A CRITICAL LOOK AT ESTIMATES OF APPARENT DIGESTIBILITY OF PROTEIN AND AMINO ACIDS

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Introduction

- Information of the apparent digestibility coefficient (ADC) of nutrients of different ingredients is increasing every year thanks to sustained research efforts
- Estimates of ADC are regularly compiled in the reference literature and increasingly used by feed manufacturers who are now formulating their feeds on a digestible protein and amino acid basis
- This progressive move from formulating on a 'total nutrient' basis to formulating on digestible nutrients is praiseworthy.
- However, increasing reliance by feed millers on published estimates of ADCs makes it critical to ensure that the information available is relevant and reliable

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

CHO C.Y. and BUREAU D.P. (1997) Reduction of waste output from salmonid aquaculture through feeds and feedings. The Progressive Fish Culturist 59, pp.155-160.

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

These estimates of apparent digestibility have been revised/ reviewed on a regular basis and proven useful

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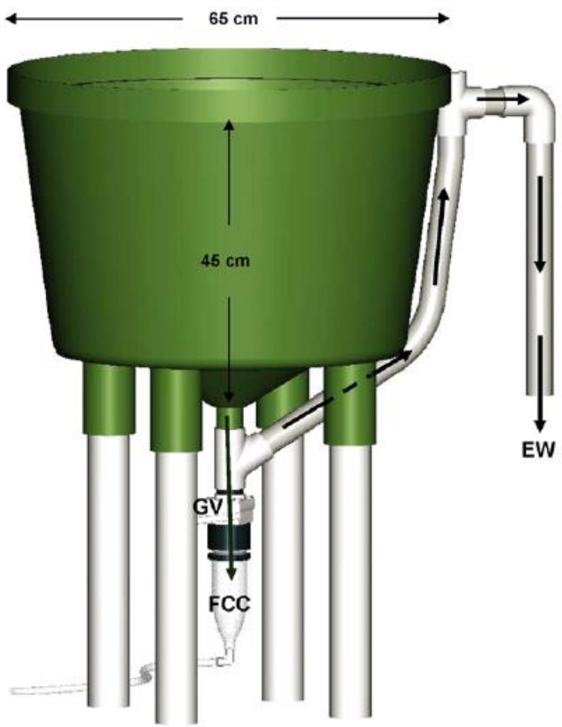
Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758)

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

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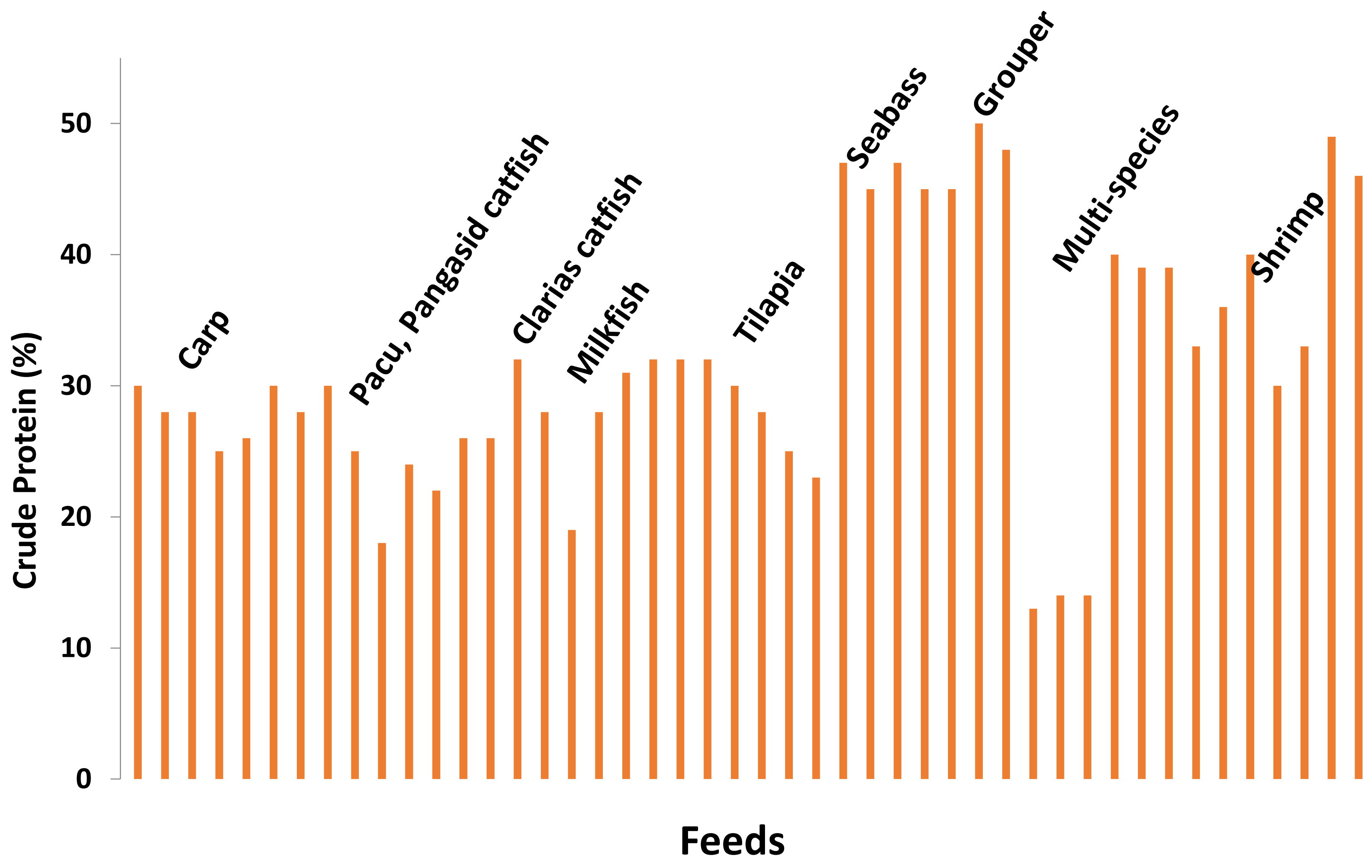
Table 1. Composition and digestibility of key feed ingredients for marine fish

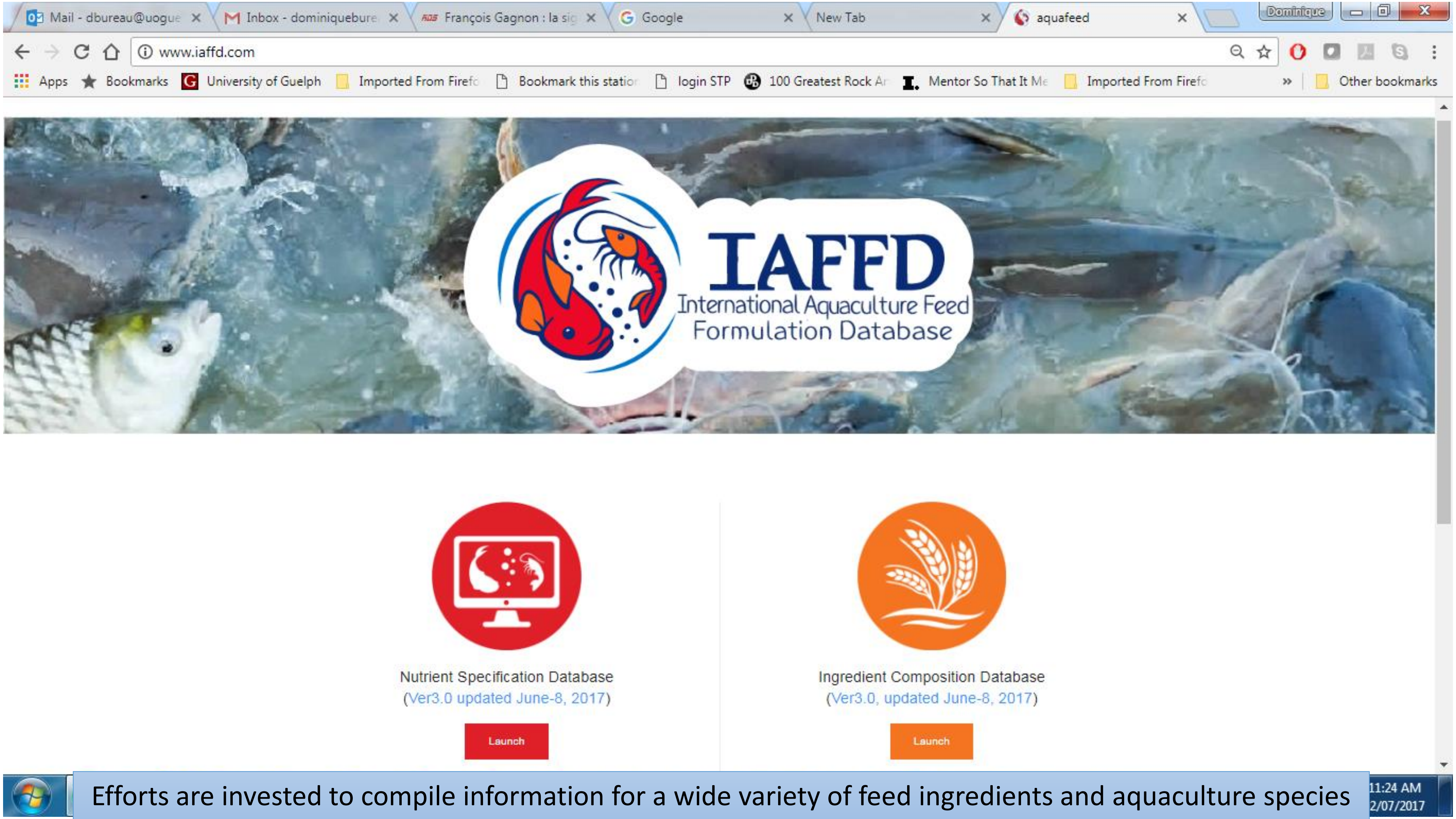
	Ingredient Specifications (all values g/kg DM)						*Ingredient Digestibility*	
	DM (g/kg)	Protein	Lipid	Ash	CHO	Energy (MJ/kg)	Protein ADC	Energy ADC
Fishmeal (CaMau - Vietnam)	903	551	125	298	26	18.4	91.9	94.6
Poultry meal (European)	919	646	127	132	95	21.9	87.8	86.5
Soybean meal (Vietnam)	883	424	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

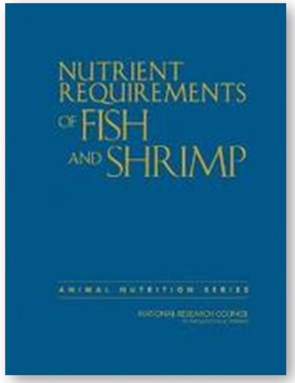
Diversity of Aquaculture Feeds Produced by a SE Asian Aquaculture Feed Manufacturer





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
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)

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 studies and species

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

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

Observations

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 issues 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

Mathematical Issues

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

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

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

which can be simplified to:

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

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%)

Methodological Issues

Feces Collection Equipment and Protocol

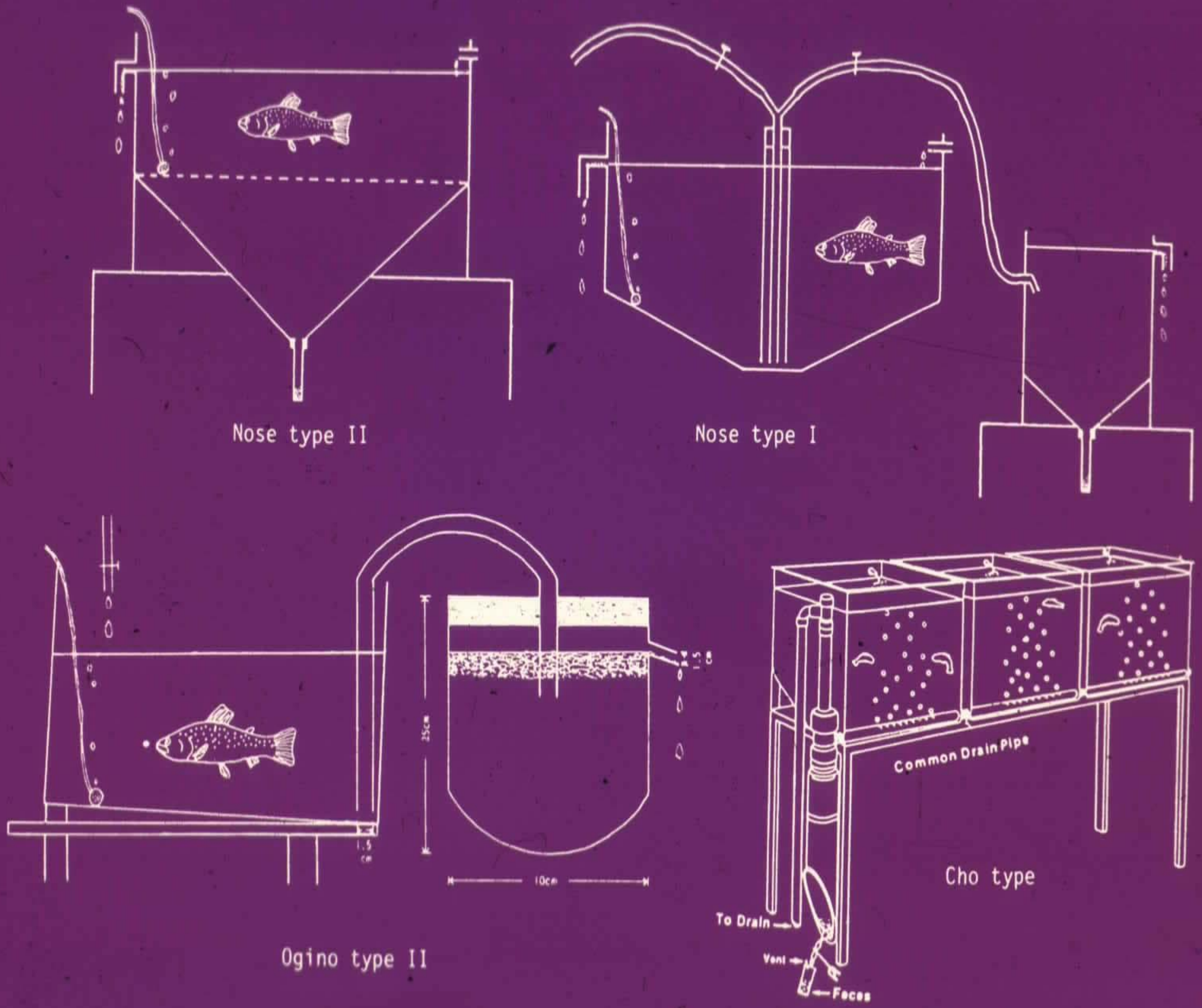
Measuring Digestibility in Fish

Several Methods:

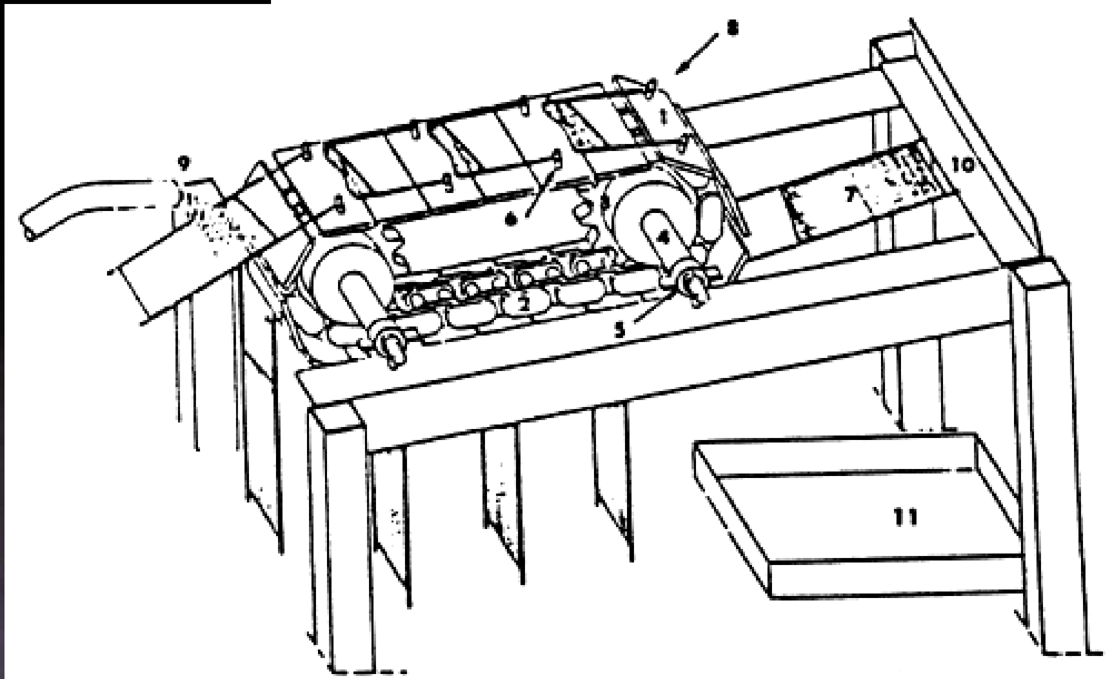
Stripping, dissection, siphoning

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
Cho type
Common Drain Pipe
To Drain
Vent
Feces

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.

Guelph Fecal Collection System (Cho et al., 1982)

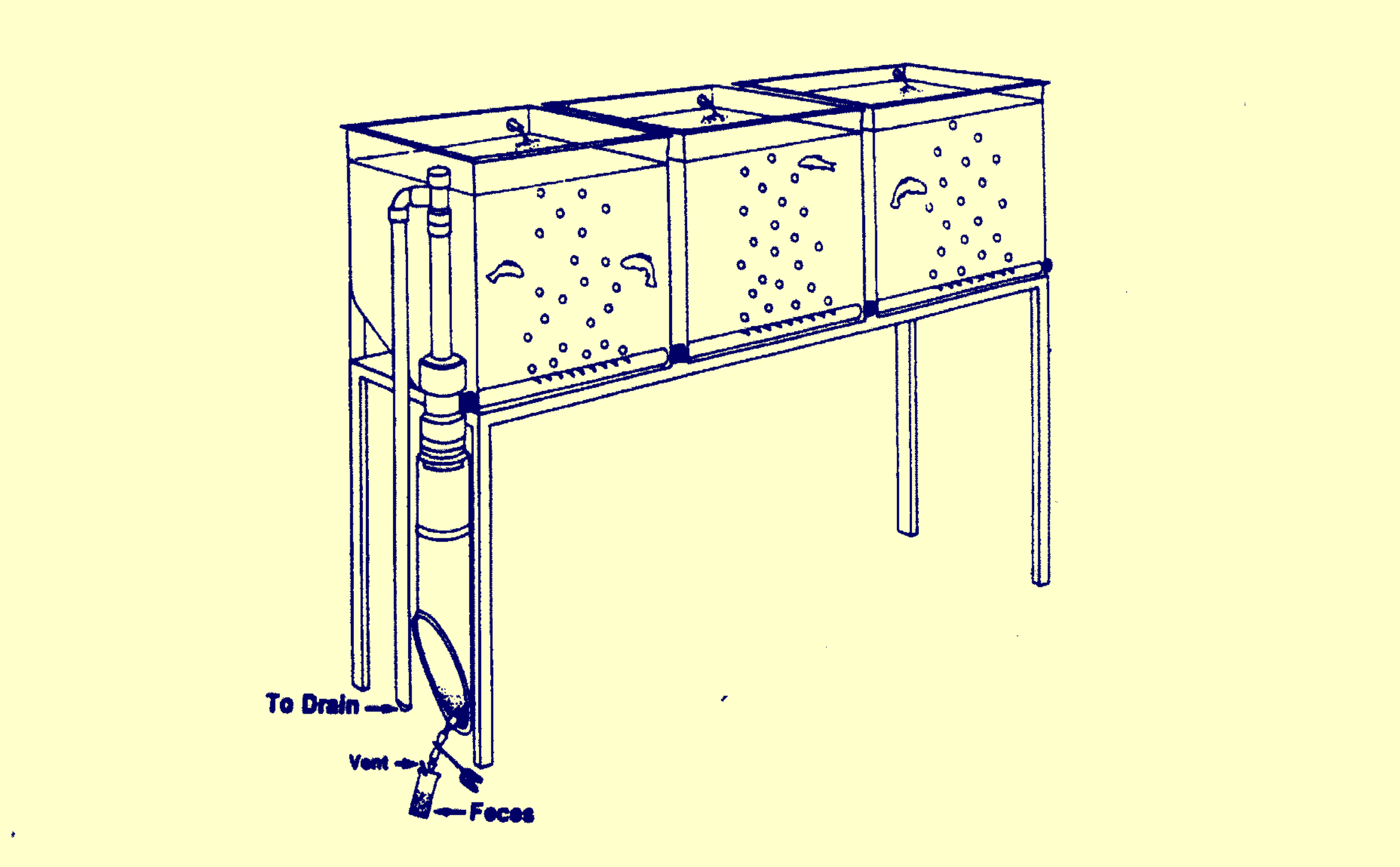


Table 1. Apparent digestibility coefficients of herring meal determined with rainbow trout (Cho & Slinger, 1979)

Feces collection method	Crude protein in herring meal	Digestibility coefficient: Dry matter	Crude protein	Lipid
	(%)	(% Mean ± SE)		
1. Metabolism chamber	75.8	—	86.7 ± 0.9	—
2. Intestinal disection	67.9	80.3 ± 1.0	90.2 ± 0.4	96.7 ± 0.5
3. Anal suction	67.9	79.1 ± 0.4	90.4 ± 0.1	97.4 ± 0.3
4. Trough netting	67.9	84.4 ± 0.1	94.6 ± 0.3	96.8 ± 0.2
5. Stripping	67.9	73.3 ± 1.6	77.5 ± 1.0	62.2 ± 5.1
6. Stripping (Guelph)	66.7	—	88.2 ± 1.7	—
7. CYAQ-2 Guelph system	66.7	80.2 ± 2.4	91.0 ± 0.8	97.3 ± 1.0

1. Smith *et al.* (1980).
2–5. Windell *et al.* (1978).

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

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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

Parameter / Method	Marker			
	Cr_2O_3	AIA	TiO_2	
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)

Methodological Issues

Chemical Analysis Issues

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)

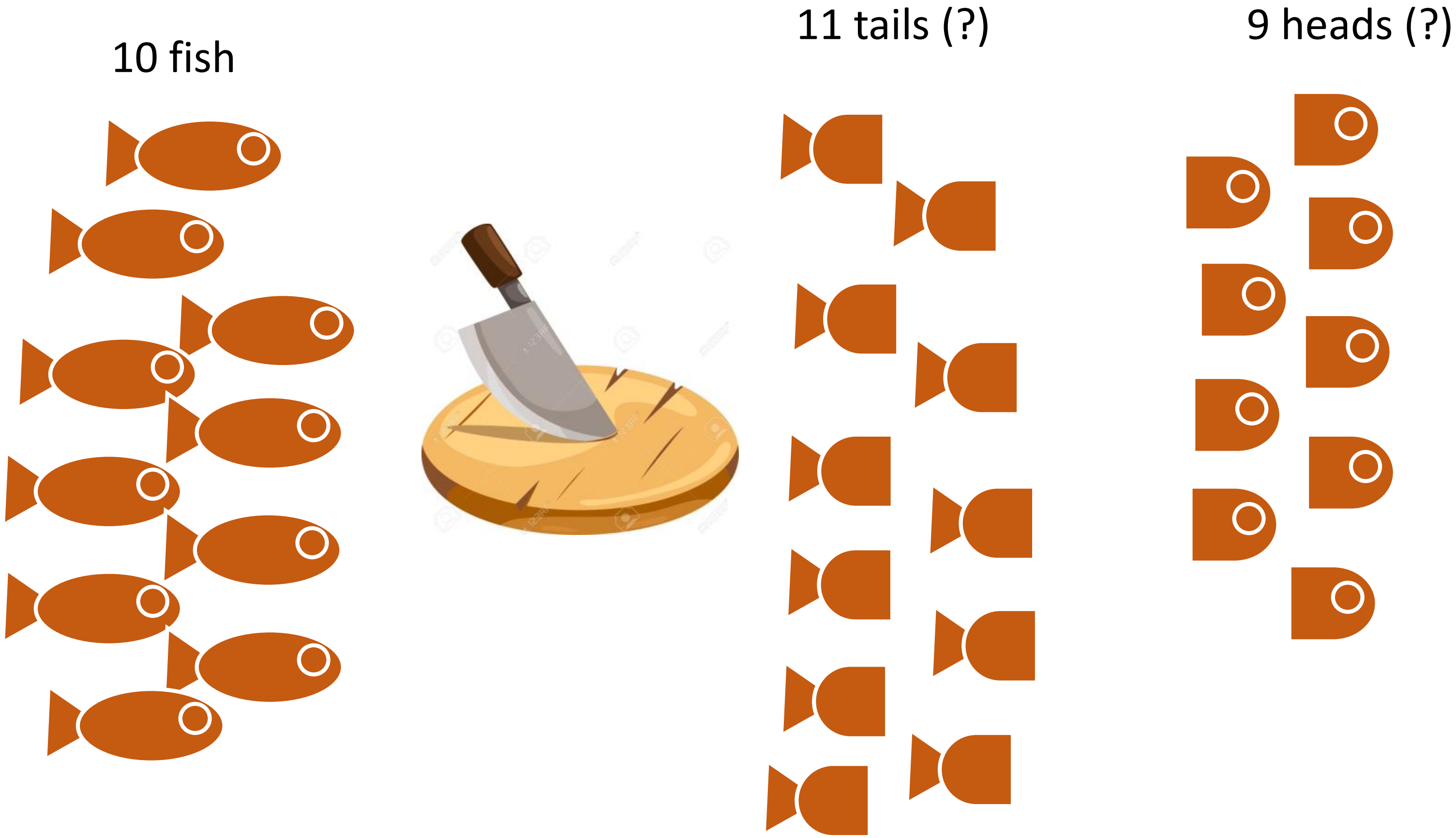
DE based on proximate = 1000*((.625*.46*23.6)+(.153*.622*39))/4.184 = 2508 kcal/kg

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	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

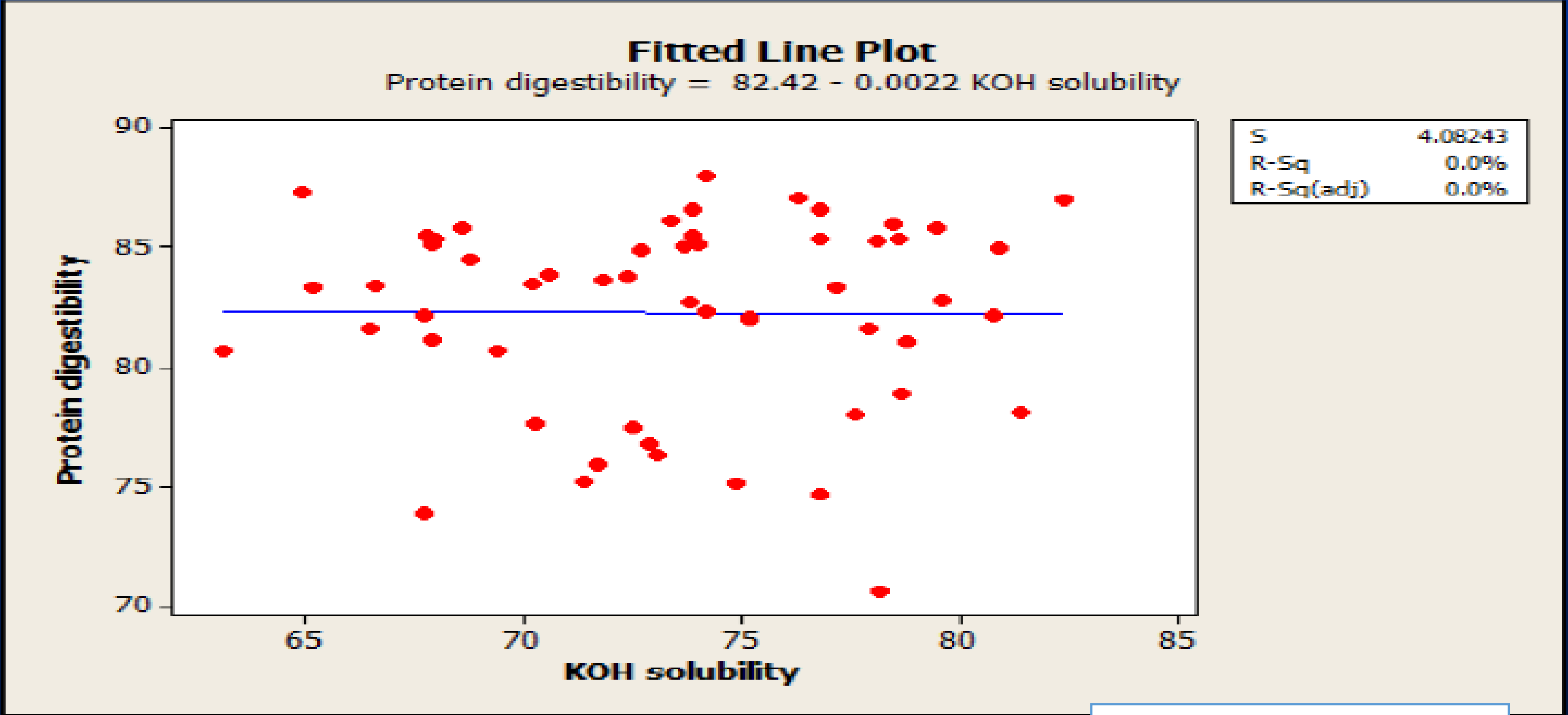
Bureau et al. (1999)

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)

Variability in the digestibility of protein of different lots of soybean meal from various origins in broiler birds and correlation with protein solubility



Ravindran et al. (2014)

P=0.97

Very significant variability even for a fairly standardized ingredient!

Massey University

Determinants of Protein Digestibility

- Processing conditions, notably heat damage, affect digestibility of crude protein and amino acids
- What chemical processes underpins these significant differences in digestibility?
- Chemically damaged amino acid should probably unlikely to be bio-available but they should, in theory, be digestible
  - Demonstrated for lysine (work on available lysine)
  - Digestibility is just a measure of disappearance not bio-availability
- Damaged amino acid are affecting proteolysis through some type of steric hindrance
  - Steric hindrance: (Definition) The stopping of a chemical reaction which might be caused by a molecule's structure)

Feather Meal Processing

Raw feather = Almost pure keratin = 0% digestible due to presence of disulfide bonds

Steam-hydrolysis breaks disulfide bonds and make the keratine digestible

Finding the sweet spot for processing

Under processing

→ High level of keratin
→ High level of disulphide bonds

Optimal processing

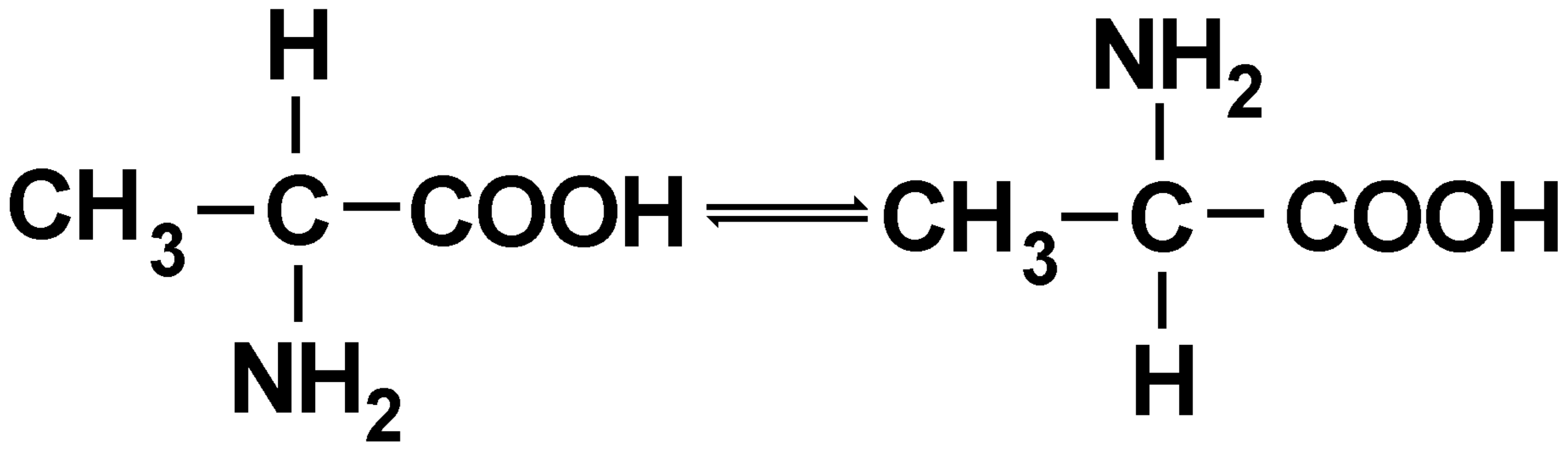
=

Optimal Bioavailability

Over Processing

High level of thiols ←
High level of cross-linked AA ←
Isomerizaton of amino acids ←
Potential new creation of disulfide bonds ←

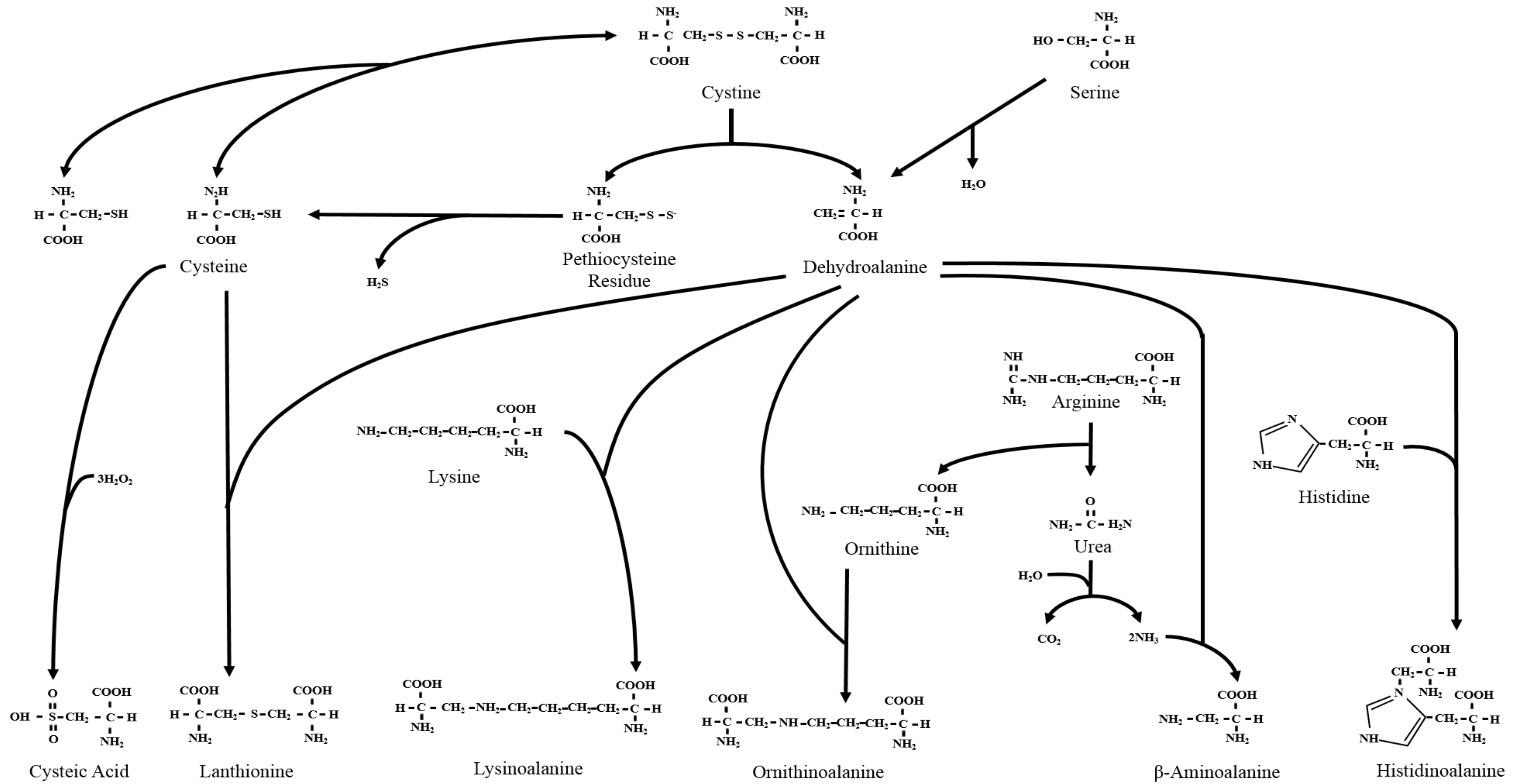
Heat Treatment can Also Induces Racemization of Amino Acids



L-Alanine

D-Alanine

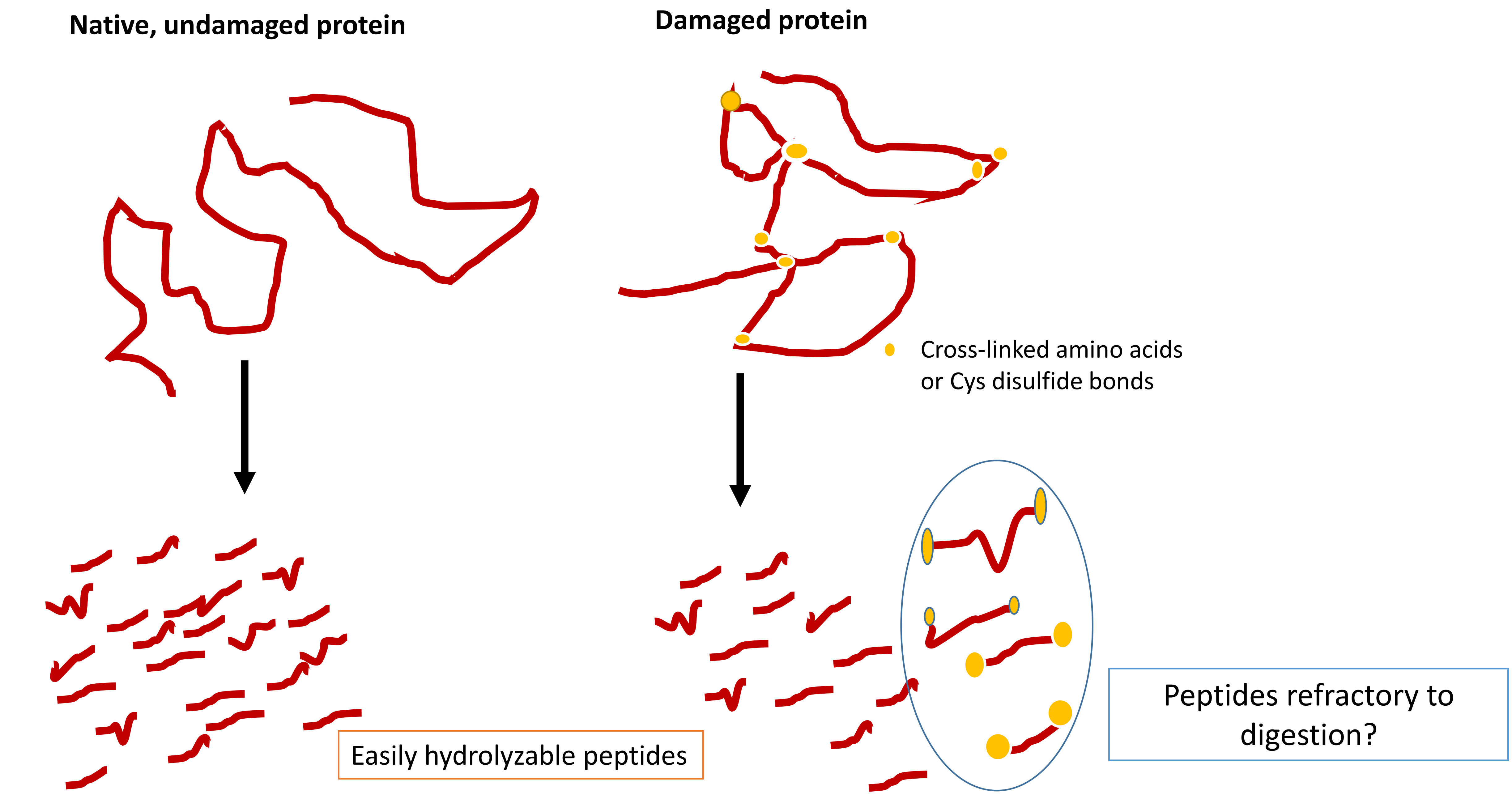
Heat Processing Promote the Formation of Cross-Linked Amino Acids



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

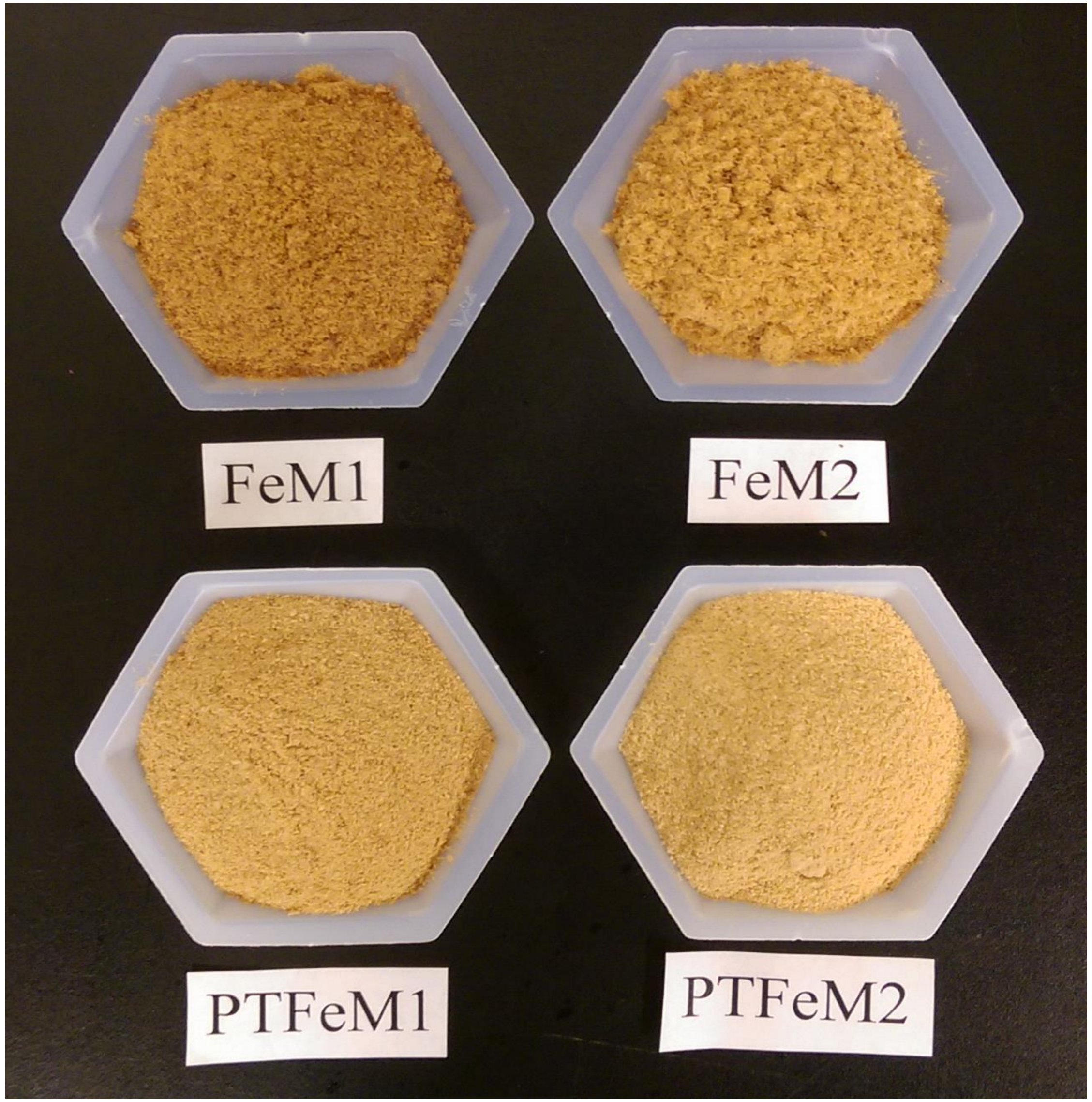
Increasing lanthionine


Native, undamaged protein
Damaged protein
Cross-linked amino acids
or Cys disulfide bonds
Easily hydrolyzable peptides
Peptides refractory to
digestion?

Feather Meal: Effectiveness of a Simple Chemical Pre-Treatment

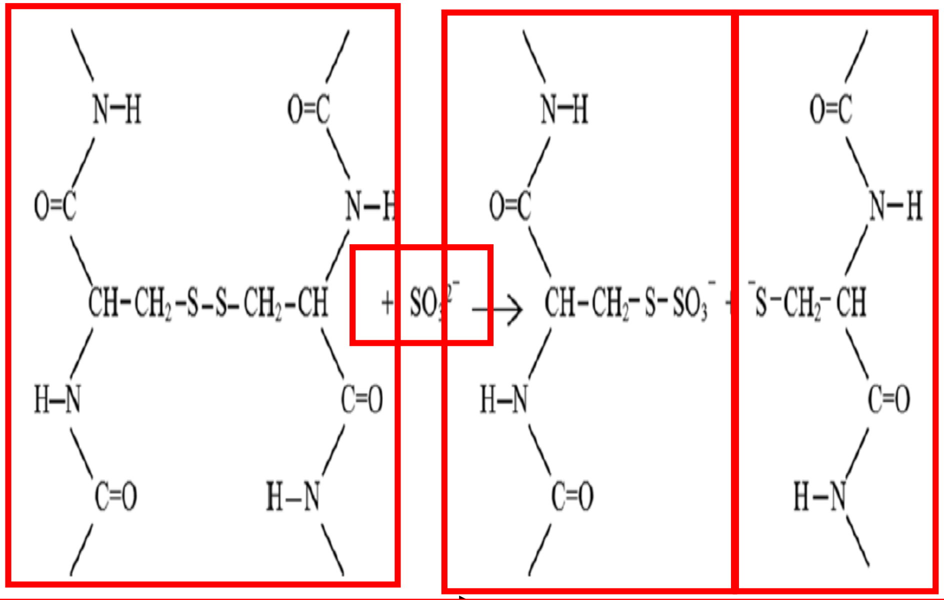
Pre-treatment of 2 commercial feather meals (FeM)

- 2% sodium sulfite (%FeM w/w)
- 0.05% Protease (%FeM w/w)
- 200% water (%FeM w/w)
- 24h incubation



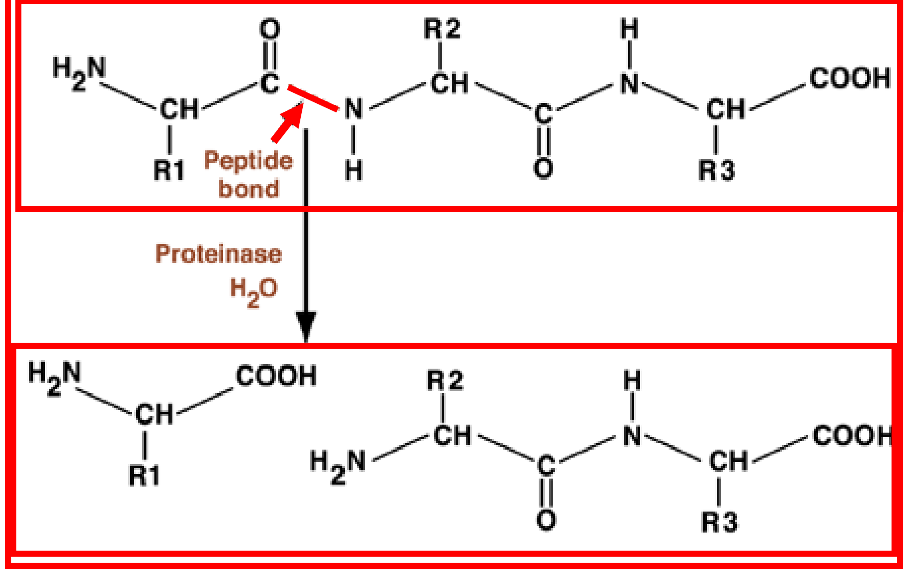
Pre-Treatment of Steam-hydrolyzed Feather Meals to Disrupt Residual Disulfide Bonds

1- Sulfitolysis using sodium sulfite (Na_2SO_3)



Cystine + Sulfite → Bunte Salt + Cysteine

2- Proteolysis using a commercial protease

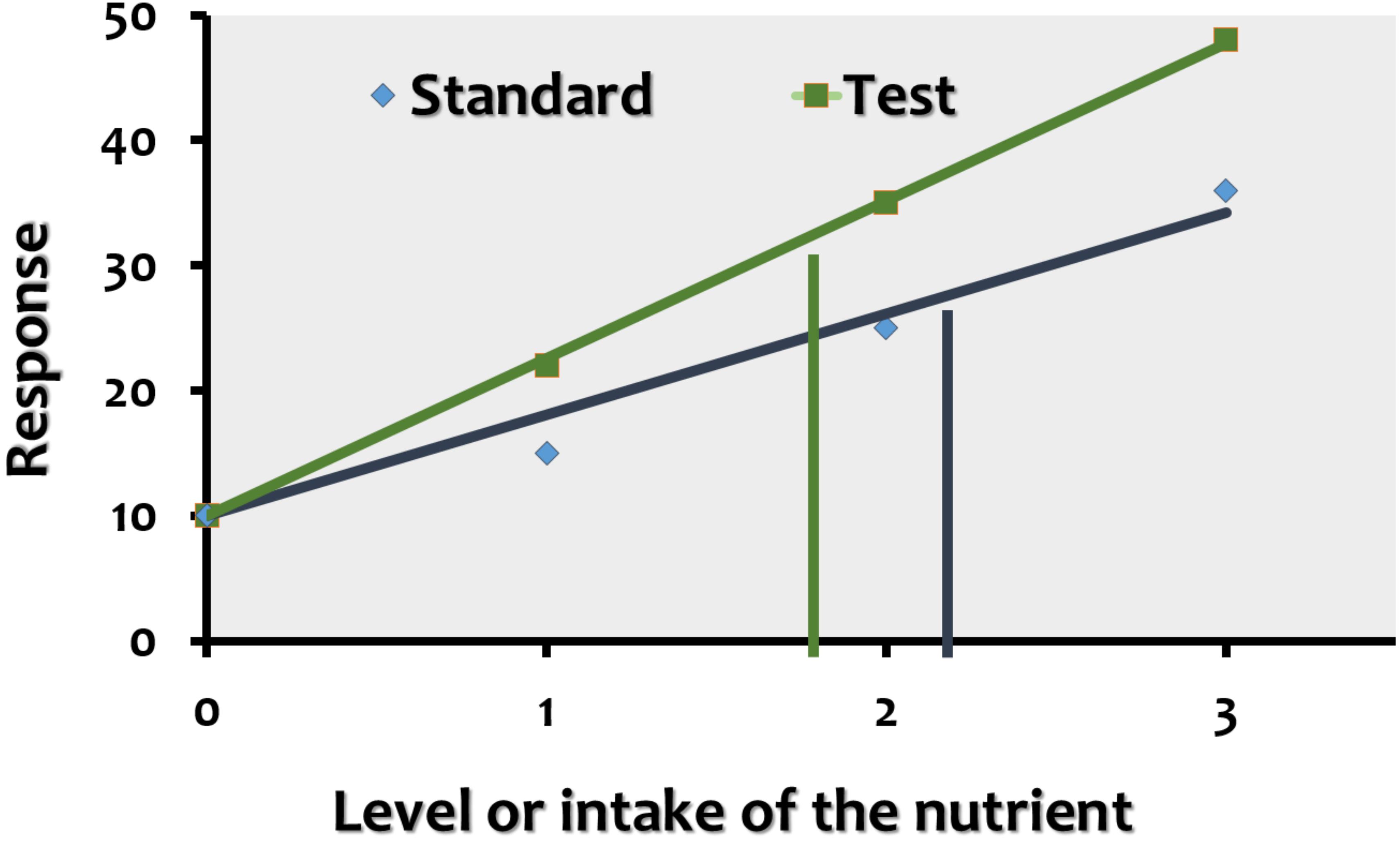


Treatment Significantly Improved Digestibility of Protein and Amino Acids

Indicating that residual disulfide bonds in steam-hydrolyzed feather meals negatively impact digestibility of protein

	Ingredients ADC (%)			
	FeM1	PTFeM1	FeM2	PTFeM2
Proximate composition (a)				
Dry matter (%)	78.3[b]	87.7[ab]	86.9[ab]	93.2[a]
Crude protein (%)	85.4[b]	94.7[a]	81.9[b]	95.5[a]
Gross energy (kJ g^{-1})[1]	78.3[b]	87.2[ab]	86.0[ab]	94.4[a]
Essential amino acids (%)				
Arginine	86.3[b]	95.6[a]	84.9[b]	95.3[a]
Histidine	53.6[b]	102.5[a]	72.8[ab]	114.8[a]
Isoleucine	86.0[b]	94.2[a]	87.9[b]	96.5[a]
Leucine	82.3[b]	96.1[a]	84.9[b]	99.4[a]
Lysine	74.1[b]	96.9[ab]	87.5[ab]	105.1[a]
Methionine	73.3[b]	87.0[ab]	88.1[a]	93.2[a]
Phenylalanine	83.0[b]	96.4[a]	85.1[b]	99.0[a]
Threonine	80.1[b]	91.0[a]	79.2[b]	91.9[a]
Valine	84.3[b]	95.3[a]	86.0[b]	96.2[a]
Non-essential amino acids and lanthionine (%)				
Alanine	81.3[b]	96.8[a]	84.0[b]	9.9[a]
Aspartic acid	80.4[c]	92.9[ab]	84.7[bc]	97.9[a]
Cyst(e)ine	78.8[b]	86.5[a]	75.4[b]	84.8[a]
Glutamic acid	82.8[b]	93.0[a]	84.8[b]	95.6[a]
Glycine	87.9[b]	96.6[a]	88.1[b]	96.0[a]
Proline	85.8[bc]	94.2[a]	83.0[c]	90.4[ab]
Serine	86.9[b]	95.0[a]	84.0[b]	94.1[a]
Lanthionine	79.8[b]	84.6[a]	66.6[c]	76.8[b]

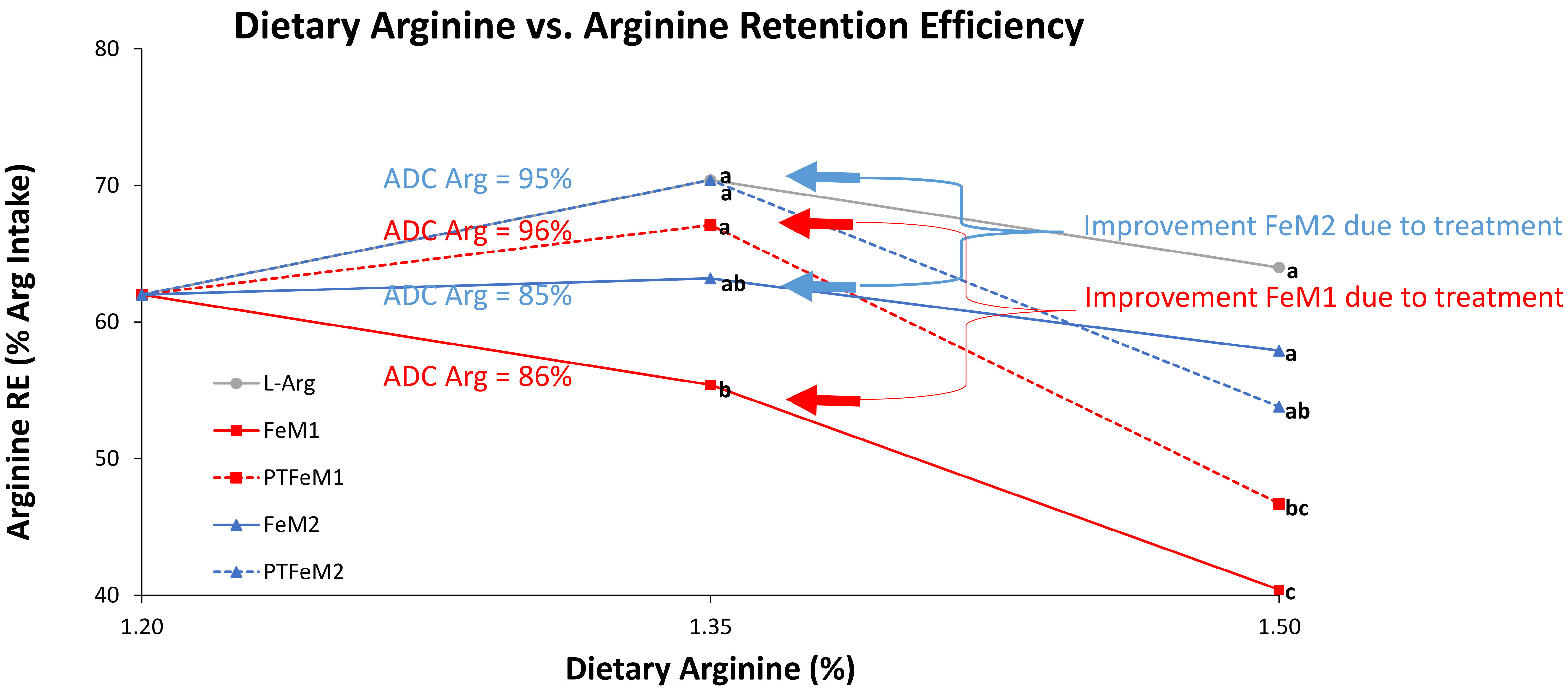
What About Bioavailability of Amino Acids?



Treatment Significant Improved Bio-Availability of Arginine

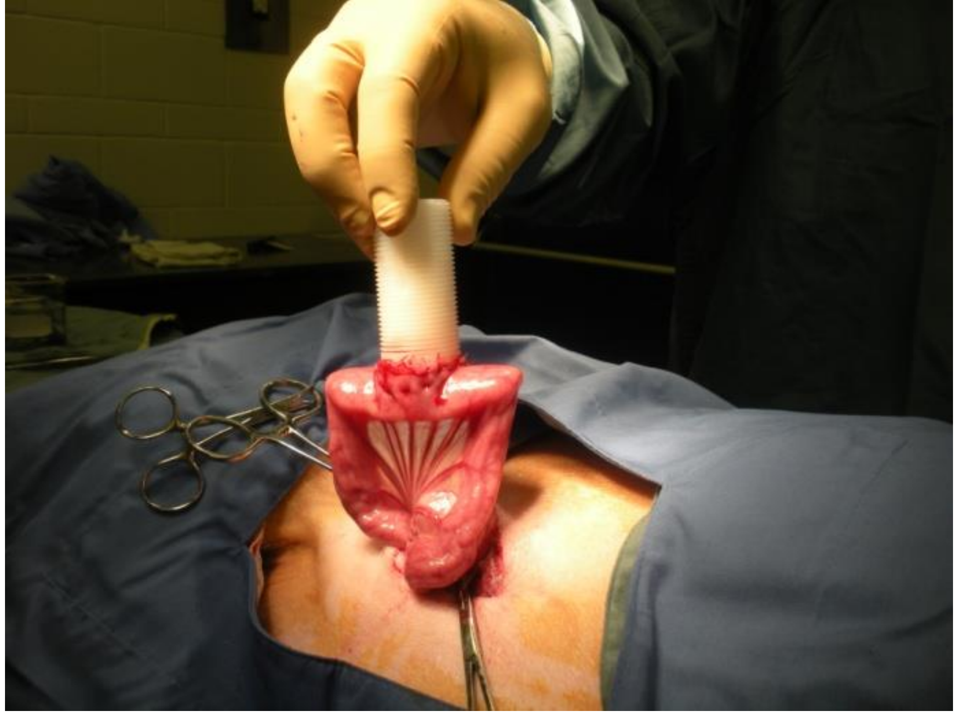
Indicates potential negative impact of residual disulfide bonds

Also indicates that digestibility is not necessarily perfect indicator of bio-availability

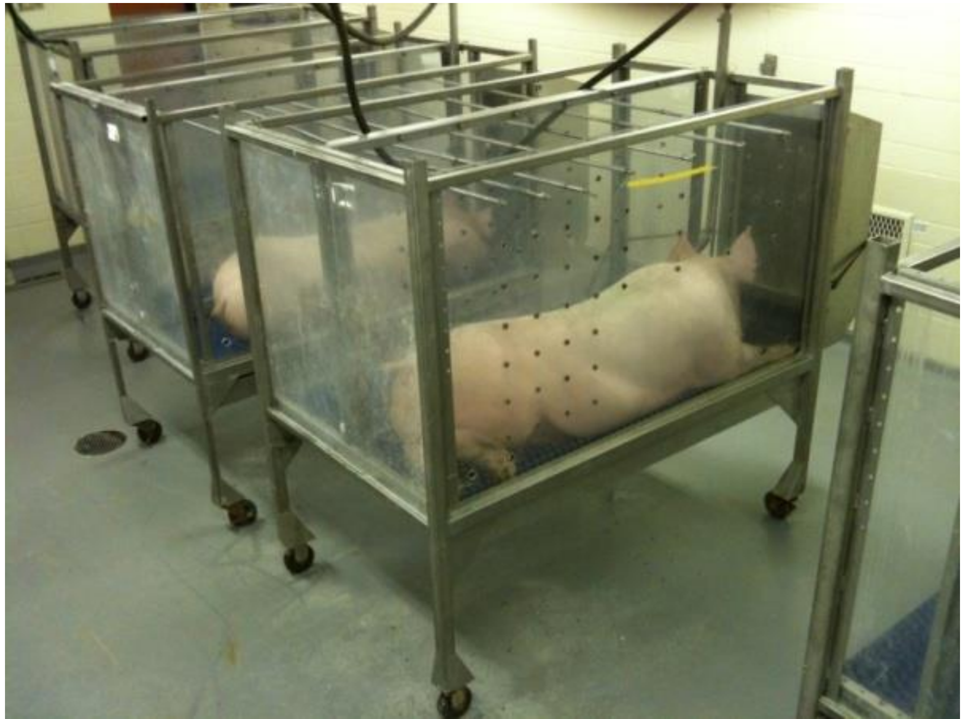


Cross-Linked Amino Acids Levels May be Inversely Correlated with Amino Acid Bioavailability

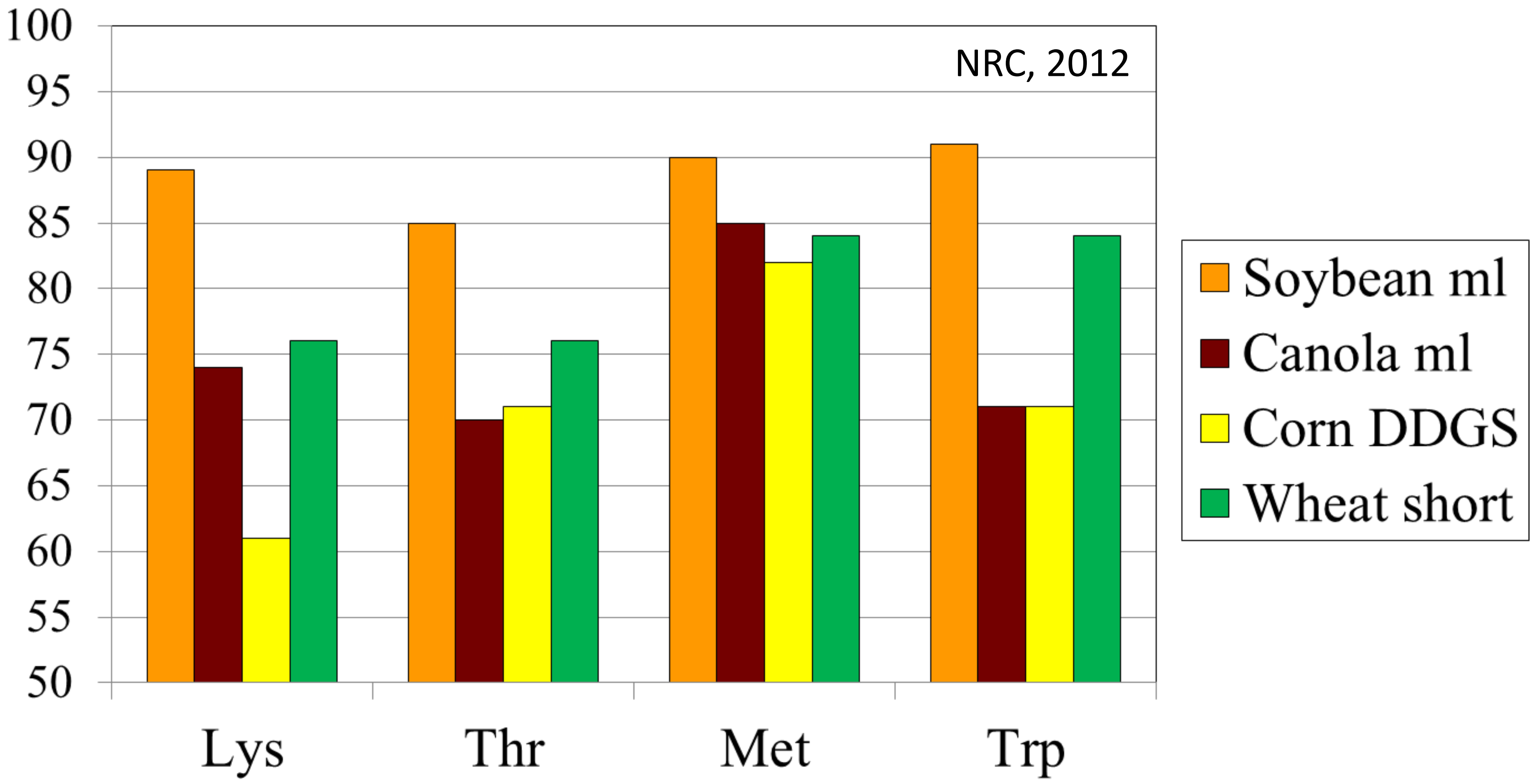
	Ingredients			
	FeM1	PTFeM1	FeM2	PTFeM2
Proximate composition (as is)				
Dry matter (%)	93.4	93.3	86.6	93.1
Crude protein (%)	81.9	80.3	76.3	81.7
Lipid (%)	8.3	7.9	6.5	6.5
Total carbohydrates (%)[1]	1.3	1.3	1.5	0.6
Ash (%)	1.9	3.8	2.3	4.3
Gross energy (kJ g^{-1})[1]	22.6	22.1	20.7	21.8
Essential amino acids (% as is)				
Arginine	5.9	5.7	5.7	6.1
Histidine	0.6	0.6	0.7	0.8
Isoleucine	4.0	3.9	3.5	3.8
Leucine	6.7	6.5	6.2	6.6
Lysine	1.8	1.8	2.2	2.3
Methionine	0.5	0.5	0.6	0.6
Phenylalanine	4.0	3.9	3.4	3.6
Threonine	3.9	3.8	3.8	4.0
Valine	6.0	5.8	5.1	5.6
Non-essential amino acids (% as is)				
Alanine	3.8	3.7	3.6	3.8
Asparatic acid	5.6	5.5	5.5	5.8
Cyst(e)ine	3.5	3.6	4.1	4.3
Glutamic acid	9.2	9.0	9.7	10.1
Glycine	6.5	6.3	5.8	6.2
Proline	8.3	7.8	6.8	7.3
Serine	9.3	8.8	8.1	8.4
Cross-linked amino acids (% as is)				
Lanthionine	3.18	3.17	2.55	2.80
DL-Lysinoalanine	0.16	0.15	0.06	0.07
B-aminoalanine	0.14	0.13	0.05	0.06



Univ. of Guelph Animal metabolism facilities



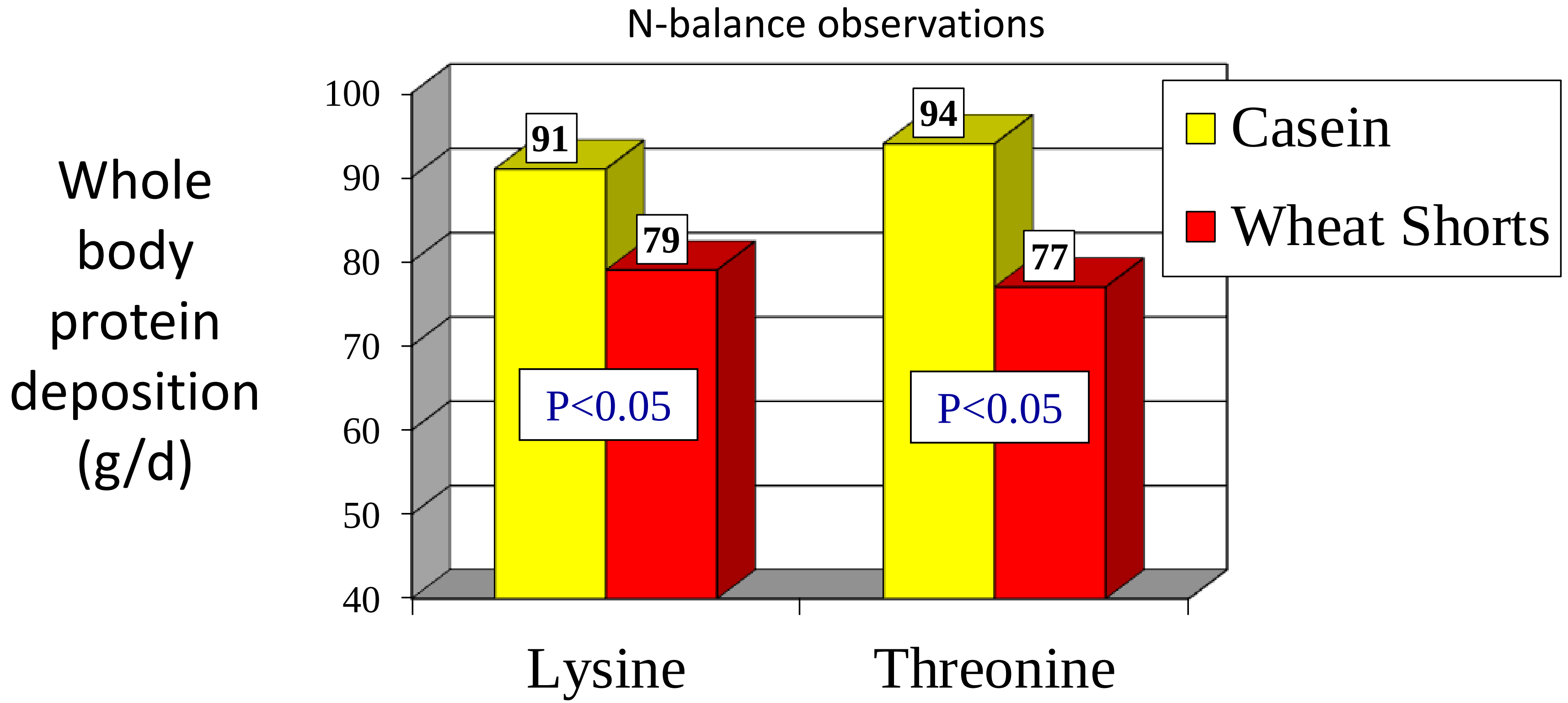
Standardized ileal digestibility (%) of key Amino Acids in Swine



Large differences in digestibility

Standardized Ileal digestibility (SID) - Swine

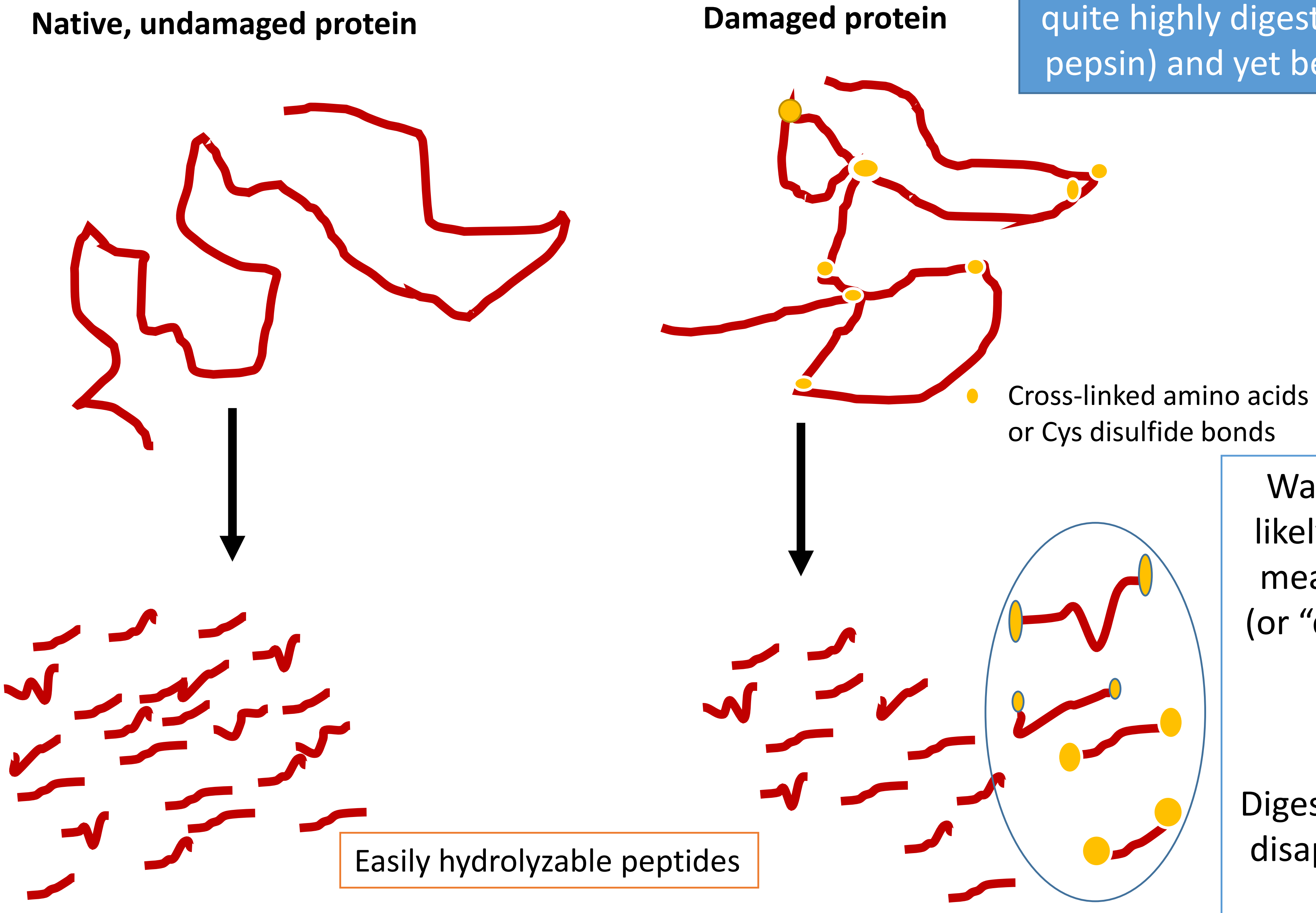
- In some instances, SID does not accurately predict bio-availability of amino acids



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

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

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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Feather processing							
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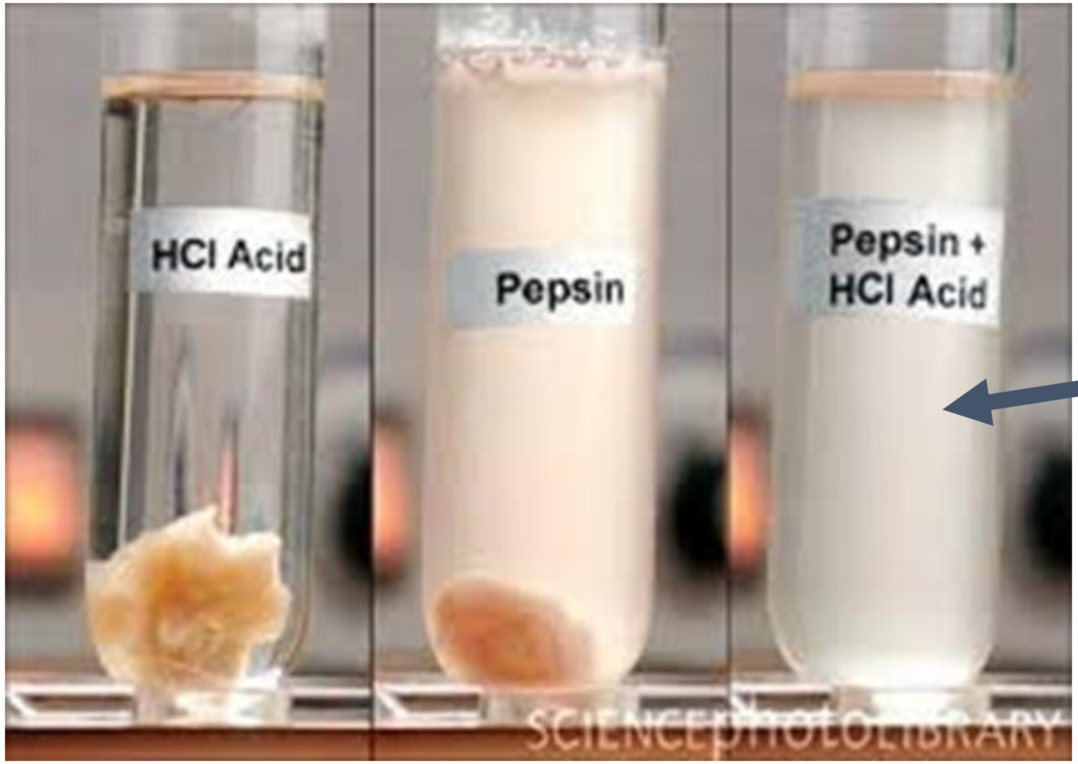
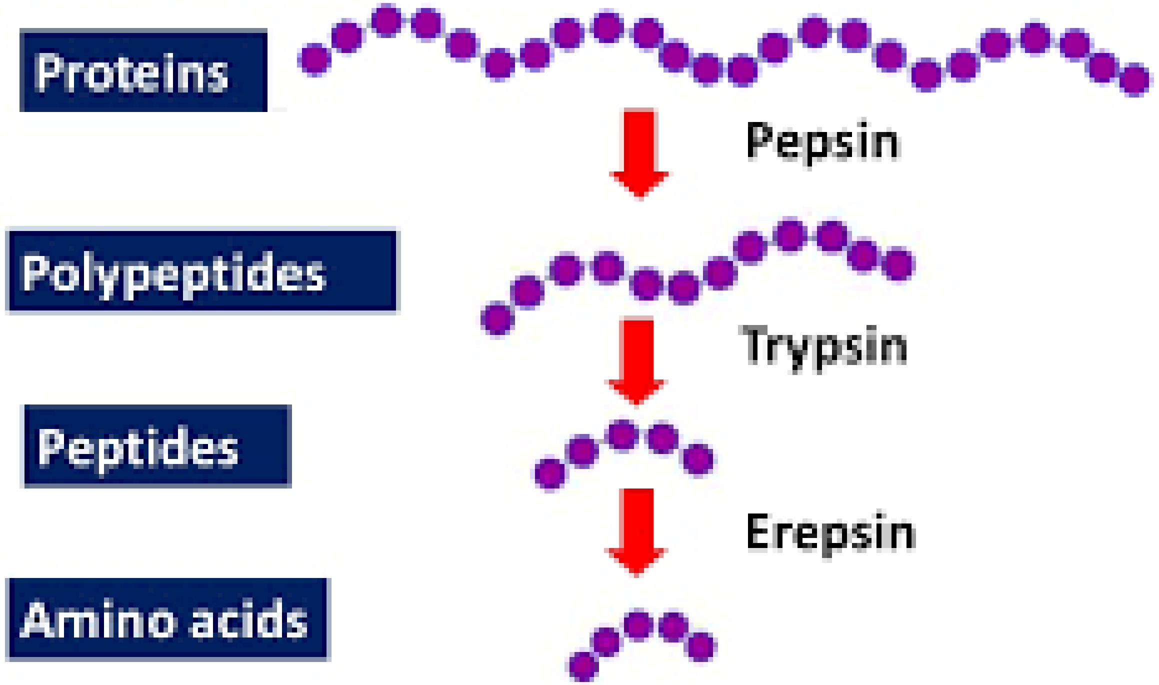
	345	89.2	88.1	79	4.00	2.18	.23

Increasing pepsin digestibility

Increasing lanthionine

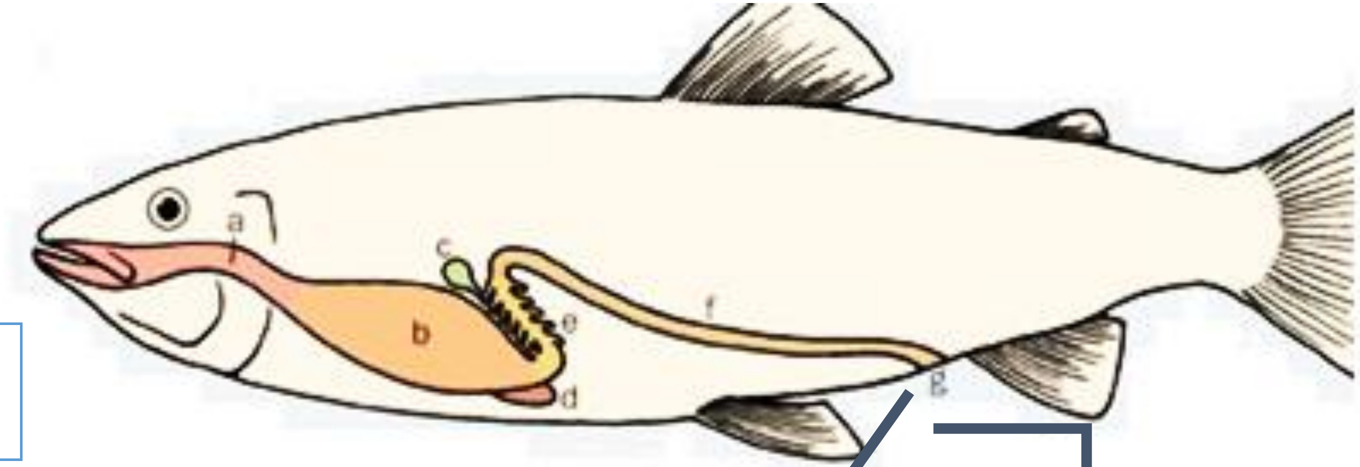

HCl Acid
Pepsin
Pepsin +
HCl Acid
SCIENCEPHOTOLIBRARY

Remember:


Proteins
Pepsin
Polypeptides
Trypsin
Peptides
Erepsin
Amino acids

What's Next?

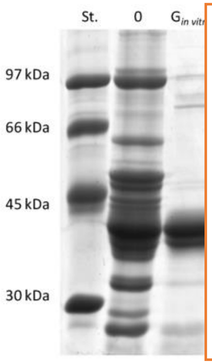
Determining digestible, non-metabolizable, and refractory elements in protein ingredients



Intake

Dietary proteins and peptides (from various ingredients)

Faeces



Proteins and peptides of dietary origin

- Not digested, refractory to digestion?
- What's their characteristics?
- Disulphide bonds? Containing damaged amino acids?

Endogenous proteins/ amino acids

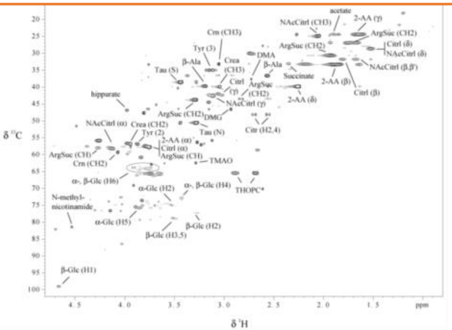
Microbial proteins/amino acids?

Urine - Difficult

Absorbed but non-metabolizable compounds

- Damaged amino acids?
- Cross-linked amino acids?
- Metabolites?

NMR 2D high-resolution liquid spectroscopy



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