
25cm
10cm
1.5 cm
Common Drain Pipe
To Drain
Vent
Feces
Cho type

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

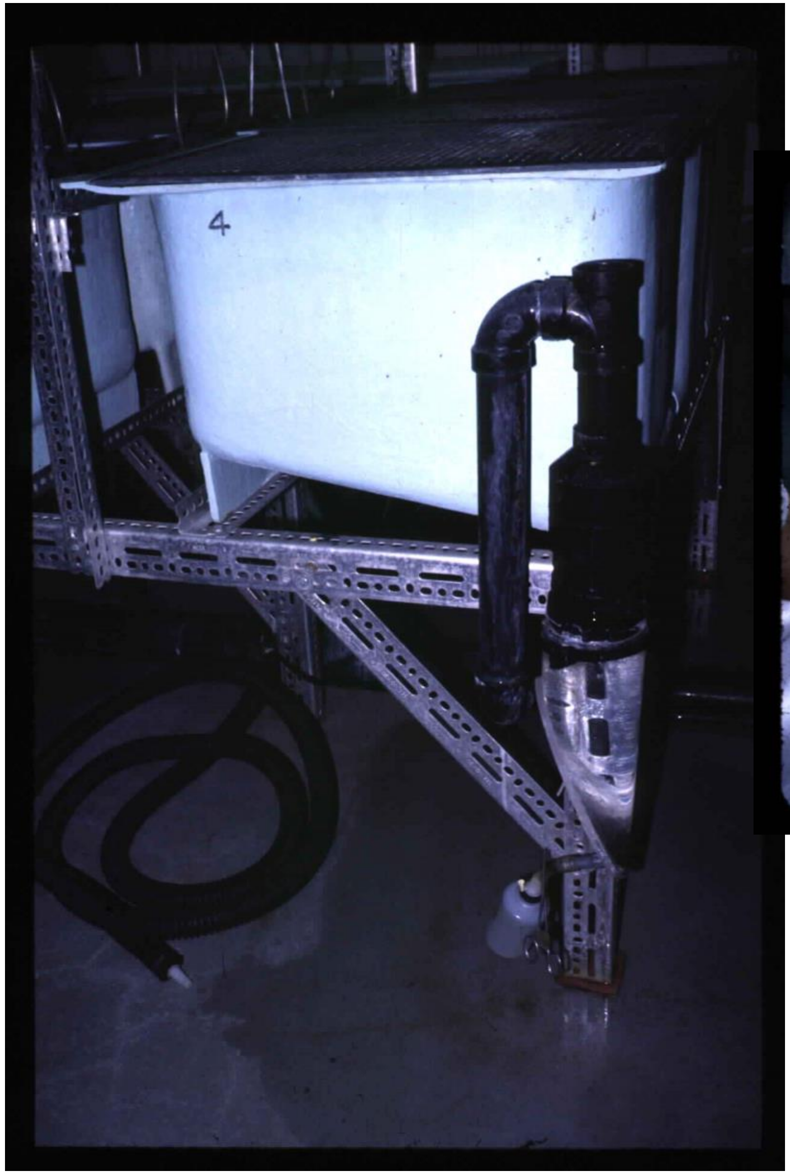


Choubert,G., de la Noue, J. and Luquet, P., 1982. Digestibility in fish: Improved device for the automatic collection of feces. Aquaculture, 29: 185-189.

The Guelph System (Cho et al., 1982)



Guelph Digestibility System



Aquaculture Nutrition 2001 7; 237–245

Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers

G.W. VANDENBERG & J. DE LA NOÜE
Groupe de recherche en recyclage biologique et aquiculture, Département des sciences animales, Université Laval, Ste-Foy, Québec G1K-7P4, Canada

Table 1 Experimental diet formulation (as-is basis)

Ingredient[1]	Inclusion ($g \cdot kg\ diet^{-1}$)
Fish meal	325.0
Wheat middlings	150.0
Soyabean meal	130.0
Corn gluten meal	100.0
Whey	125.0
Blood meal	40.0
Fish oil	80.0
Carboxymethyl cellulose	20.0
Vitamin premix[2]	5.0
Mineral premix[3]	5.0
Chromic oxide[4]	5.0
Sipernat 50[5]	10.0
Titanium dioxide[4]	5.0

	Marker			
Parameter / Method	**Cr2O3**	**AIA**	**TiO2**	
ADC Dry Matter				
St-Pee System	68.3	68.5	71.8	Middle
Guelph-Style Column	75.5	73.8	78.3	Higher
Stripping Method	48.0	58.1	64.4	Lower
ADC Crude Protein				
St-Pee System	87.4	88.2	89.7	Middle
Guelph-Style Column	91.9	90.9	91.9	Slightly higher
Stripping Method	80.0	83.1	85.7	Lower
ADC Lipids				
St-Pee System	84.3	85.1	86.9	Similar
Guelph-Style Column	81.7	84.3	86.8	Similar
Stripping Method	75.0	75.4	81.8	Lower

Vandenberg and de la Noue (2001)

Which technique is the best?

Focus on collecting a “representative” fecal sample free of uneaten feed

Beware of leaching / break-up of fecal material

Use a technique consistently

Recognize the limitations

Poultry By-Products Meal

Guelph System	ADC	
	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%

Data obtained using the same facilities and methodology. There is value in using standard methodological approaches consistently over many years.

FISHERIES SCIENCE 2002; **68**: 325–331

Original Article

Changes of phosphorus absorption from several feed ingredients in rainbow trout during growing stages and effect of extrusion of soybean meal[†]

SHUICHI SATOH,[1]* MINORU TAKANEZAWA,[1] ATSUSHI AKIMOTO,[2] VISWANATH KIRON[1] AND TAKESHI WATANABE[1]

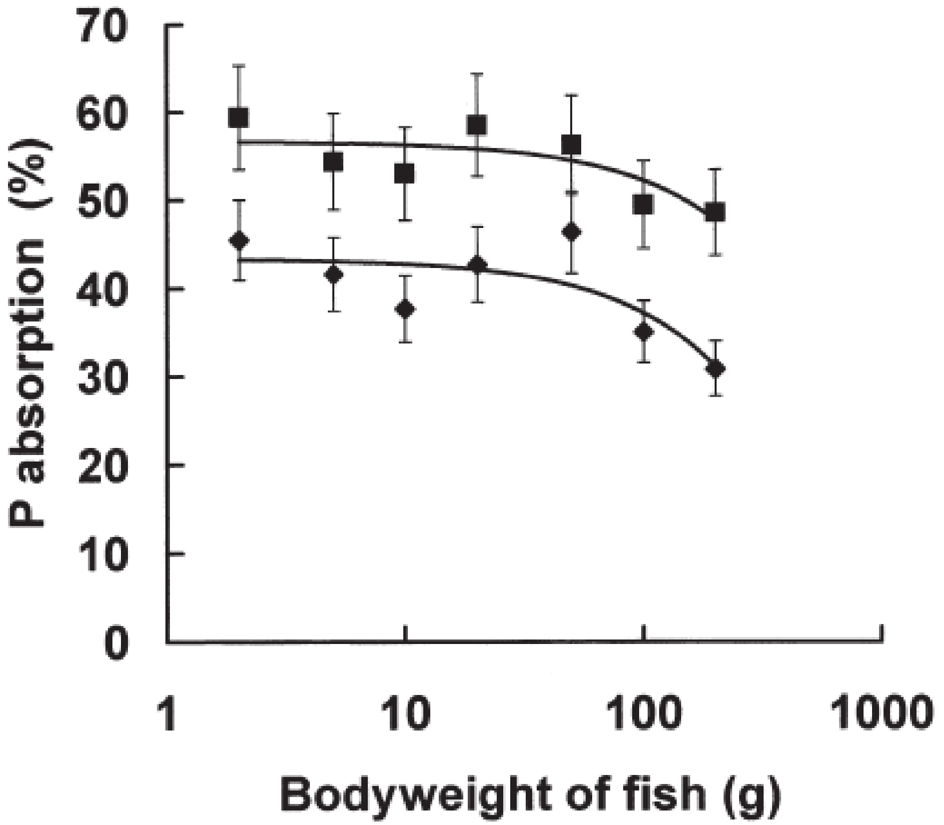


Fig. 1 Changes in phosphorus (P) absorption from (◆) pollock meal and (■) sardine meal by rainbow trout during their growing stages.

Differences in Digestibility Between Animals of Different Sizes or Simple Methodological Artefact due to Differences in Surface Area of Fecal Material ???

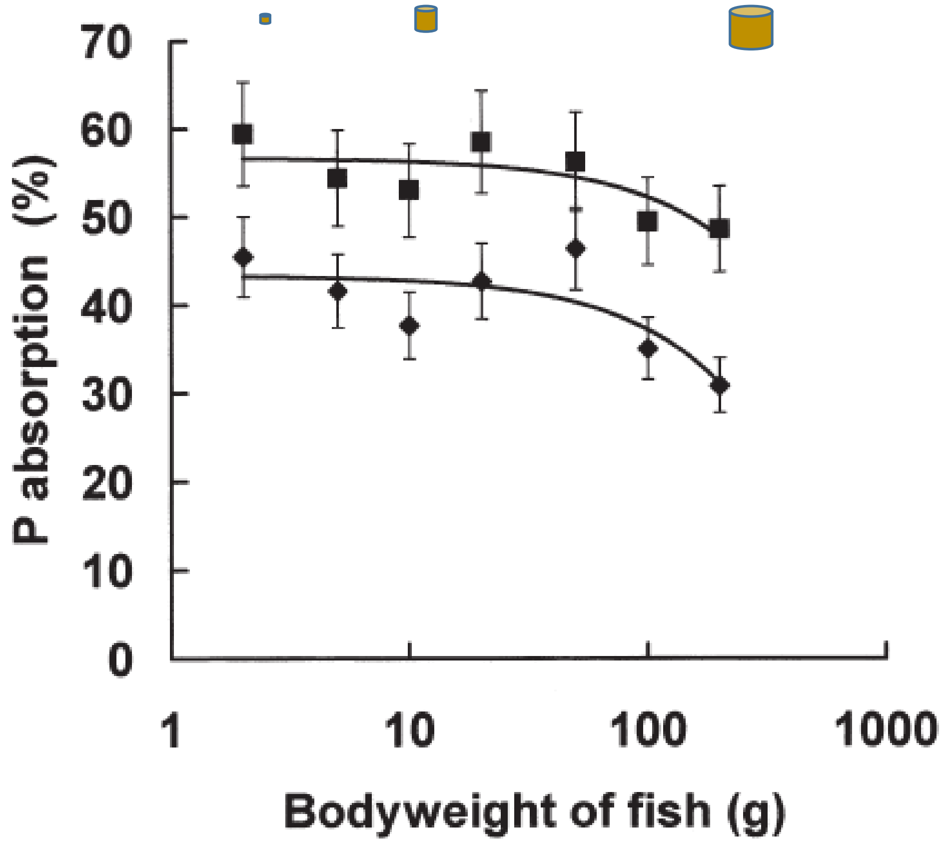
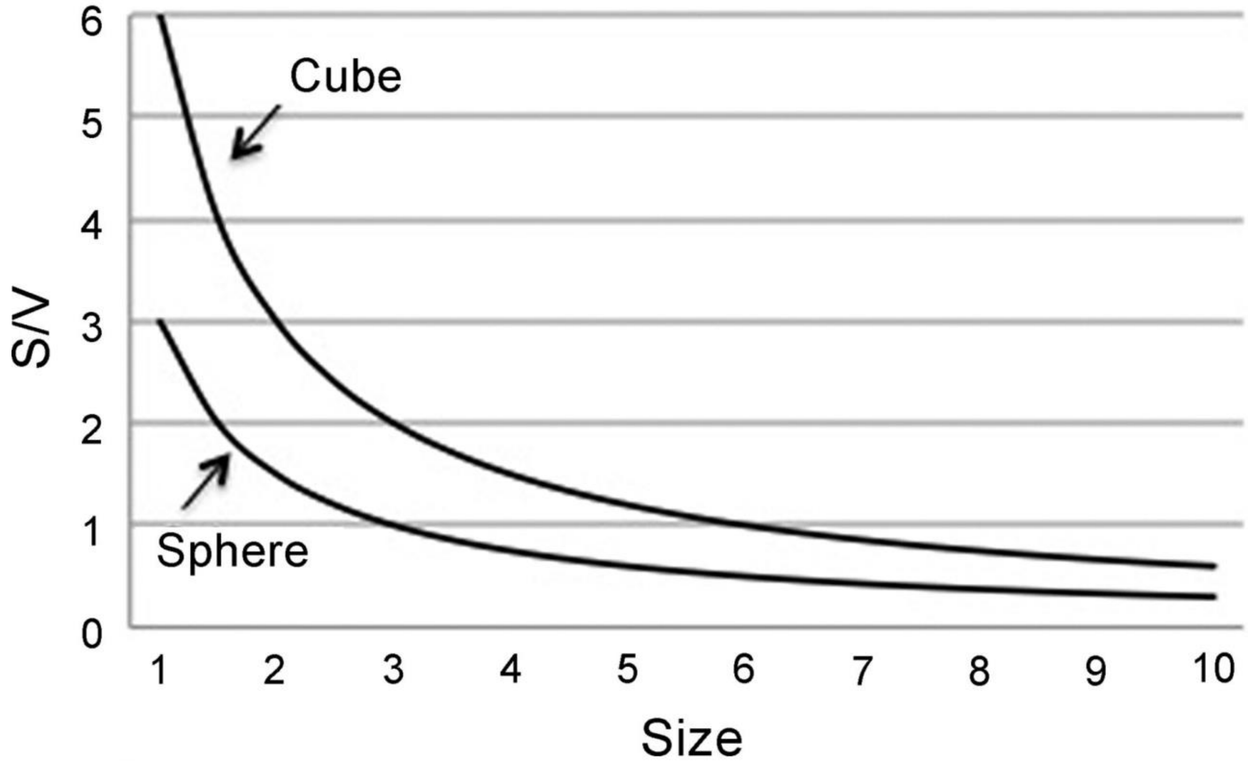


Fig. 1 Changes in phosphorus (P) absorption from (◆) pollock meal and (■) sardine meal by rainbow trout during their growing stages.



Smaller fecal particles =
Greater surface area =
More prone to leaching
And also a lot more difficult to collect!

Differences Between Species

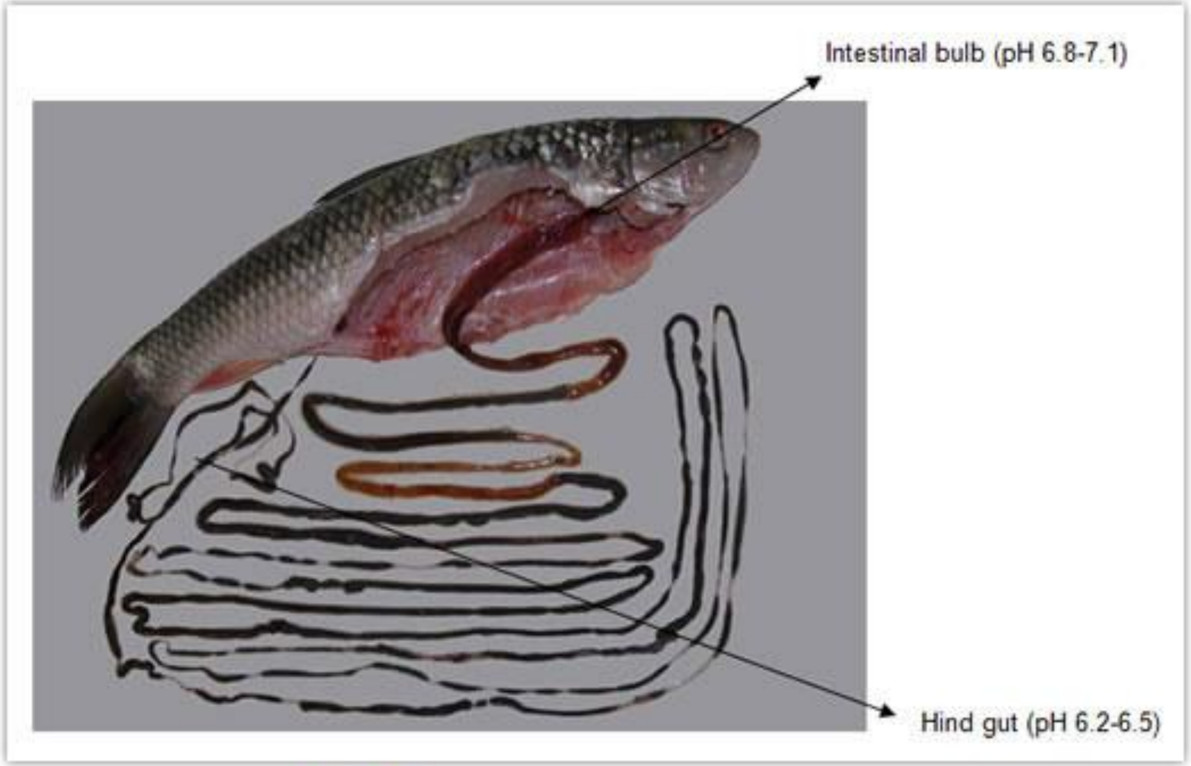


Figure 5. The uncoiled digestive tract of mrigal.
The long and thin-walled intestine provides a large surface area for nutrient absorption.

Apparent Digestibility of Four Practical Diets in Two Fish Species

	ADC (%)			
	DM	CP	GE	L
Lake trout				
Diet 1, DP/DE = 24	84.3	94.3	88.7	89.3
Diet 2, DP/DE = 22	81.9	94.3	86.0	86.8
Diet 3, DP/DE = 20	78.8	93.8	82.3	81.9
Diet 4, DP/DE = 18	79.3	94.6	82.4	83.8
Significance[1]				
Linear	$P<0.05$	NS	$P<0.05$	NS
Quadratic	NS	NS	NS	NS
Atlantic salmon				
Diet 1, DP/DE = 24	82.9	93.6	86.5	84.9
Diet 2, DP/DE = 22	79.2	93.4	82.3	80.8
Diet 3, DP/DE = 20	76.0	92.5	78.7	76.3
Diet 4, DP/DE = 18	77.8	93.9	80.0	78.8
Significance[1]				
Linear	$P<0.05$	NS	$P<0.05$	NS
Quadratic	$P<0.05$	$P<0.05$	NS	NS
SEM[2]	1.2	0.3	1.6	3.1
Effects of				
Species	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$
Diet	$P<0.05$	$P<0.05$	$P<0.05$	NS
Species * Diet	NS	NS	NS	NS

Aquaculture 435 (2015) 92–99

Contents lists available at ScienceDirect

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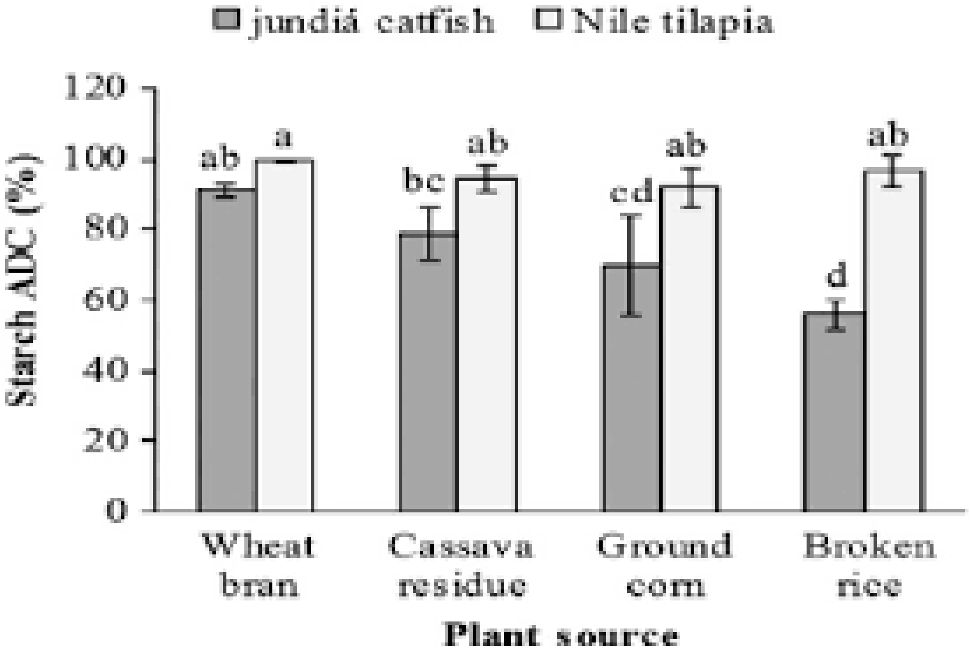
journal homepage: www.elsevier.com/locate/aqua-online

Comparison between the omnivorous jundiá catfish (*Rhamdia quelen*) and Nile tilapia (*Oreochromis niloticus*) on the utilization of dietary starch sources: Digestibility, enzyme activity and starch microstructure

Maria do Carmo Gominho-Rosa [a,1], Ana Paula Oeda Rodrigues [b], Bruna Mattioni [c], Alicia de Francisco [c], Gilberto Moraes [d], Débora Machado Fracalossi [a,*]



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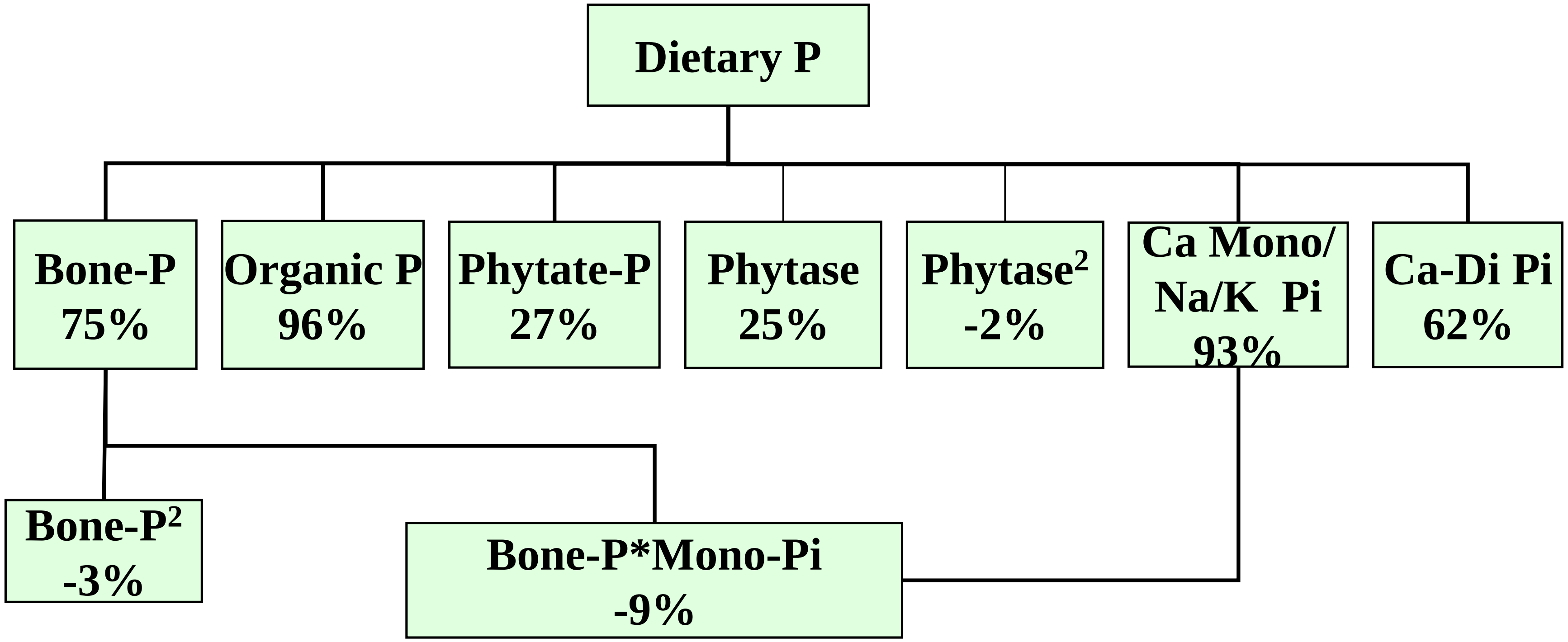
Quantification of differences in digestibility of phosphorus among cyprinids, cichlids, and salmonids through a mathematical modelling approach

K. Hua *, D.P. Bureau

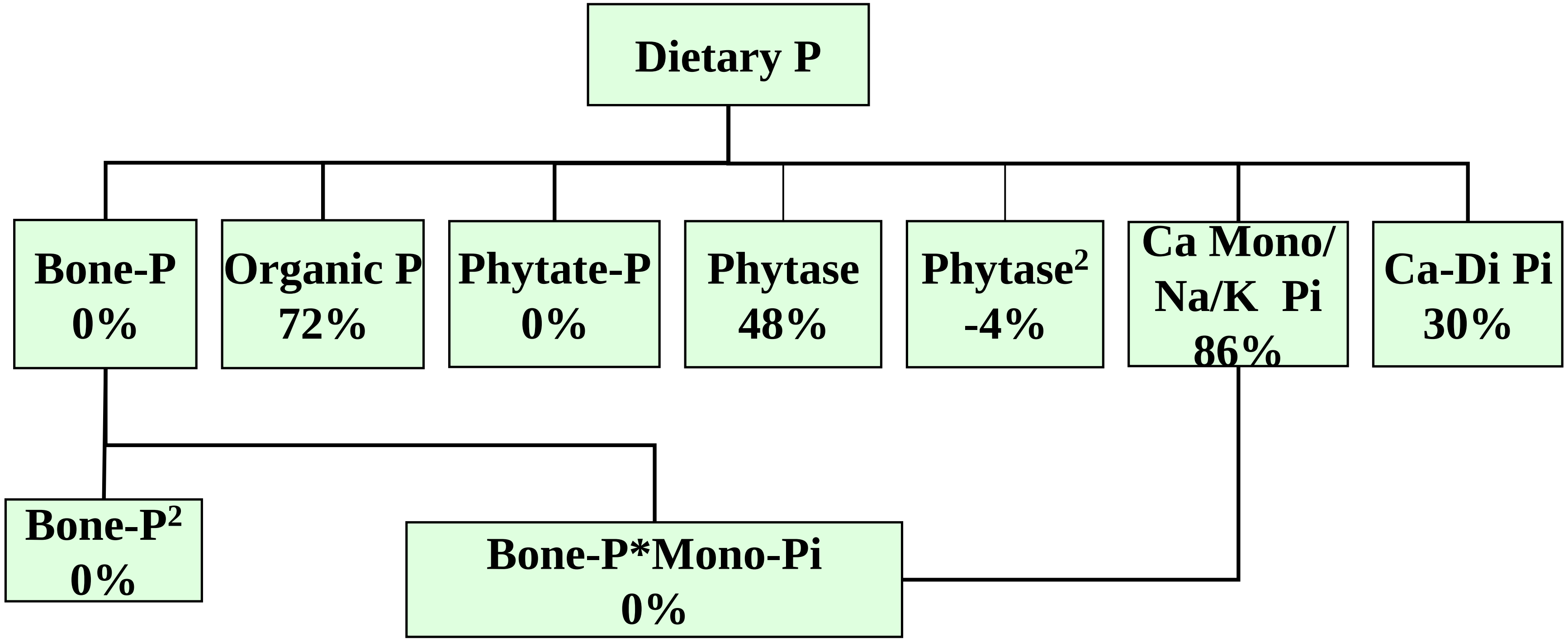
UG/OMNR Fish Nutrition Research Laboratory, Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

P Digestibility Model for Tilapia



$$
\begin{aligned}
\text{Digestible P} = {} & 0.75\ \text{bone}-\text{P} \\
& + 0.27\ \text{phytate}-\text{P} \\
& + 0.95\ \text{organic P} \\
& + 0.93\ \text{Ca monobasic /Na/ K Pi supplement} \\
& + 0.62\ \text{Ca dibasic Pi supplement} \\
& + 0.25\ \text{phytase/phytate} \\
& - 0.02\ (\text{phytase/phytate})^2 \\
& - 0.03\ (\text{bone}-\text{P})^2 \\
& - 0.09\ \text{bone}-\text{P} \\
& \times {}^{*}\text{Ca monobasic /Na/ K Pi supplement}
\end{aligned}
$$

P Digestibility Model for Common carp



$$\begin{aligned}\text{Digestible P} = {} & 0 \text{ bone} - \text{P} + 0 \text{ phytate} - \text{P} + 0.72 \text{ organic P} \\ & + 0.86 \text{ Ca monobasic /Na/ K Pi supplement} \\ & + 0.30 \text{ Ca dibasic Pi supplement} \\ & + 0.48 \text{ phytase/phytate} - 0.04 \,(\text{phytase/phytate})^2\end{aligned}$$

Historical Ingredient Digestibility Data

Table 8. Digestible and metabolizable energy and ratio measured with rainbow trout (Smith *et al.*, 1980 and NRC-NAS, 1981b)

Ingredient name	International feed number	Digestible energy* (MJ/kg)	Metabolizable energy (MJ/kg)	ME/DE*
Alfalfa meal	1-00-023	8.1	5.8	0.72
Blood meal, spray-dried	5-00-381	19.4	16.8	0.87
Corn gluten meal	5-09-318	16.9	14.9	0.88
Corn dist. solubles	5-02-844	10.3	9.6	0.93
Cotton seed meal	5-07-874	12.4	10.3	0.83
Fish meal, anchovy	5-01-985	19.1	16.8	0.88
herring	5-02-000	19.8	17.3	0.87
salmon	5-02-012	16.8	14.9	0.89
whitefish	5-02-025	14.6	12.4	0.85
Fish solubles, dehy.		15.5	14.0	0.90
Rapeseed meal, sol. extracted	5-03-871	12.5	11.3	0.90
Soybean meal, dehulled	5-04-612	12.5	10.7	0.86
Soybean, fullfat,	5-04-597			
roasted, 232°C, 8 min.		18.1	16.4	0.91
Jetsploder, 204°C		18.6	17.1	0.92
Wheat, hard, clears		7.9	6.6	0.84
Wheat middlings	4-05-205	10.3	9.4	0.91
Wheat germ meal	5-05-218	12.6	11.5	0.91
Whey, dehydrated	4-01-182	11.3	10.0	0.88
low lactose	4-01-186	11.1	9.5	0.86
Yeast, brewers	7-05-527	15.9	12.2	0.77
torula	7-05-534	15.4	14.1	0.92

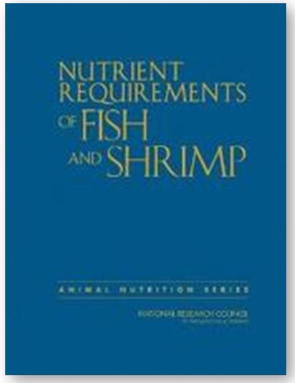
CHO C. Y. & SLINGER S. J. (1979) Apparent digestibility measurement in feedstuffs for rainbow trout. Proc. World Symp. on Finfish Nutrition and Fishfeed Technoloqy, Hamburg, Germany, Vol. II, pp. 239 247.

NRC-NAS (1981b) Nutrient Requirements of Coldwater Fishes. Nutrient Requirement of Domestic Animals No. 16, 63 p. National Academy Press, Washington, D.C.

CHO, C.Y., SLINGER S.J. and BAYLEY H.S. (1982) Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, pp. 25-41

Estimates of apparent digestibility of protein and energy of practical ingredients have been available for about 40 years

Apparent Digestibility Coefficient (ADC) of Crude Protein of Different Ingredients



Ingredients	Salmon	Rainbow Trout	Atlantic Cod	Silver Perch	Tilapia	Gilthead Sea Bream	Rockfish	Penaid Shrimp
Blood meal	30	84 – 99		90		90	87	66-71
Casein	100	92–95						96
Canola meal	79	91	76-79	83	85			80
Corn gluten meal	92	92–97	86	95	89–97	90	92	59
Feather meal	71-80	77–87	62	93	79	58	79	64
Fish meal, Anchovy	91	94–97	92		91		95	83-89
Fish meal, Menhaden	83-88	86–90			85			84-89
Meat and bone meal	85	83–88		73	78	72-90	91	60–88
Poultry by-products meal	74–94	83–96	80	85	74–90	82		79
Soybean meal	77–94	90–99	92	95	87– 94	87–91	84	89–97
Soy protein concentrate	90	98–100	99					93
Soy protein isolate	97	98	97					94
Wheat gluten	99	100	100	100				96

NRC (2011)

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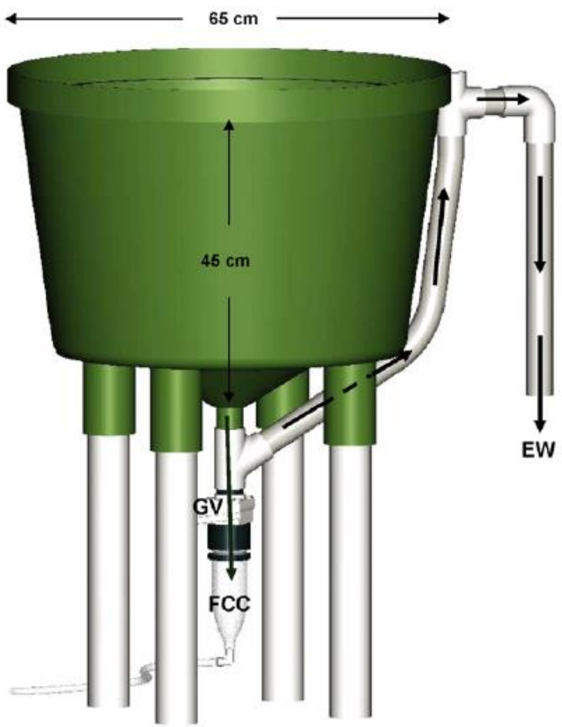
Aquaculture 261 (2006) 1314–1327
www.elsevier.com/locate/aqua-online

Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758)

Sean M. Tibbetts, Joyce E. Milley, Santosh P. Lall *



Ingredient	Protein ADC	Energy ADC	DE[a]
Reference diet	91.2	80.7	16.5
Fish meals			
Herring meal	93.3±0.6	92.8±0.1	19.3±0.0
Anchovy meal	92.2±0.5	86.4±0.7	16.5±0.1
Crustacean by-product meals			
Whole krill meal	96.3±0.6	96.3±0.6	18.1±0.1
Crab meal	89.4±0.7	82.4±0.7	13.0±0.1
Shrimp meal	66.7±1.4	41.4±4.0	5.1±0.5
Animal by-product meals			
Poultry by-product meal	80.2±0.7	71.0±1.1	15.6±0.2
Hydrolyzed feather meal	62.4±0.3	58.9±0.3	13.3±0.1
Oilseed meals			
Soybean meal	92.3±1.5	88.1±0.3	15.3±0.1
Soy protein concentrate	98.6±0.6	94.9±0.3	18.0±0.1
Soy protein isolate	97.4±0.6	92.1±0.8	19.5±0.2
Canola meal	76.0±1.6	60.6±1.7	11.0±0.3
Canola protein concentrate	88.8±0.4	83.3±0.3	16.1±0.1
Flaxseed meal (period 1)	50.2±1.6	21.2±0.3	4.0±0.1
Flaxseed meal (period 2)	55.0±1.1	37.4±0.1	7.0±0.0
Pulse meals			
Pea protein concentrate	89.8±0.8	76.7±0.3	14.2±0.1
White lupin meal	89.7±3.8	75.3±1.3	14.3±0.2
Cereal grain meals			
Corn gluten meal	86.3±1.0	82.7±0.7	17.2±0.1
Wheat gluten meal	99.9±0.3	95.4±0.7	21.5±0.2

Values are mean±SE (n=4 except for flaxseed meal where n=2).

Estimates from large-scale or sustained efforts are available for different species

ASSESSMENT OF THE NUTRITIONAL VALUE OF INGREDIENTS FOR FEED DEVELOPMENT FOR ASIAN SEABASS, Lates calcarifer

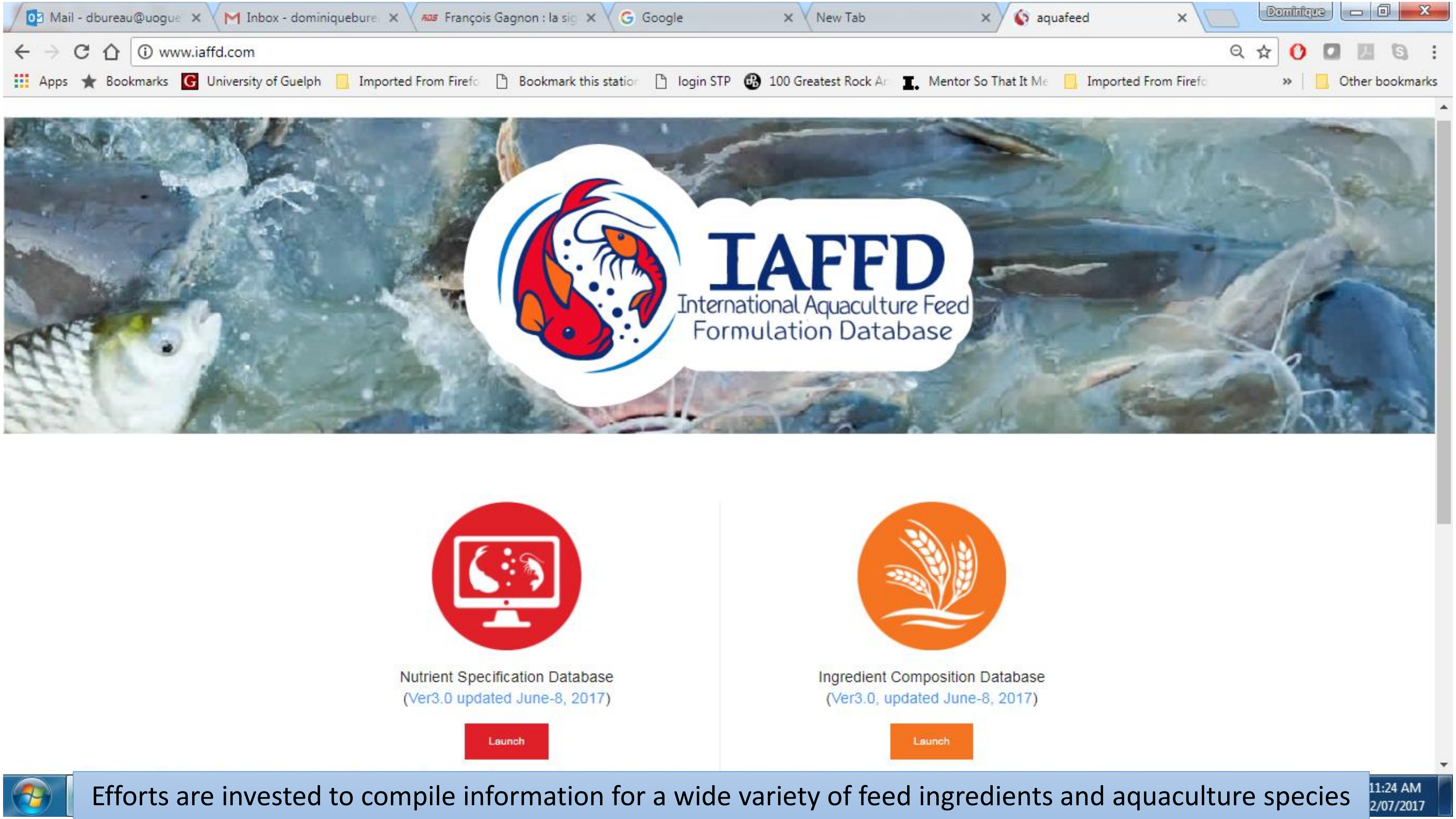
Tran Quoc Binh*, Vu Anh Tuan, David Smith and Brett Glencross Minh Hai Sub-Institute for Fisheries Research (Research Institute for Aquaculture No.2), Ca Mau City, Ca Mau Province, Vietnam. tranquocbinhaquaculture@yahoo.com.vn

Table 1. Composition and digestibility of key feed ingredients for marine fish

	Ingredient Specifications (all values g/kg DM)						*Ingredient Digestibility*	
	DM (g/kg)	Protein	Lipid	Ash	CHO	Energy (MJ/kg)	Protein ADC	Energy ADC
Fishmeal (CaMau - Vietnam)	903	551	125	298	26	18.4	91.9	94.6
Poultry meal (European)	919	646	127	132	95	21.9	87.8	86.5
Soybean meal (Vietnam)	883	424	215	51	310	23.8	88.7	80.6
Soybean meal (Argentina)	871	521	35	71	373	20.1	92.7	68.8
Cassava (Vietnam)	864	29	7	26	938	17.2	78.9	71.2

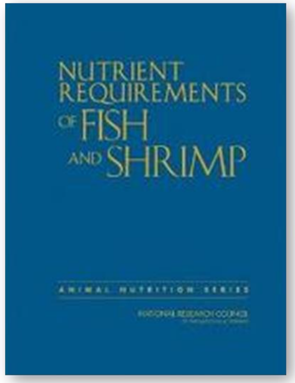
DM : Dry matter, ADC: Apparent Digestibility Coefficient, CHO: Carbohydrate

Estimates are available for Asian feed ingredients and aquaculture species
These are highly valuable to Asian aquaculture feed manufacturers



Efforts are invested to compile information for a wide variety of feed ingredients and aquaculture species with the needs of aquaculture feed manufacturers in mind

Apparent Digestibility Coefficient (ADC) of Crude Protein of Different Ingredients



Ingredients	Salmon	Rainbow Trout	Atlantic Cod	Silver Perch	Tilapia	Gilthead Sea Bream	Rockfish	Penaid Shrimp
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Casein	100	92–95						96
Canola meal	79	91	76-79	83	85			80
Corn gluten meal	92	92–97	86	95	89–97	90	92	59
Feather meal	71-80	77–87	62	93	79	58	79	64
Fish meal, Anchovy	91	94–97	92		91		95	83-89
Fish meal, Menhaden	83-88	86–90			85			84-89
Meat and bone meal	85	83–88		73	78	72-90	91	60–88
Poultry by-products meal	74–94	83–96	80	85	74–90	82		79
Soybean meal	77–94	90–99	92	95	87– 94	87–91	84	89–97
Soy protein concentrate	90	98–100	99					93
Soy protein isolate	97	98	97					94
Wheat gluten	99	100	100	100				96

NRC (2011)

Plant Protein Ingredients of Similar Botanical Origins with Different Nutritional Compositions

	Sunflower Meals				Canola/Rapeseed Meals/ Concentrates		
	HPSFM Fino	**HPSFM Bunge**	**SFM Chile**	**SFM USA**	**CM Canada**	**HPRSM Bunge**	**CPC Bunge**
Dry matter, %	91.0	91.5	90.8	93.9	90.0	92.3	95.6
Crude protein, %	41.8	45.5	38.7	18.5	35.0	39.3	60.9
Lipids, %	3.2	0.8	0.7	25.5	2.5	1.1	0.0
Ash, %	8.8	8.2	7.3	8.4	7.4	7.1	8.1
Total carbohydrates, %	37.3	37.0	44.0	41.5	45.1	44.9	26.7
Gross energy, KJ/g	17.5	17.4	17.0	21.6	17.0	17.4	19.0
Total phosphorous, %	2.0	1.6	1.3	0.9	1.1	1.3	1.7
Arginine	5.7	6.0	5.6	2.3	4.3	5.7	8.4
Histidine	1.0	1.0	0.9	0.4	1.0	1.2	1.7
Isoleucine	1.5	1.5	1.4	0.6	1.3	1.7	2.5
Leucine	2.6	2.6	2.4	1.3	2.5	3.3	5.2
Lysine	1.5	1.6	1.4	0.6	2.1	2.3	3.4
Phenylalanine	1.9	1.9	1.8	0.8	1.5	1.9	3.1
Threonine	1.5	1.6	1.5	0.7	1.6	2.0	2.9
Valine	1.8	1.8	1.8	0.8	1.7	2.2	3.2

	HPSFM Fina	HPSFM Bunge	SFM Chile	SFM USA	CM Canada	HPRSM Bunge	CPC Bunge
ADC (%) of proximate components, gross energy, and total phosphorous							
Dry matter	71	79	64	57	73	80	76
Crude protein	100	96	99	73	95	95	87
Lipids	-	-	-	-	-	-	-
Ash	31	42	47	52	56	64	64
Total carbohydrates	42	62	35	44	53	68	54
Gross energy	80	88	71	62	79	86	81
Total phosphorous	15	18	28	52	40	49	67
ADC (%) of essential amino acids							
Arginine	100	98	100	93	100	100	92
Histidine	100	100	100	88	100	100	94
Isoleucine	100	100	100	93	100	100	93
Leucine	100	95	100	88	99	98	92
Lysine	100	96	100	82	99	100	93
Phenylalanine	99	97	100	92	99	99	92
Threonine	100	99	100	95	100	100	94
Valine	100	96	100	89	98	99	93

Plant protein ingredients from various origins can be very highly digestible to rainbow trout (carnivorous fish)
Difference in nutritional composition (protein and fibre levels) don’t appear to play a major role. Manufacturing does.

Observations Regarding Available Data

Digestibility very high (> 90%) for "high quality", standardized, feed ingredients (e.g. casein, wheat gluten, spray-dried blood, low temperature fish meal, krill, soy protein concentrate, etc.) across <u>studies</u> and <u>species</u>

Significant differences (10-20%) across species for certain ingredients

Significant variability (10-20%) in the estimate of digestibility of ingredients across <u>studies</u> but also <u>within</u> studies

Implications: If formulating on digestible protein (DP) and digestible methionine levels:

10% variation in estimates of ADC = USD 5 to 10/tonne of feed

3. Limitations / Pitfalls

Systematic compilation of data from published digestibility trials as well as many years of carrying out peer-review of scientific manuscripts and review/auditing of diverse research efforts of academic and industry partners highlighted the following <u>issues</u> in terms of estimation of ADC of crude protein:

1) Methodological Issues
    1) Mathematical Issues*
    2) Equipment/ Approach Used (Fecal Collection*)
    3) Chemical analysis Issues*
    4) Statistical Issues

2) Nutritional Issues
    1) Characterization of ingredient origin/ type*
    2) Digestibility vs. bio-availability

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 x Di/Fi)

	Feed %	Feces %	Digestibility	%
Dry matter	95	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**

Digestibility of Single Ingredients

Most ingredients cannot be fed alone

- Acceptance (palatability)
- Pelletability
- Nutritional quality

Test diet

- 70% Reference diet
- 30% Test ingredient

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Aquaculture 252 (2006) 103–105

Aquaculture

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Letter to the Editor of *Aquaculture*

The dietary protocol of Cho and Slinger (1979) is one of the most widely used protocols for determining the digestibility of test ingredients for fish. In this protocol 7 parts (as is) of reference diet mash are mixed with 3 parts (as is) test ingredient to form a test diet. The following equation has been used by many laboratories for many years to calculate the apparent digestibility coefficients (ADC) for nutrients of test ingredient based on the ADC of reference and test diets (Cho and Slinger, 1979; Cho et al., 1982).

$$ADC_{\text{test ingredients}} = [ADC_{\text{test diet}} - (0.7 \times ADC_{\text{reference diet}})]/0.3 \quad (1)$$

Mathematically incorrect / illogical except for Dry Matter

Forster (1996) and Sugiura et al. (1996) demonstrated that Eq. (1) was mathematically incorrect since it did not account for the real nutrient contribution of the reference diet and the test ingredient. A revised equation to calculate ADC of the test ingredient was first presented by Forster (1996) and published in peer-reviewed publications a few years later (Sugiura et al.,1998; Forster, 1999):

$$ADC_{\text{ingredient}} = [(ADC_{\text{test diet}} \times D_{\text{test}}) - (0.7 \times D_{\text{ref}} \times ADC_{\text{reference diet}})]/(0.3 \times D_{\text{ing}}) \quad (2)$$

Mathematically Correct/ Logical

where D_{ref}=% nutrient (or kJ/g gross energy) of reference diet (as is); D_{test}=% nutrient (or kJ/g gross energy) of test diet (as is); D_{ing}=% nutrient (or kJ/g gross energy) of test ingredient (as is).

This can be simplified to:

$$ADC_{\text{ingredient}} = [ADC_{\text{test diet}} \times D_{\text{test}} \times (0.7 \times DM_{\text{ref}} + 0.3 \times DM_{\text{ingr}}) - (0.7 \times D_{\text{ref}} \times ADC_{\text{reference diet}})]/(0.3 \times D_{\text{ingr}}) \quad (3)$$

Mathematically Correct/ Logical Adjusted for different dry matter

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$$ADC_{\text{ingredient}} = [(ADC_{\text{test diet}} \times D_{\text{test}}) - (0.7 \times D_{\text{ref}} \times ADC_{\text{reference diet}})]/(0.3 \times D_{\text{ing}}) \tag{2}$$

$$ADC_{\text{ingredient}} = [ADC_{\text{test diet}} \times D_{\text{test}} \times (0.7 \times DM_{\text{ref}} + 0.3 \times DM_{\text{ingr}}) - (0.7 \times D_{\text{ref}} \times ADC_{\text{reference diet}})]/(0.3 \times D_{\text{ingr}}) \tag{3}$$

$$ADC_{\text{test ingr}} = [(ADC_{\text{test diet}} \times (0.7 \times D_{\text{ref}} + 0.3 \times D_{\text{test}})) - (0.7 \times D_{\text{ref}} \times ADC_{\text{ref. diet}})]/(0.3 \times D_{\text{ingr.}})$$

which can be simplified to:

$$ADC_{\text{test ingredient}} = ADC_{\text{test diet}} + [(ADC_{\text{test diet}} - ADC_{\text{ref. diet}}) \times (0.7 \times D_{\text{ref}}/0.3 \times D_{\text{ingr}})] \tag{4}$$

All these equations are "mathematically" correct / logical so they should be giving the same answer, right?

Real-Life Comparison of the Results of Three Mathematically Correct Equations

Ingredient : Blood Meal 2 – Bureau et al (1999)	Values
ADC Crude Protein - Test ingredient	90.2%
ADC Crude Protein - Reference diet	92.3%
Dry Matter - Reference diet mash – Analyzed	92.8%
Dry Matter – Test ingredient – Analyzed	89.5%
Crude protein – Reference diet – Analyzed	45.0% (as is mash); 48.5% (DM) ; 46.5% (pellet, 95% DM)
Crude protein – Test ingredient – Analyzed	84.6% CP (as is) ; 94.5% (DM)
Crude protein – Test diet (70:30) – Expected	58.8% (as is 95.1% DM); 61.9% (DM)
Crude protein – Test diet (70:30) - Analyzed	57.1% (as is, 95.1% DM); 60.0% (DM)

Equation	ADC protein Expected diet composition	ADC protein Analyzed diet composition
Equation 2	90.7	84.6
Equation 3	87.3	81.3
Equation 4	87.5	87.5

Why???

Because we are compounding of all errors/discrepancies onto the term we are solving for (i.e. the ADC of test ingredient)

Equation – Digestibility (Equation 4)

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

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%)

Trial on the Digestibility of Crude Protein of Three Commercial Common Carp Feeds

	DM	CP	Lipid	TC	Ash	Cr	Cr
						Analyzed level	**Theoretical level**
Feed A	95.3	30.2	6.3	49.5	9.2	0.53	**0.42**
Feed B	94.4	31.5	6.5	44.9	11.4	0.64	**0.42**
Feed C	96.3	27.8	6.4	50.4	11.7	0.54	**0.42**

Digestion indicator incorporation level = 0.6% Cr_2O_3 (0.42% Cr)

	ADC CP	ADC CP	Difference
	Calculated based on analyzed Cr	Calculated based on theoretical Cr (in diets)	% point
Feed A	67.7	74.4	6.7
Feed B	64.1	76.4	12.3
Feed C	68.7	75.6	6.9

Digestion indicator analysis is frequently an issue. Identifying a problem for diet is easy but for fecal material it is very difficult

Real-Life Comparison of Results of Ingredient and Test Diet Analyses

	Dry Matter	Crude Protein	
Ingredients		Analyzed	Expected
Reference diet - mash	93.2	44.6	-
Canola meal – regular (CM)	90.0	32.7	-
Rapeseed meal - High Protein (HPRSM)	92.3	38.2	-
Canola Protein Concentrate (CPC)	95.6	53.1	-
Diets			
Test diet CM (70%Ref:30% CM)	94.9	40.4	41.3
Test diet HPRSM (70%Ref:30%HPRSM)	94.9	42.0	42.5
Test diet CPC (70%Ref:30%CPC)	94.7	46.5	49.0

Analytical errors are also very common
Data should add up

Importance of Being Rational and Critical in Review of Scientific Literature
Even if data is from a reputed laboratory and published in reputed journal!

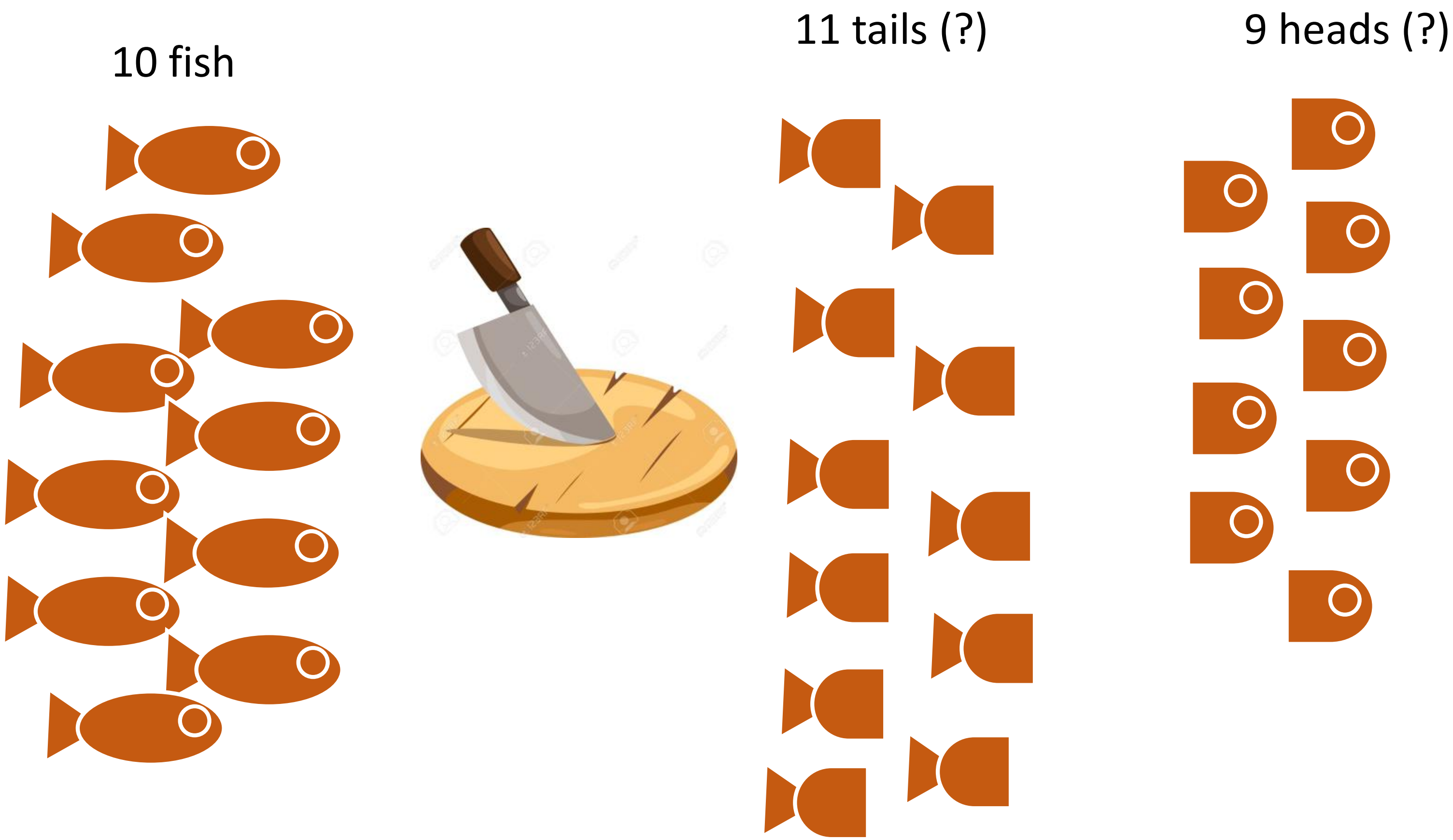
Table 3
Percent apparent digestibility coefficient (ADC) and phosphorus availability values of practical feedstuffs determined for a marine fish species

Ingredient	Organic matter ADC	Crude protein ADC	Lipid ADC	Gross energy ADC		Phosphorus availability
Select menhaden fish meal	93 9^{a} (4.9)	87.9^{ab} (1.4)	87.2^{a} (2.4)	95.0^{a} (2.7)	??	50.3^{ab} (6.7)
Regular menhaden fish meal	93.7^{a} (10.7)	76.9^{ab} (9.0)	67.6^{ab} (7.5)	92.1^{ab} (8.9)	??	47.9^{ab} (11.9)
Poultry by-product meal	75.6^{ab} (11.8)	48.7^{c} (5.3)	59.0^{b} (7.1)	71.7^{abc} (9.6)	????	26.5^{b} (4.7)
Meat and bone meal	86.2^{a} (11.7)	78.9^{ab} (6.7)	66.5^{b} (8.5)	86.0^{abc} (11.2)	??	65.5^{a} (11.7)
Soybean meal, dehulled	65.2^{ab} (14.4)	86.1^{ab} (4.7)	62.7^{b} (8.3)	63.3^{bc} (12.4)		46.8^{ab} (13.7)
Cottonseed meal	70.2^{ab} (8.4)	84.5^{ab} (4.1)	75.4^{ab} (4.1)	70.4^{abc} (7.1)		40.2^{ab} (19.1)
Wheat	46.9^{b} (11.6)	96.8^{a} (2.7)	87.9^{a} (0.9)	61.6^{c} (4.7)		78.8^{a} (5.9)

DE based on proximate = 1000*((.625*.46*23.6)+(.153*.622*39))/4.184 = 2508 kcal/kg
DE based on analyzed gross energy = 4993*0.717 = 3580 kcal/kg
Clearly a problem somewhere! ADC crude protein? **Diff: 1000 kcal !!!**

10 Heads and 10 Tails:

Dr. Young Cho's Parable About Making Sure Results are Adding Up



May be only wrong by 10% but illogical!

Test Material Issues

Characterization of Test Ingredients

Blood Meals – Same Name but Very Different Ingredients!

Guelph System	ADC Protein	ADC Energy
Spray-dried	96-99%	92-99%
Ring-dried	85-88%	86-88%
Steam-tube dried	84%	79%
Rotoplate dried	82%	82%

Different drying technique

Bureau et al. (1999)

Apparent Digestibility Coefficient (ADC) of Crude Protein of Different Ingredients – NRC 2011

Ingredients	Salmon	Rainbow Trout	Atlantic Cod	Silver Perch	Tilapia	Gilthead Sea Bream	Rockfish	Penaid Shrimp
Blood meal **(that's it???)**	30	82 – 99		90		90	87	66-71
Casein	100	92–95						96
Canola meal	79	91	76-79	83	85			80
Corn gluten meal	92	92–97	86	95	89–97	90	92	59
Feather meal	71-80	77–87	62	93	79	58	79	64
Fish meal, Anchovy	91	94–97	92		91		95	83-89
Fish meal, Menhaden	83-88	86–90			85			84-89
Meat and bone meal	85	83–88		73	78	72-90	91	60–88
Poultry by-products meal	74–94	83–96	80	85	74–90	82		79
Soybean meal	77–94	90–99	92	95	87– 94	87–91	84	89–97
Soy protein concentrate	90	98–100	99					93
Soy protein isolate	97	98	97					94
Wheat gluten	99	100	100	100				96

NRC (2011)

4. Determinants of the digestibility of nutrients: It's a matter of chemistry?

Poultry By-Products Meal

Guelph System	ADC	
	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%

Data obtained using the same facilities and methodology. There is value in using standard methodological approaches consistently over many years.

Exploring the value of a *in vitro* pH-stat digestibility assay

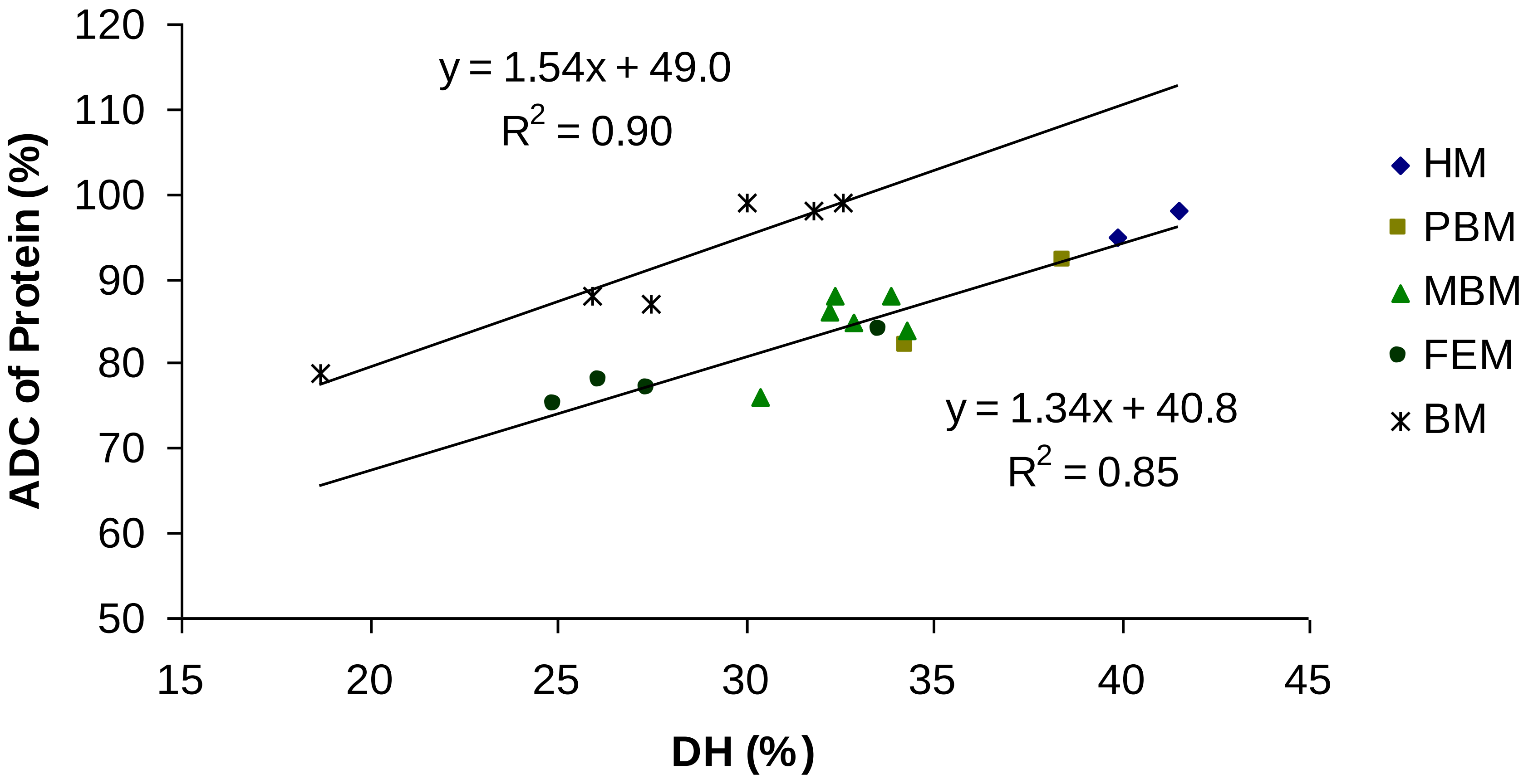
Collaboration with Dr. Adel El Mowafi, Shur-Gain AgResearch

Automated Titrator

TitraLab 854 pH-Stat
Titration Workstation

http://www.labsearch.ie/prod_pages/radiometer/TitraLab/ti_index.html#article1

Relationship between degree of hydrolysis (DH) with pH-Stat assay and digestibility of protein (ADC of protein) of animal proteins.



The results suggest that there is rational "chemical" bases to differences in apparent digestibility of proteins

Legends: HM= herring meal, PBM= poultry by-products meal, MBM = meat and bone meal, FEM=feather meal, BM = blood meal

El Mowafi et al. 1999

Thermal Processing of Protein Ingredients

Under-Processing

High level of moisture
High level of anti-nutritional factors
Susceptibility to microbial spoilage
High volume
Problems with handling and storage

Over- Processing

Heat damage
Chemical changes
Amino acids destruction
Lower nutritional value

Optimal Processing

Heat Treatment of Soybean Meal (SBM)

Control (Not heated)	Autoclaved SBM to 125°C for 15 min	Autoclaved SBM to 125°C for 30 min

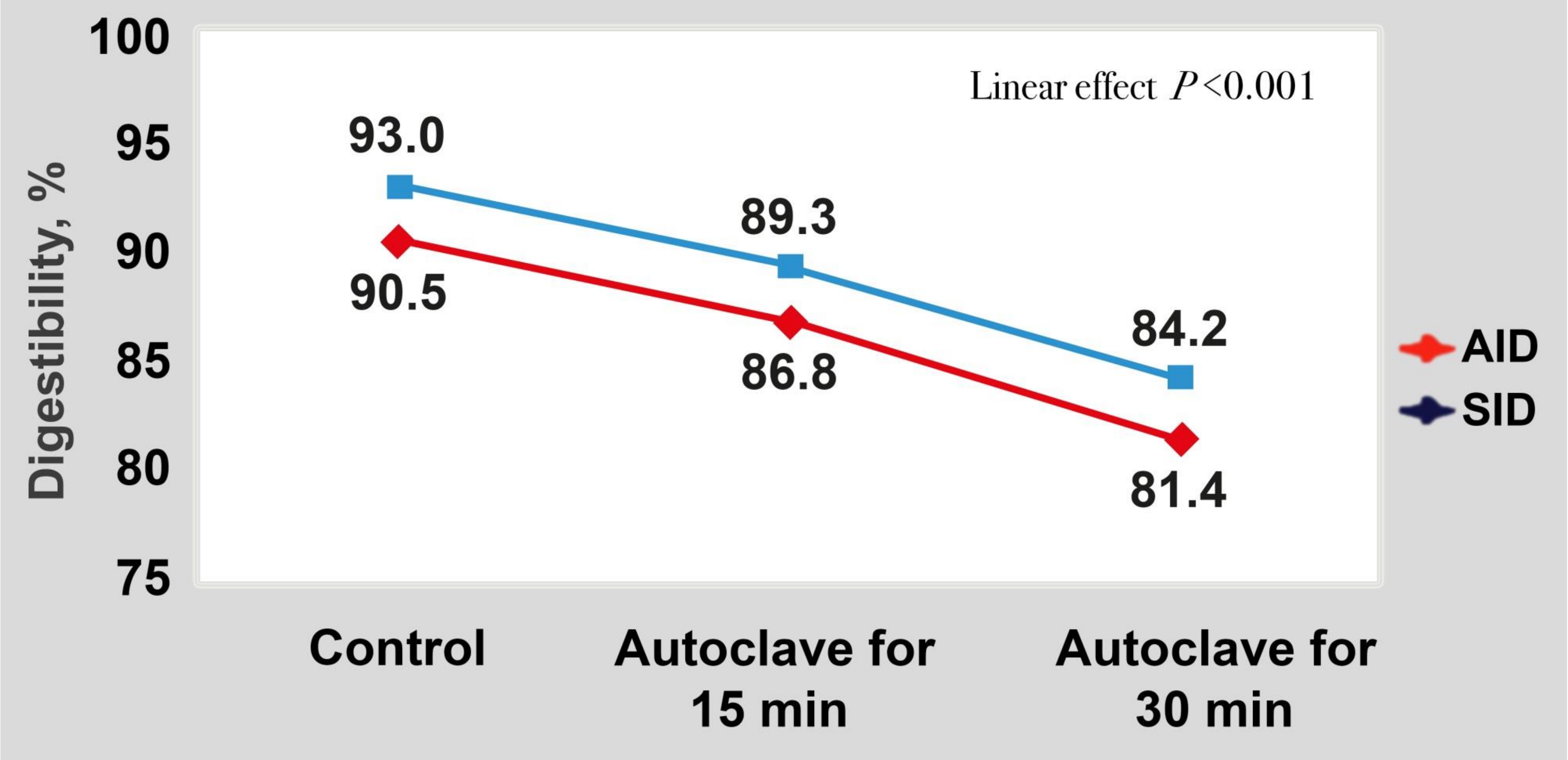
L*	76.7	**61.7**	**52.5**
a*	3.4	**10.0**	**12.5**

L* : Indication of the lightness of the product
a*: Measurement of the redness of the colors

Gonzalez- Vega et al., 2011

Heat Damage in SBM
Impact of Overheating on Digestibility of Lysine

Effect of autoclaving time on apparent ileal digestibility (AID)
and standardized ileal digestibility (SID) of lysine
in pigs fed soybean treated products in their diets **(Temperature: 125 °C)**

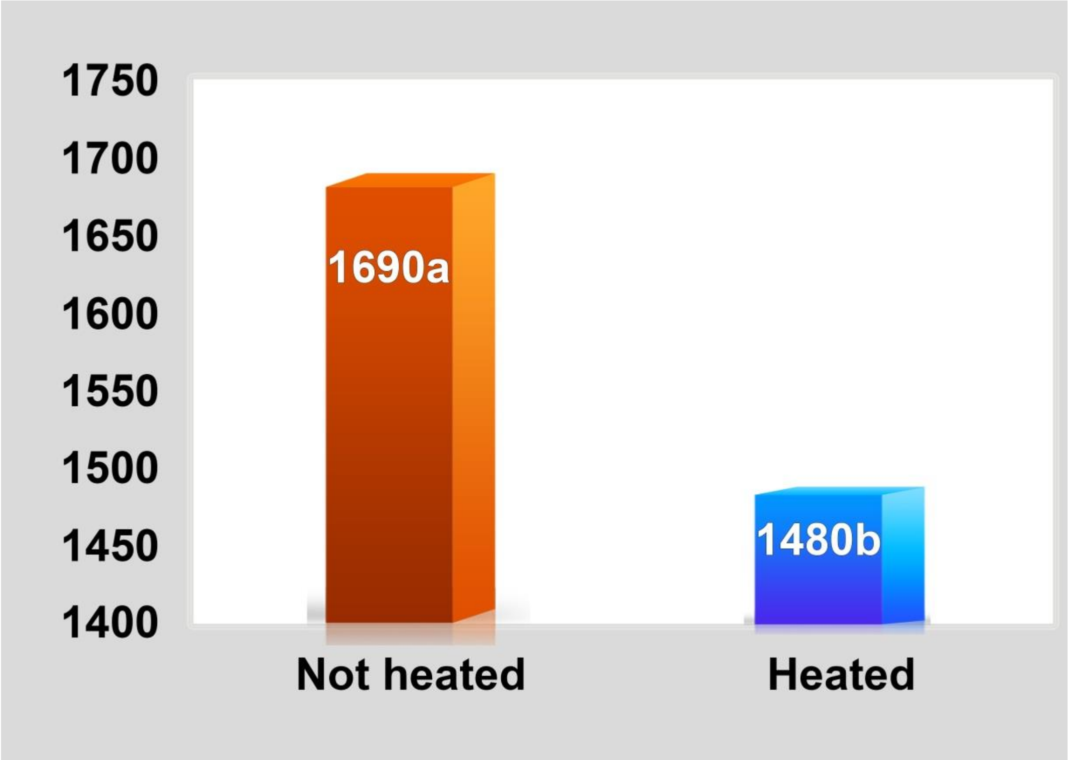


Gonzalez- Vega et al., 2011

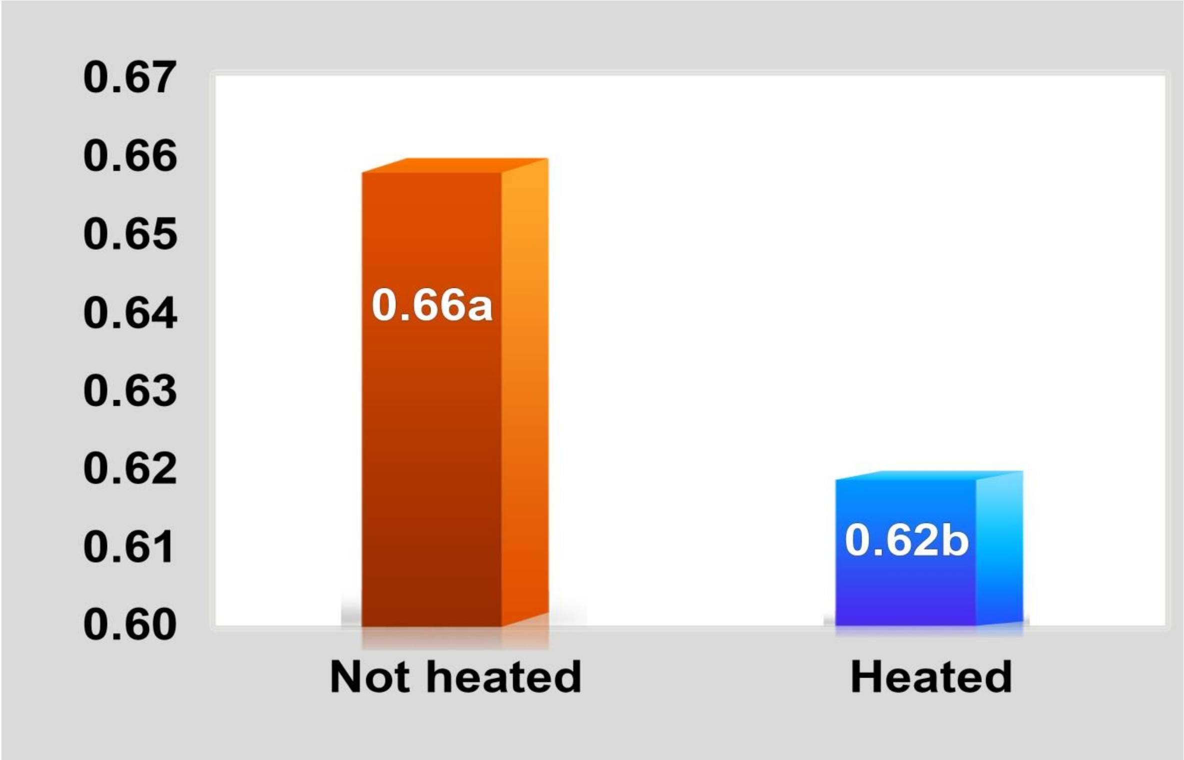
Practical Impact of Heat Damage

Heat Damaged SBM fed to Broiler Chicks

BW Gain, Day 10 to 28, g



Gain: Feed Ratio, Day 10 to 28

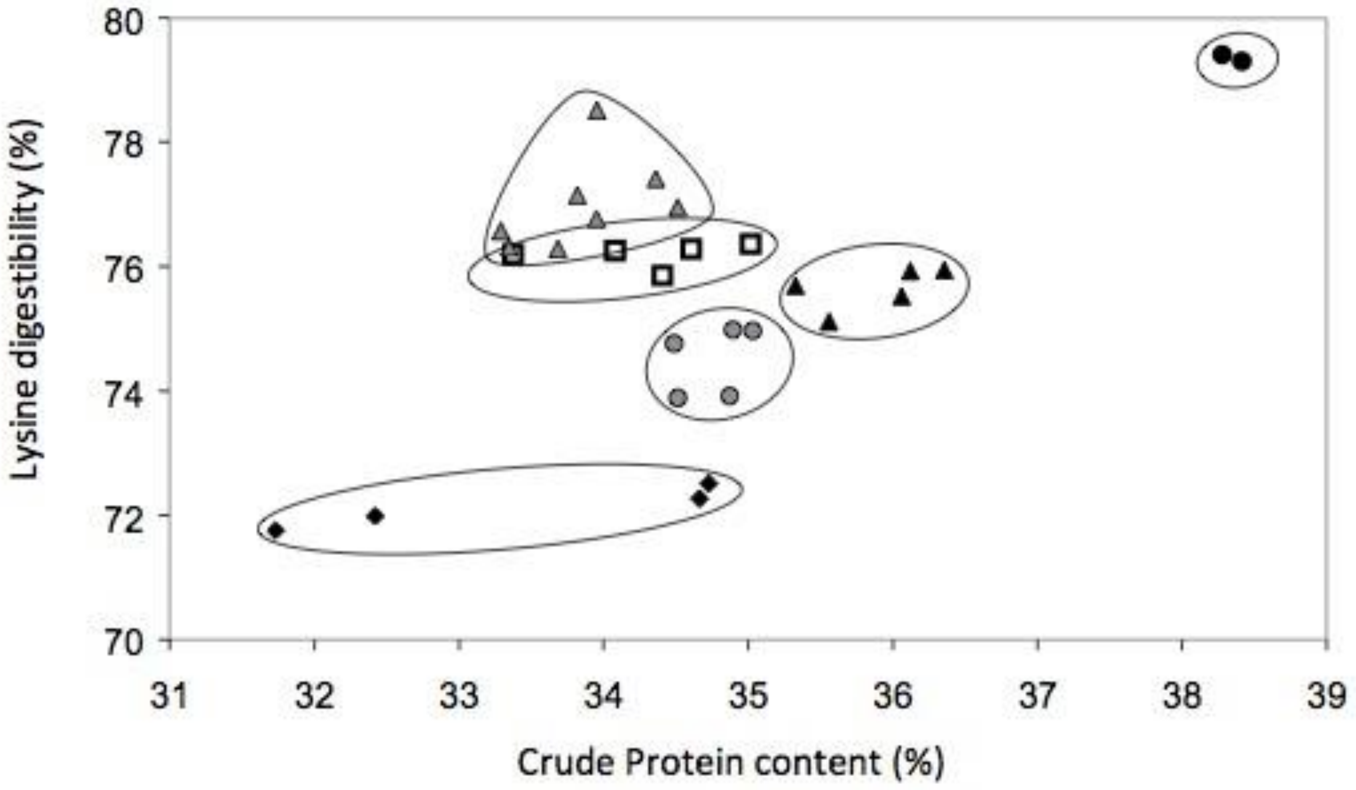


Heat Damaged Soybean Meal Through Autoclaving at 130°C for 60 minutes

Redshaw et al., 2010

Figure 3 Rapeseed meal digestibility is pretty much affected by the manufacturing process

Different symbols represent rapeseed meals from different crushing plants (29 samples from 6 crushing plants)



http://gfmt.blogspot.ca/2013/04/adisseo-survey-on-nutritional-value-of.html

Processing (manufacturing process) is a key determinant of amino acid digestibility

Apparent digestibility of corn gluten meal and wheat gluten meal-based diets with deficient and marginal adequate lysine level

Diet	Lysine %	Protein Source	CP %	Lipid %	TC %	GE %
1	1.2	Corn Gluten Meal	89^{a}	82^{a}	47^{a}	78^{a}
3	2.0	Corn Gluten Meal	89^{a}	89^{b}	47^{ab}	78^{a}
7	1.2	Wheat Gluten Meal	96^{b}	82^{a}	37^{bc}	79^{a}
9	2.0	Wheat Gluten Meal	96^{b}	86^{b}	30^{c}	78^{a}
Pooled SEM			0.3	0.3	0.7	0.1
Prot source			****	N.S.	****	N.S.
Lys level			N.S.	****	*	N.S.
Prot source*Lys level			N.S.	N.S.	N.S.	N.S.

Lower ADC

Higher ADC

N.S. = Not statistically significant (P>0.05); *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001

Gholami (2015)

Chemical Reactions Resulting from Thermal Processing

1. Protein oxidation (Protox)

2. Pyrolysis of amino acids and carbohydrates

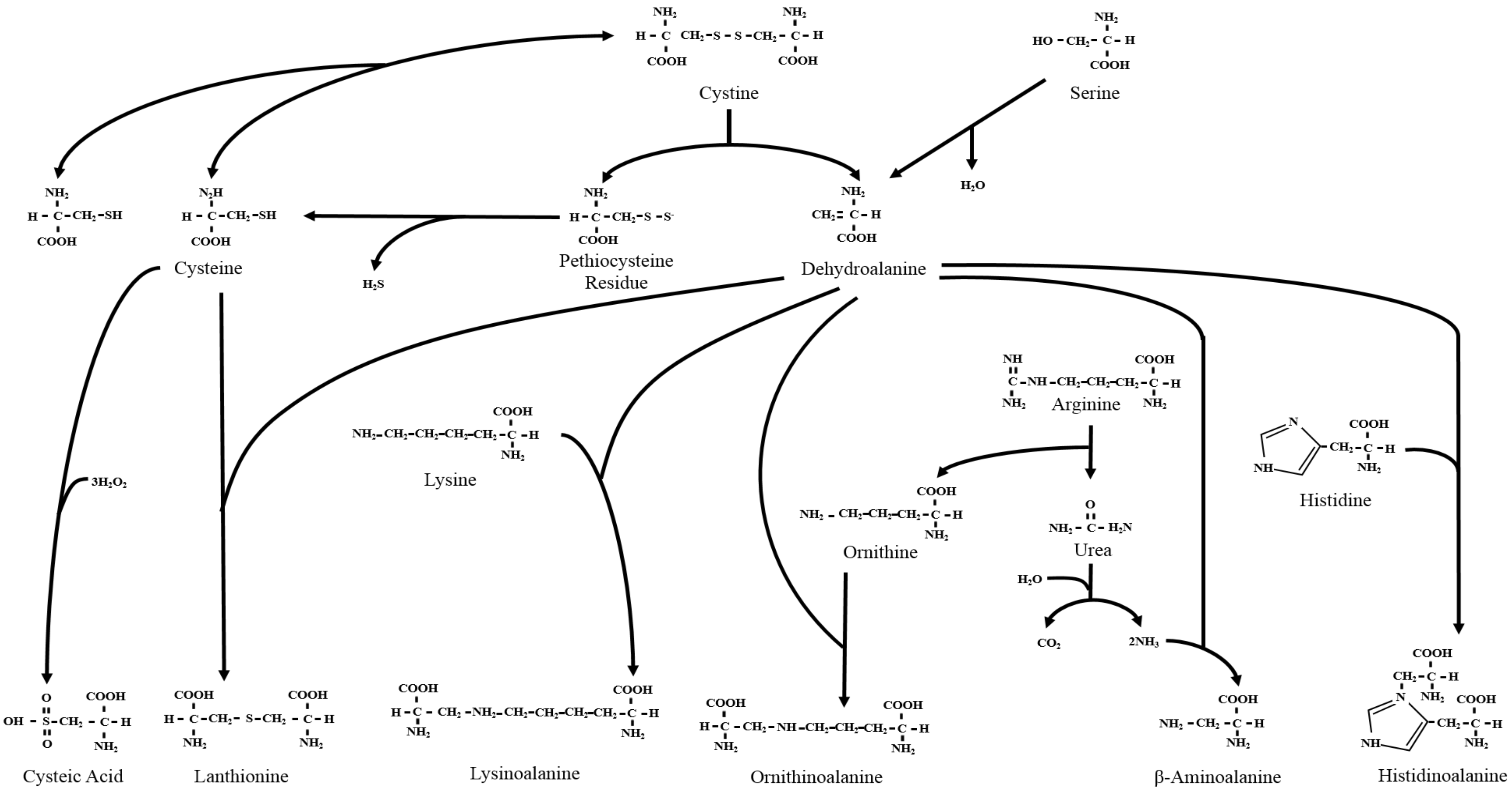
3. Racemization of amino acids

4. Amino acids- reducing carbohydrates reactions (Maillard reactions)

5. Protein Cross-Linkage (Protein- protein interactions)

a) Disulfide bonds
b) Cross-linked amino acids

Heat Processing Promote the Formation of Cross-Linked Amino Acids

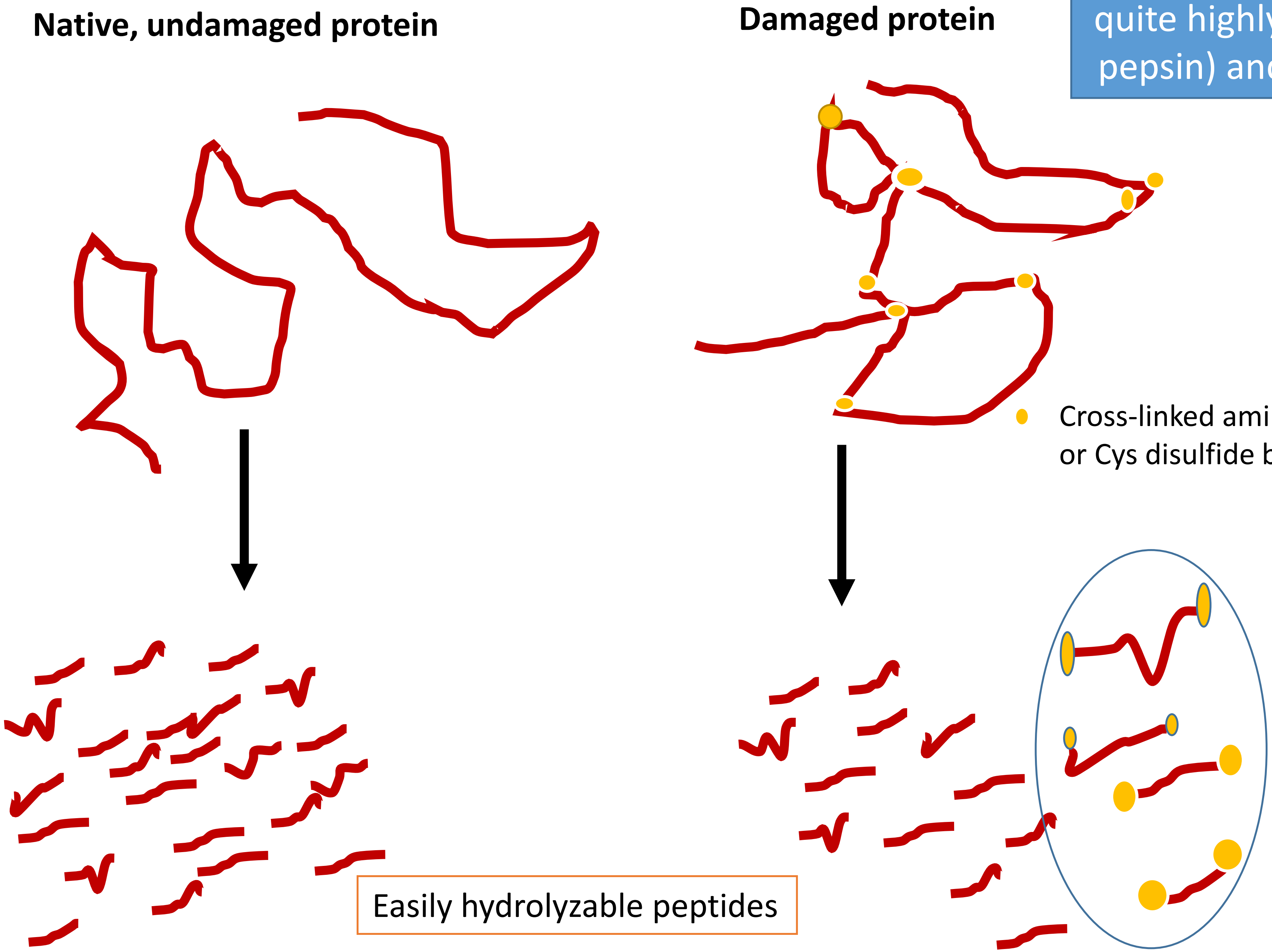


Increase in Cross-Linked Amino Acid (Lanthionine) in Feather Meal Processed Under Increasing Harsh Conditions - Latshaw et al. (2001)

Feather processing							
pH	Steam pressure	Dry matter	Crude protein	Pepsin-digestibility	Half cystine[1]	Lanthionine[1]	Methionine[1]
	(kPa)	(%)	(% of sample)	(% of CP)	(% of sample)		
5	207	90.2	89.9	38	6.71	.66	.43
	276	89.6	89.2	48	6.31	.81	.46
	345	89.4	88.7	66	5.61	1.46	.42
7	207	90.0	88.5	52	6.14	1.07	.51
	276	89.4	88.8	66	5.83	1.51	.36
	345	88.3	88.4	71	4.42	1.63	.24
9	207	89.3	88.4	59	6.31	1.14	.30
	276	89.3	89.3	66	4.59	1.68	.36
	345	89.2	88.1	79	4.00	2.18	.23

Increasing lanthionine

How could something be measured as quite highly digestible or degradable (by pepsin) and yet be not so bio-available?



Water-soluble peptides, likely not bioavailable but measured as “digestible” (or “degradable” by pepsin digestibility test).

Remember:
Digestibility is a measure of disappearance, not one of “utilization”

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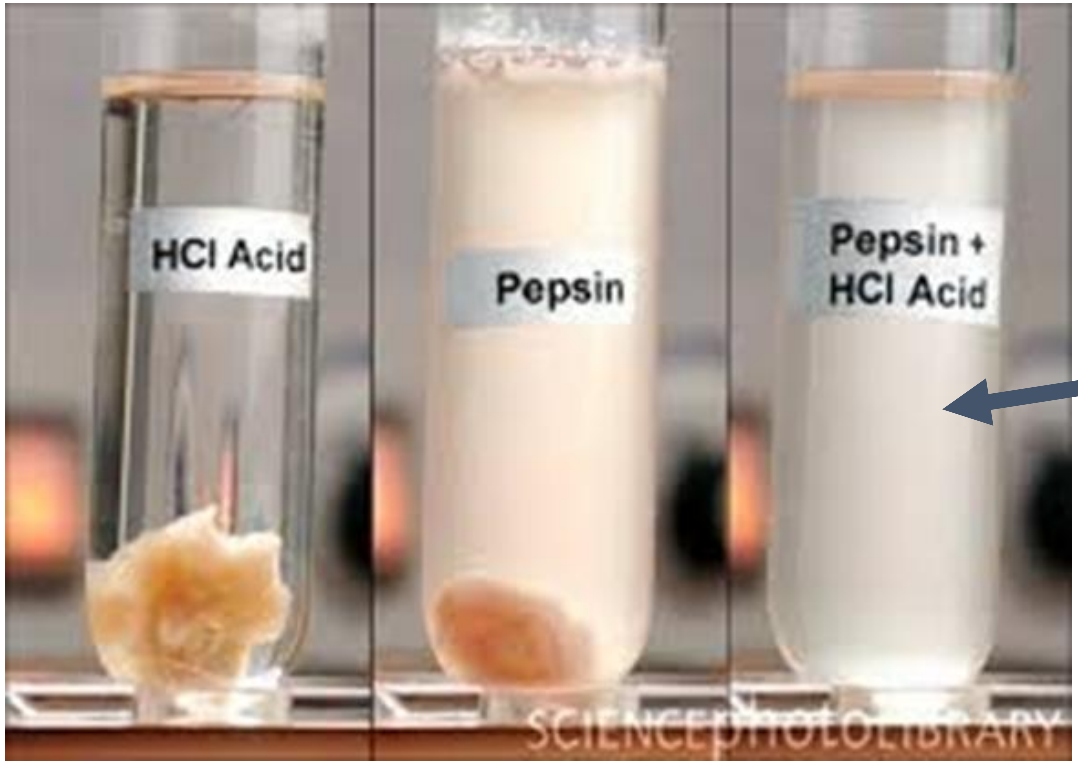
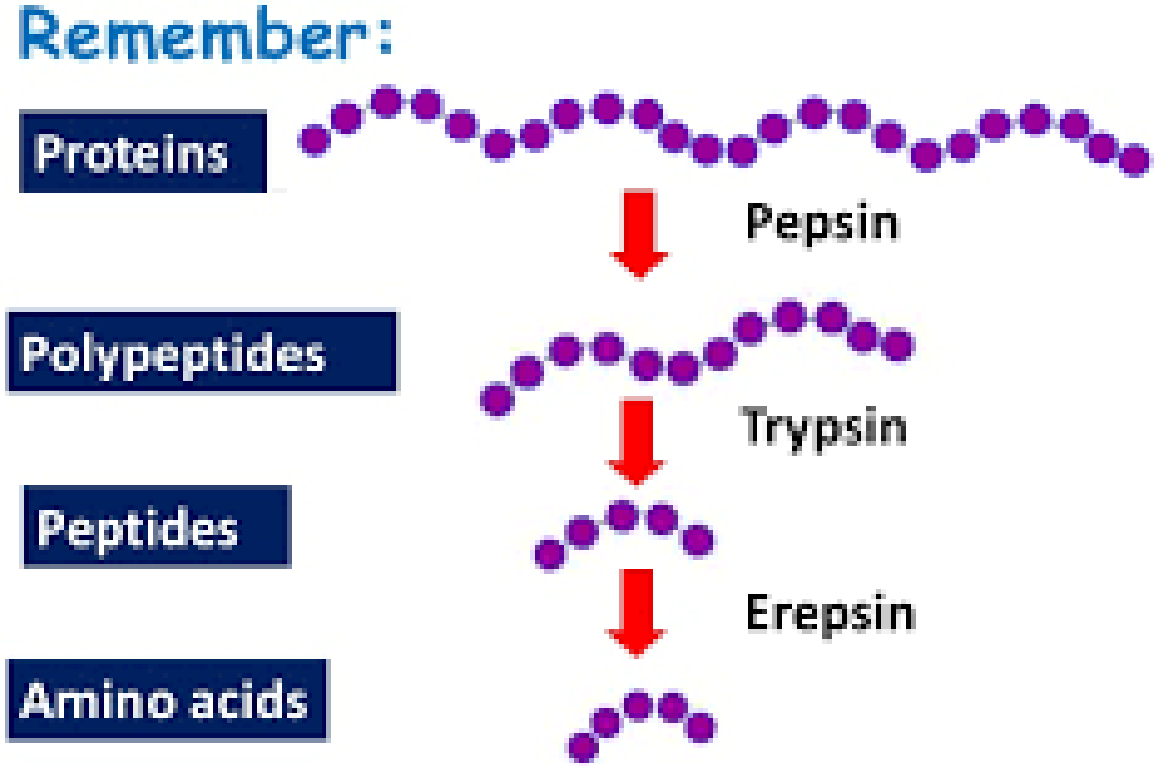
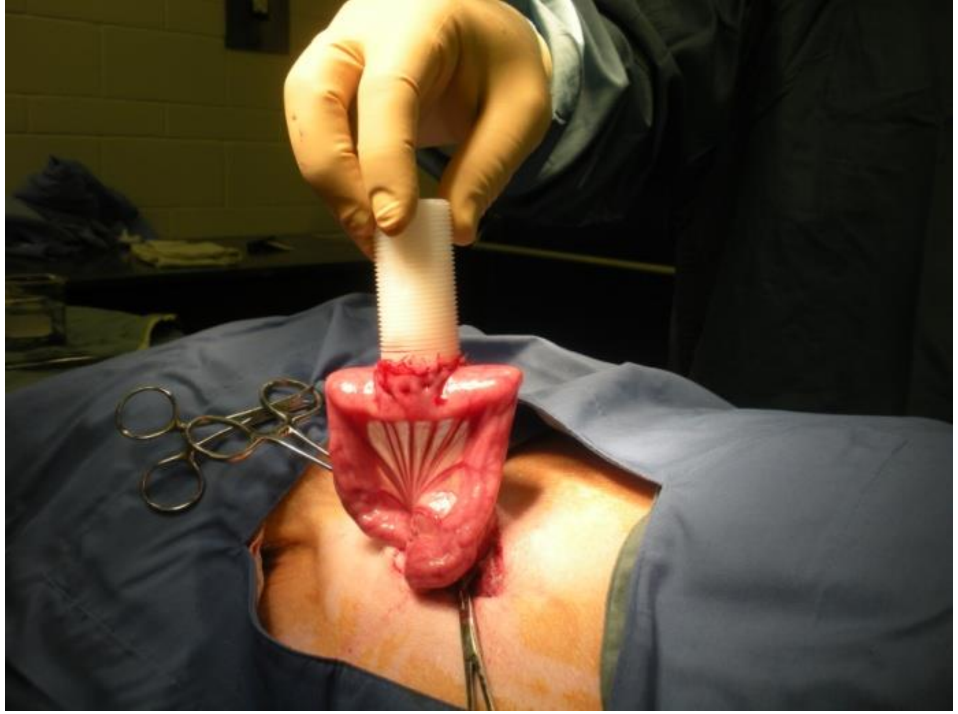
Increasing pepsin digestibility

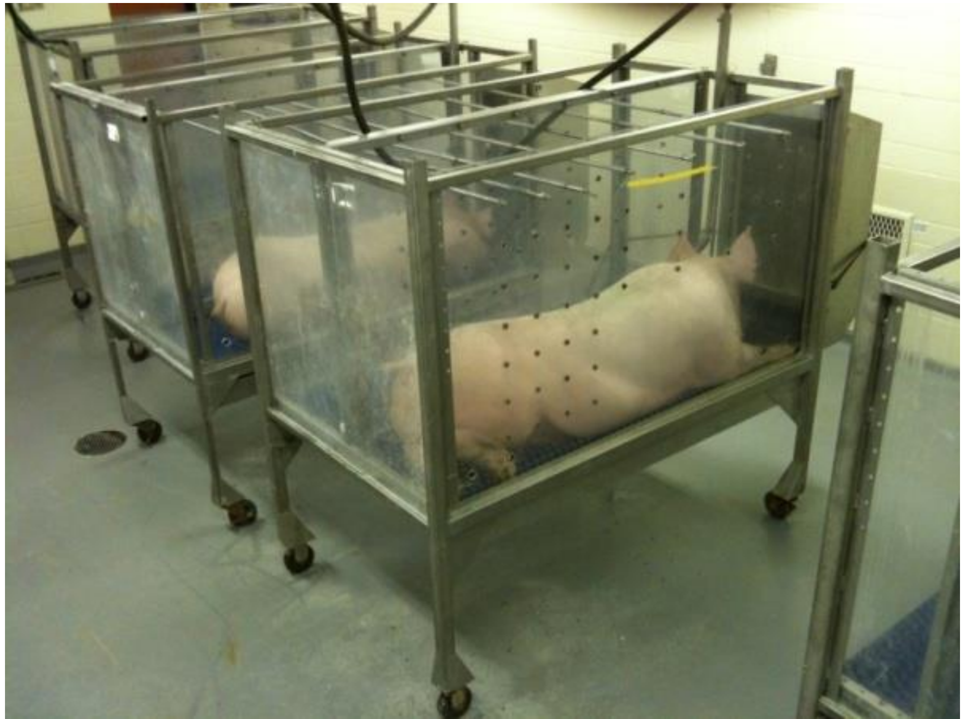
Increasing lanthionine


HCl Acid
Pepsin
Pepsin +
HCl Acid
SCIENCEPHOTOLIBRARY

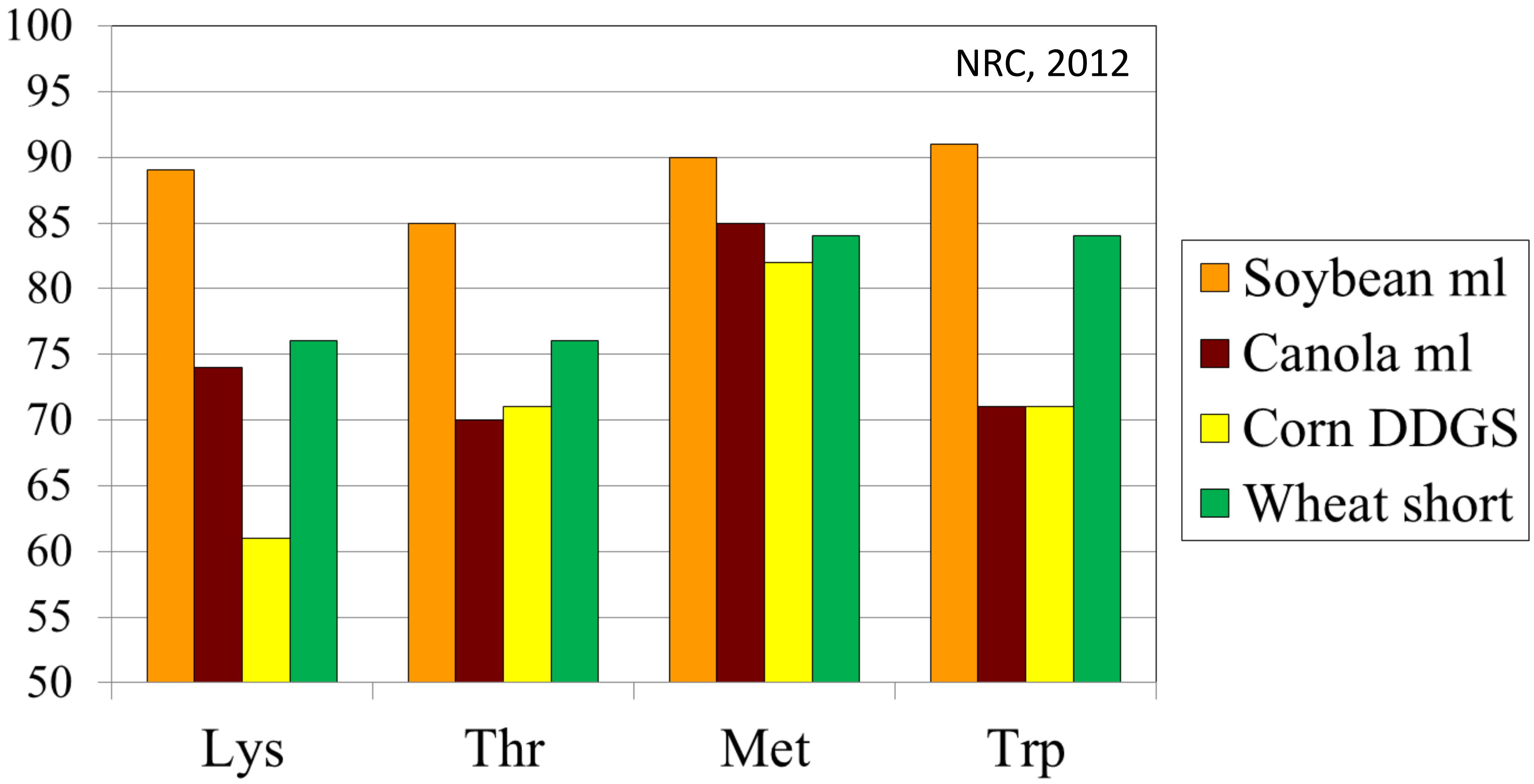

Remember:
Proteins
Pepsin
Polypeptides
Trypsin
Peptides
Erepsin
Amino acids



Univ. of Guelph Animal metabolism facilities



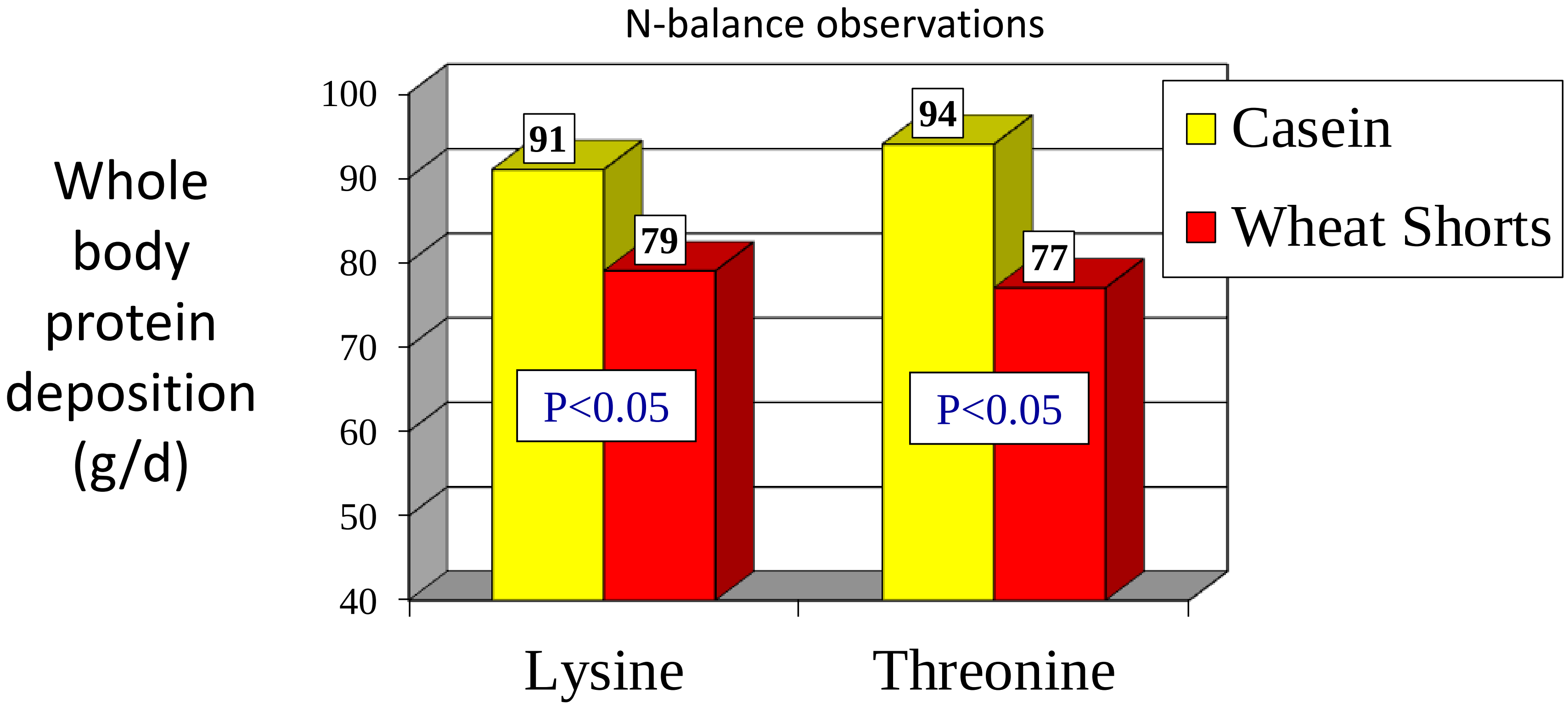
Standardized ileal digestibility (%) of key Amino Acids in Swine



Large differences in digestibility

Standardized Ileal digestibility (SID) - Swine

- In some instances, SID does not accurately predict bio-availability of amino acids



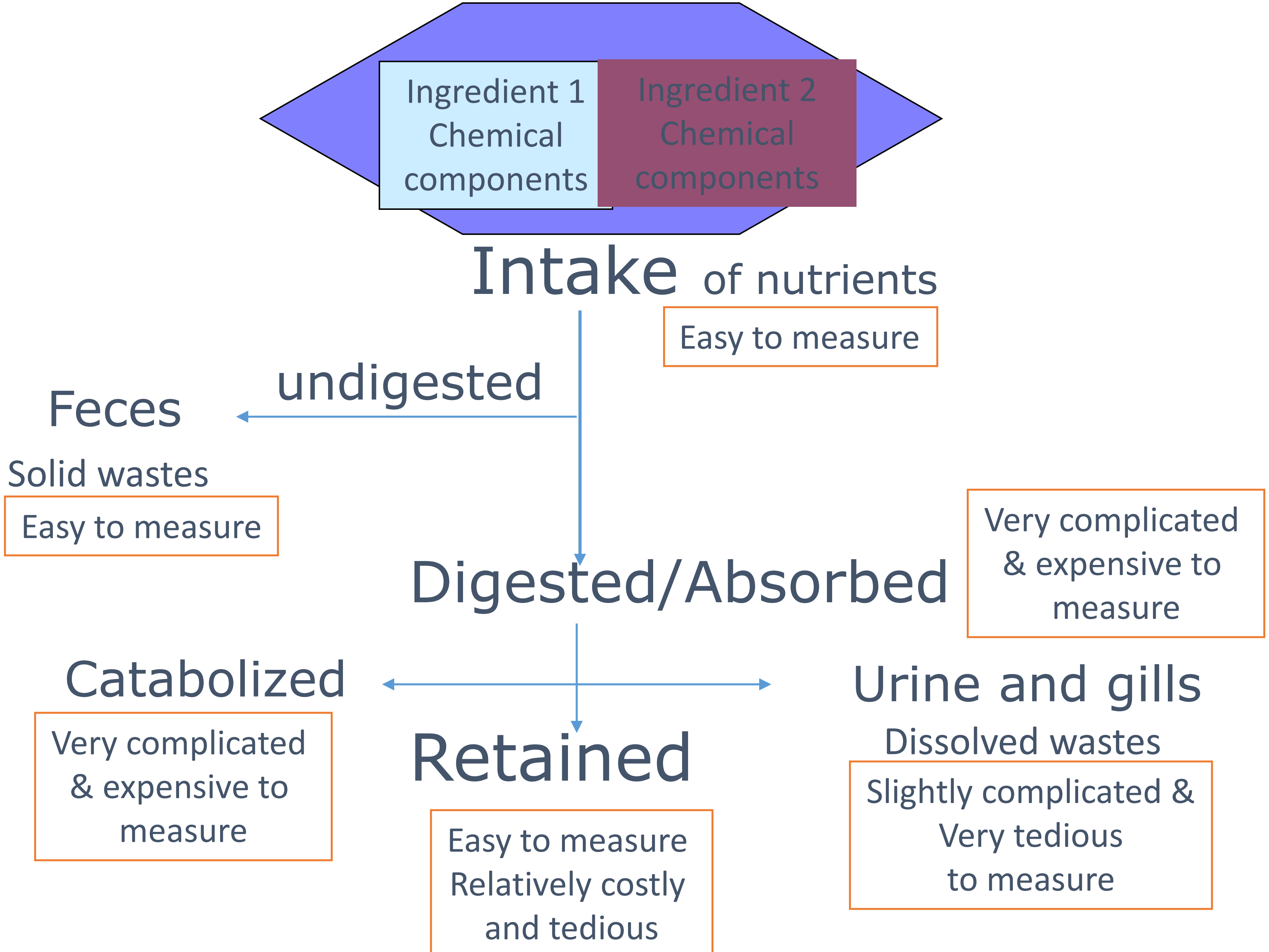
Growing pigs fed threonine or lysine limiting diets; equal intakes of SID Lys and Thr

Libao-Mercado et al., 2006; Univ. of Guelph

Take Home Message

- Digestibility is a measure of disappearance from the intestine not a measure of utilization
- High digestibility does not always mean “high bioavailability”
- Heat or chemically damaged amino acids may be measured as digestible but may not be bio-available
- Must often “back up” measure of digestibility with measure of bio-availability : The proof of the pudding is in the eating

Assessing the Nutritive Value of Feed and Feed Ingredients



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