Rationally Approaching the Estimation of the Nutritive Value of Feed Ingredients

1. Chemical composition and nutritive value

2. Digestibility and bio-availability of nutrients

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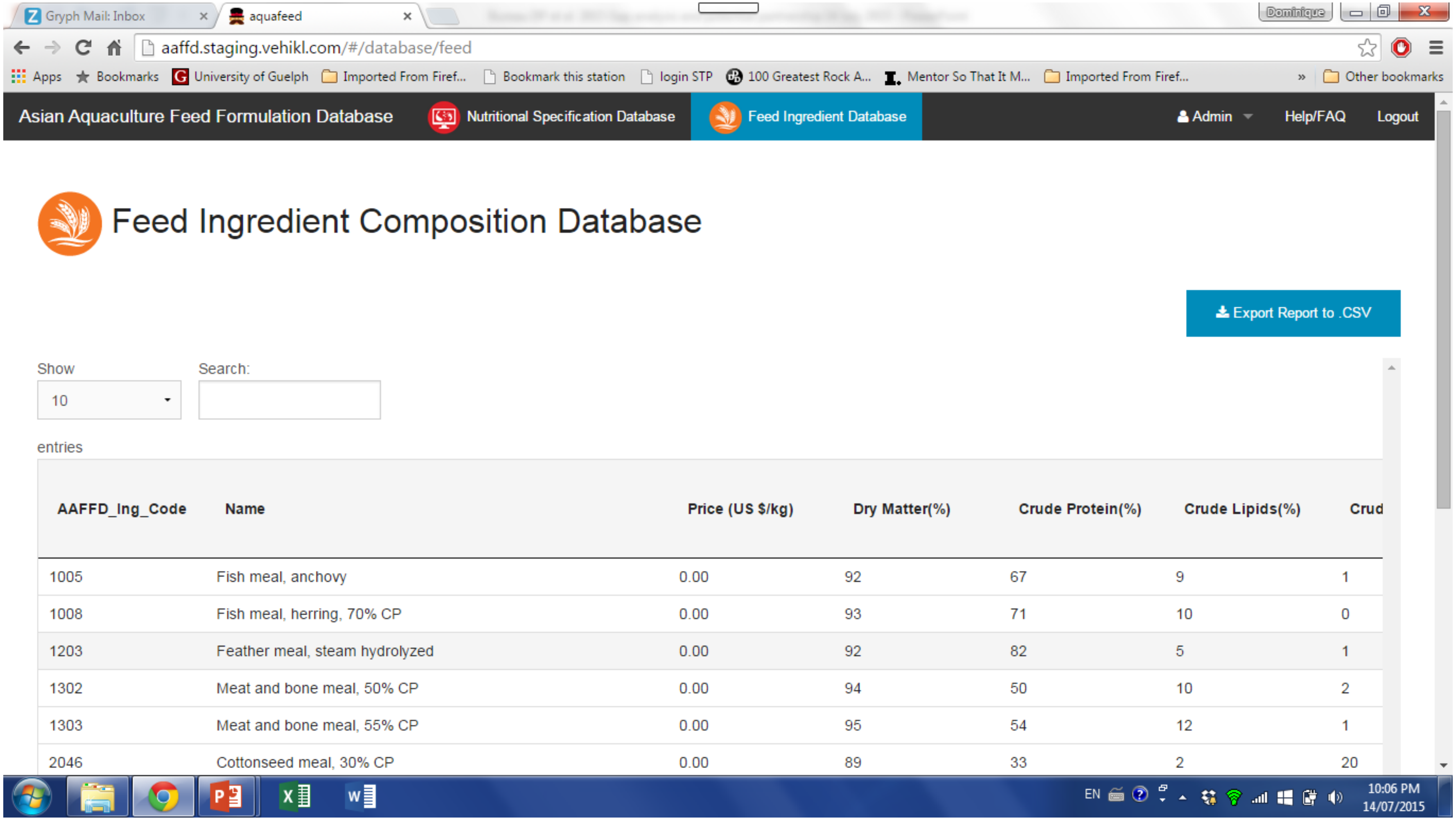
IAFFD
International Aquaculture Feed Formulation Database

FICD

Feed Ingredient Compositions

This part of the project involved compiling or generating information on chemical and nutrient compositions and nutritive value of a large number of feed ingredients that could potentially be used in the manufacturing of aquaculture feeds



Asian Aquaculture Feed Formulation Database | Nutritional Specification Database | Feed Ingredient Database | Admin | Help/FAQ | Logout

Feed Ingredient Composition Database

Export Report to .CSV

Show 10 entries Search:

AAFFD_Ing_Code	Name	Price (US $/kg)	Dry Matter(%)	Crude Protein(%)	Crude Lipids(%)	Crud
1005	Fish meal, anchovy	0.00	92	67	9	1
1008	Fish meal, herring, 70% CP	0.00	93	71	10	0
1203	Feather meal, steam hydrolyzed	0.00	92	82	5	1
1302	Meat and bone meal, 50% CP	0.00	94	50	10	2
1303	Meat and bone meal, 55% CP	0.00	95	54	12	1
2046	Cottonseed meal, 30% CP	0.00	89	33	2	20

- Compiled information on about 500 generic ingredients for 239 parameters (!?)
- No single study / document contained all this massive amount of information
- Multiple observations for same ingredients (protein, lipid, amino acids, etc.)
- Many “blank” for many/most parameters that had to be estimated

Animals Utilize **NUTRIENTS**
not "Ingredients"

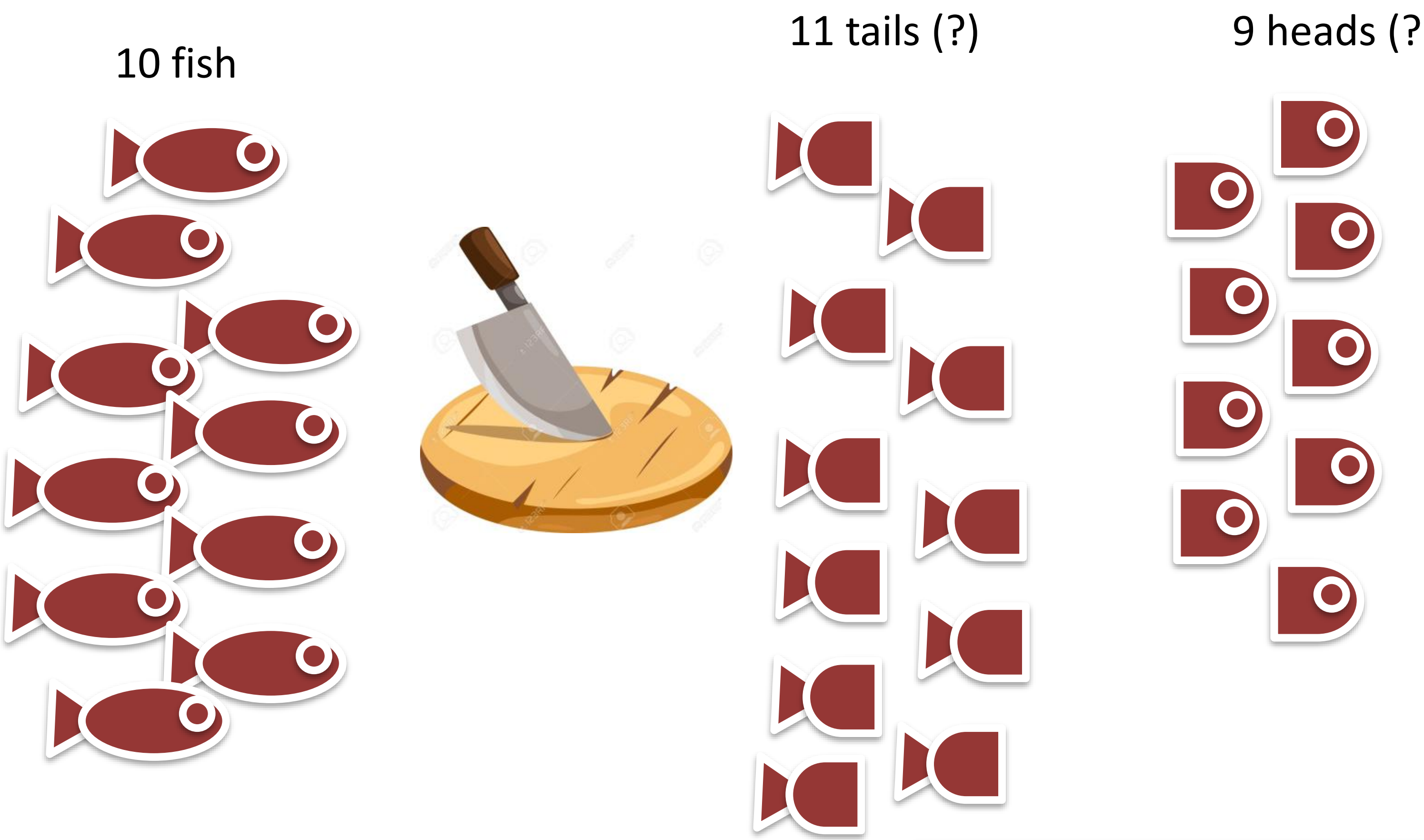
What's important in feed formulation?

- Individual nutrient requirements of animals (with adequate safety margins)
- Nutrient content of feed ingredients and associated variability
- Digestibility and bio-availability of nutrients
- Potential limitations (e.g. contaminants, anti-nutritional factors)
- Impacts (e.g. physical properties, waste outputs, final product quality) of the ingredients

General "mind-frame" underlying the development of the International Aquaculture Feed Formulation Database

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!

Law of Conservation of Mass

Nothing is lost, nothing is created, everything is transformed.

"Rien ne se perd, rien ne se crée, tout se transforme."
—Antoine Lavoisier

26 August 1743 – 8 May 1794

General "mind-frame" underlying the development of the International Aquaculture Feed Formulation Database

Proximate Analysis + Carbohydrates

Ingredient	PA01 Dry Matter	PA03 Crude Protein	PA04 Crude Lipids	PA05 Crude Fibre	PA06 Ash	PA07 NFE	PA08 NDF	PA09 ADF	PA10 Total CHO	PA11 Starch	PA12 Sugars
	%	%	%	%	%	%	%	%	%	%	%
Fish meal	90.8	74.2	5.0	0.5	10.0	1.2	0.0	0.0	1.7	0.0	0.0
Wheat middlings	90.0	15.8	3.0	7.0	3.6	60.6	3.0	13.0	67.5	31.5	3.0
Canola meal, exp.	89.9	35.2	7.5	11.9	7.0	28.4	33.3	26.0	40.3	0.9	6.0

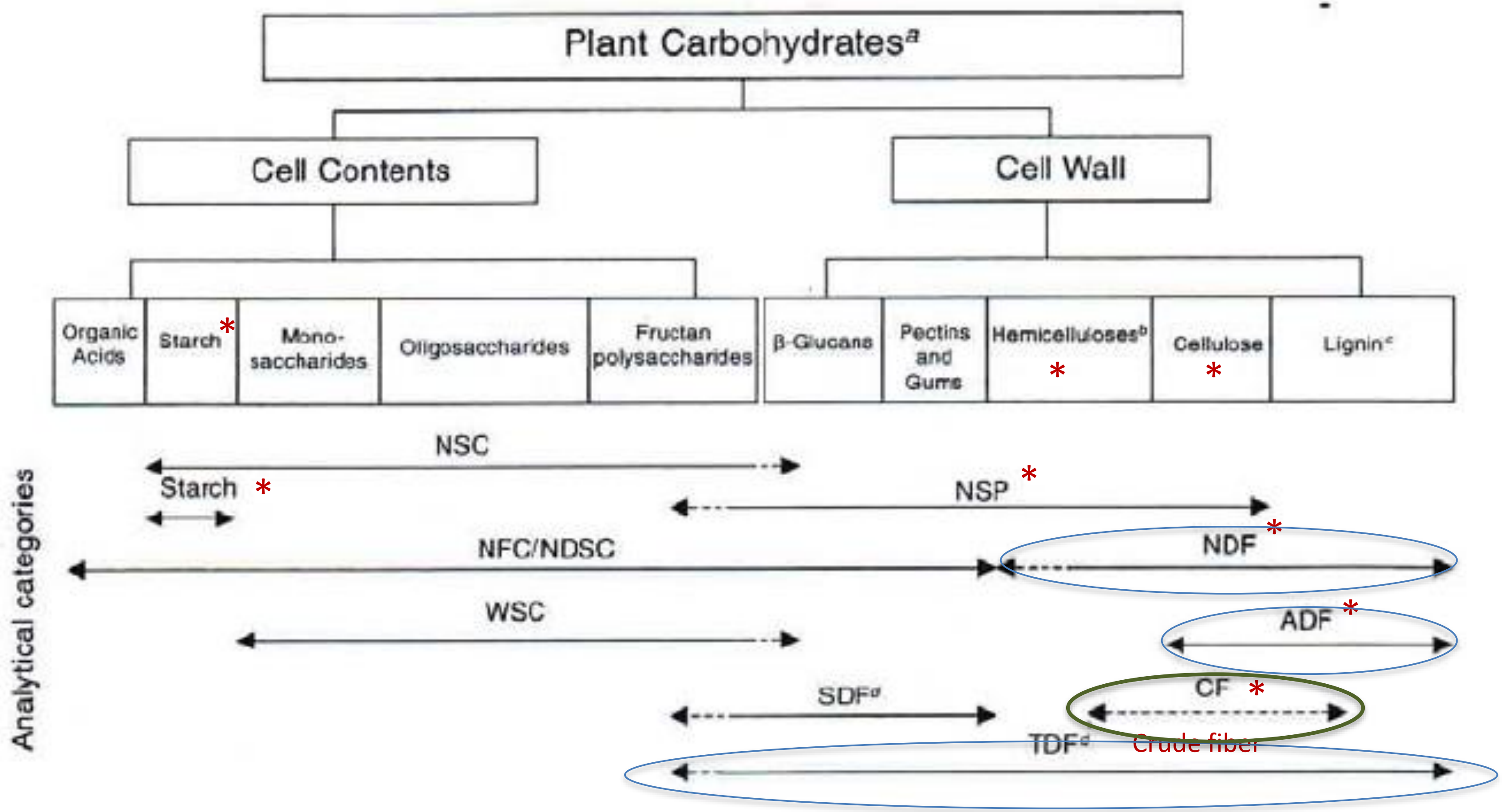
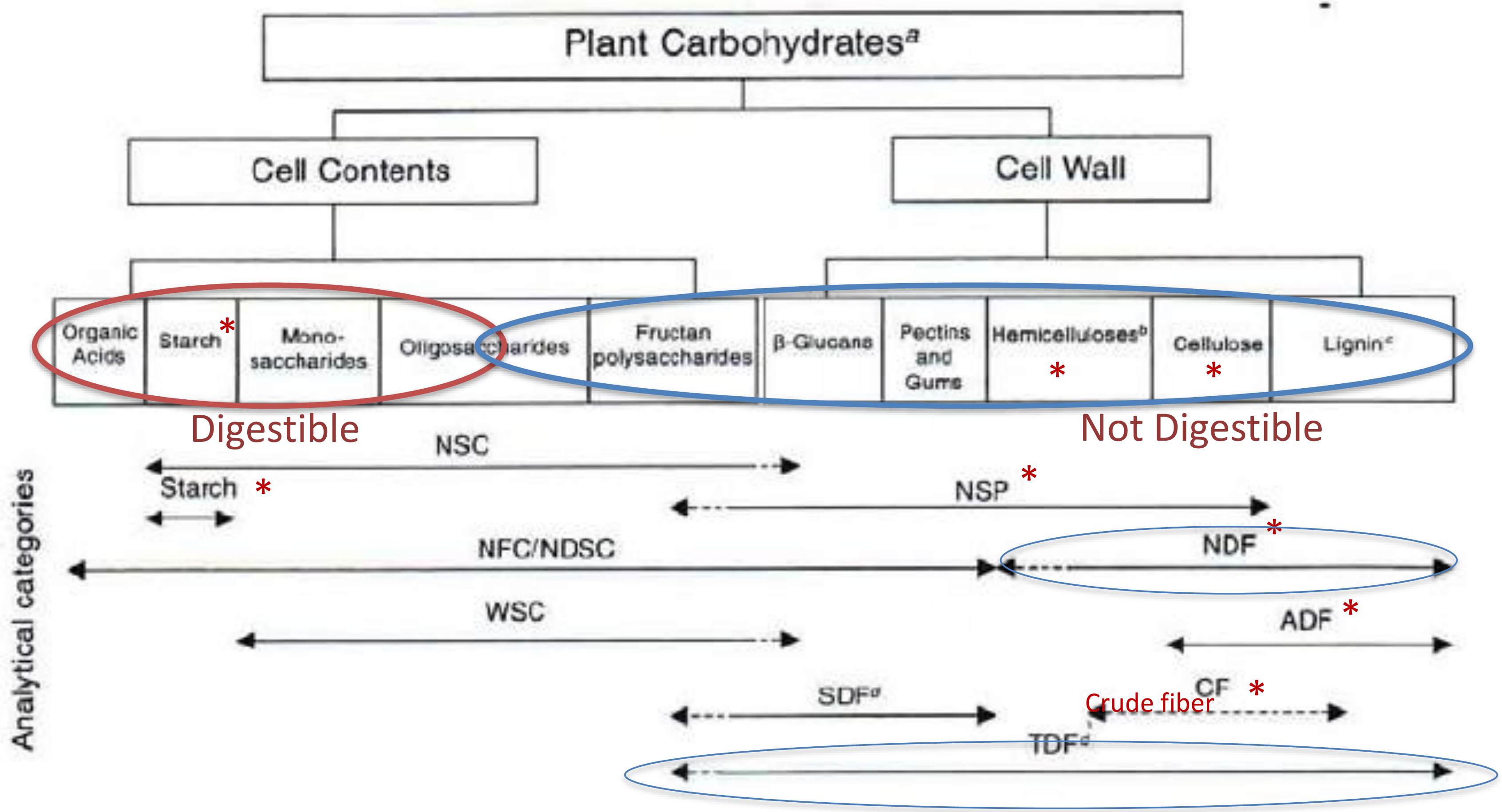


FIGURE 7-5 Categories of dietary carbohydrates based on current analytical methods.
ABBREVIATIONS: ADF = acid detergent fiber; CF = crude fiber; NDF = neutral detergent fiber; NDSC = neutral detergent soluble carbohydrates; NFC = nonfiber carbohydrates; NSC = nonstructural carbohydrates; NSP = non-starch polysaccharides; SDF = soluble dietary fiber; TDF = total dietary fiber; WSC = water-soluble carbohydrates. Dashed lines indicate that recovery of included compounds may be incomplete.

NRC (2011)



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NRC (2011)

IAFFD ASNS Database- v 4.4 - 14 June 2019 final - Excel

	Species	**Tilapia**									
						Specifications for Stage / Live Weight Range (g)					
						101_1	**101_2**	**101_3**	**101_4**	**101_5**	**101_6**
						Starter	**Fry**	**Pre-grower**	**Grower1**	**Finisher**	**Brood**
Code_Species	**Code_NutrSpec**	**NutrSpecification**	**Short Name**	**Unit**	**Restriction Type**	**< 5 g**	**5-50 g**	**50-200 g**	**200-500 g**	**500-1500 g**	**>1500 g**
101	TX06	Gossypol	GOS	mg/kg	Maximum	1052.60	1315.79	1447.37	1447.37	1578.95	1578.95
101	TX07	Phytic Acid	PHY	g/kg	Maximum	5.00	7.50	10.00	10.00	10.00	10.00
101	TX08	Glucosinolates	GLSNT	mmol/kg	Maximum	1.00	2.00	3.00	4.00	5.00	5.00
101	TX09	Sinapine	SINAP	mg/kg	Maximum	1578.95	2105.26	4210.52	4210.52	4210.52	4210.52
101	TX10	Tannins	TANN	mg eq cathin/kg	Maximum	2105.26	2900.00	3157.89	3157.89	3157.89	3157.89
101	TX11	Lectins	LECTI	mg/Kg	Maximum	526.32	1052.63	1052.63	1052.63	1052.63	1052.63
101	TX12	Cyanogens	CYANG	mg/kg	Maximum	21.06	25.26	31.58	42.10	42.10	52.64
101	TX13	PCBs	PCB	ngWHOTEQ/kg	Maximum	1.89	2.11	2.11	2.42	2.95	3.47
101	TX14	Dioxins	DXN	ngWHOTEQ/kg	Maximum	0.63	0.95	1.26	1.26	1.37	1.37
101	TX15	Soyasaponins	SSAP	mg/kg	Maximum	1052.63	1578.95	2105.26	3157.89	3157.89	3157.89
101	TX16	Isoflavones	ISOFLV	mg/kg	Maximum	2105.26	2631.58	2631.58	3157.90	3157.90	3157.90

Tilapia (June2019) | N-Tilapia-Semi (June2019) | N-Tilapia-Intens (June2019) | N-Tilapia- RAS (June2019) | Pangasius (June2019) | Milkfish (June2019) | Asian sea bass (June2019) | Asian ...

Reconciling Elemental and Individual Nutrient Analyses to Improve the Characterization of the Nutritive Value of Protein Sources

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Rational

Limited systematic efforts to critical examine estimates of individual nutrient concentrations of practical ingredients. This is especially important since 1) results of analysis of individual nutrients (e.g. amino acids) are often costly & difficult to objectively evaluate and 2) true nutrient content of ingredients has an important impact on animal performance

Tools (equations) allowing the comparison of results from proximate or elemental mass analysis and individual nutrient analysis could provide a rational basis for critically evaluating the reliability of results of individual nutrient analysis and examining nutritive value of ingredients

This first part of this project involves an effort to carry out an elemental nitrogen (N) mass balance effort and initiate work on developing elemental carbon (C) balance equations

Preliminary Results

Ingredients	Total N	EAA-N	NEAA-N	Total NPN		Missing N balance	"Missing" N
	% DM	% DM	% DM	% DM	% of Total N	% DM	%
Fish meal, herring	11.1	4.7	4.9	0.06	0.51	1.42	13
Meat and bone meal	8.0	3.2	3.9	0.03	0.37	0.90	11
Poultry by-products meal, low ash	11.2	4.9	5.1	0.05	0.43	1.02	9
Poultry by-products meal, high ash	11.2	4.8	5.2	0.05	0.46	1.16	10
Hydrolyzed feather meal	15.6	5.8	6.6	0.16	1.06	3.02	19
Spray-dried blood meal	16.4	7.5	4.8	0.01	0.08	4.20	26
Porcine meat meal	9.9	4.5	5.1	0.04	0.40	0.27	3

Animals Utilize **NUTRIENTS**

not "Ingredient", and not "Proximate Components"

What's important in feed formulation?

- Individual nutrient requirements of animals (with adequate safety margins)
- Nutrient content of feed ingredients and associated variability
- Digestibility and bio-availability of nutrients
- Potential limitations (e.g. contaminants, anti-nutritional factors)
- Impacts (e.g. physical properties, waste outputs, final product quality) of the ingredients

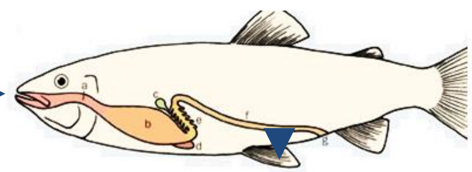
Ingredients	Total C	CHO	Fat	EAA-C	NEAA-C	CHO-C[1]	Fat-C	DNA and RNA-C	Difference C balance	Missing C
	% DM	% DM	% DM	% DM	% DM	%DM	%DM	% DM	% DM	%
Fish meal, herring	48.5	2.3	16.4	14.5	15.7	1.0	12.6	0.01	4.68	9.6
Meat and bone meal	37.9	11.2	12.3	9.4	12.1	4.9	9.5	0.02	1.94	5.1
Poultry by-products meal, low ash	51.0	3.7	17.7	15.0	16.6	1.6	13.6	0.01	4.14	8.1
Poultry by-products meal, high ash	48.6	3.7	13.5	14.5	16.6	1.6	10.4	0.01	5.51	11.3
Hydrolyzed feather meal	50.4	5.9	2.3	19.1	21.2	2.6	1.8	0.00	5.74	11.4
Spray-dried blood meal	51.0	1.7	1.1	24.3	14.9	0.7	0.8	0.00	10.20	20.0
Porcine meat meal	43.7	8.4	13.7	13.1	n/a	3.7	10.5	0.01	n/a	n/a

Determinants of Digestibility and Bio-Availability of Nutrients in Feed Ingredients:

How much is determined by ingredient characteristics and how much is associated with species?

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

Intake



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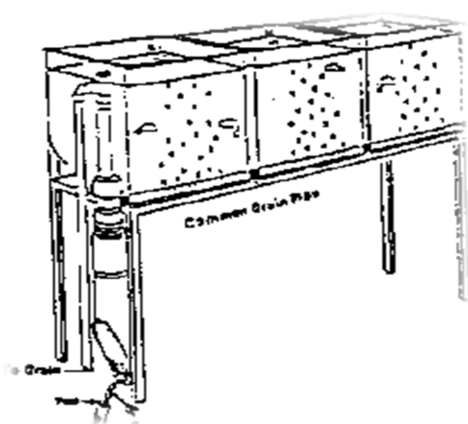
Guelph System (Developed in Early 1970's)



Digestible Nutrient as a Rational Basis for Feed Formulation

- 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

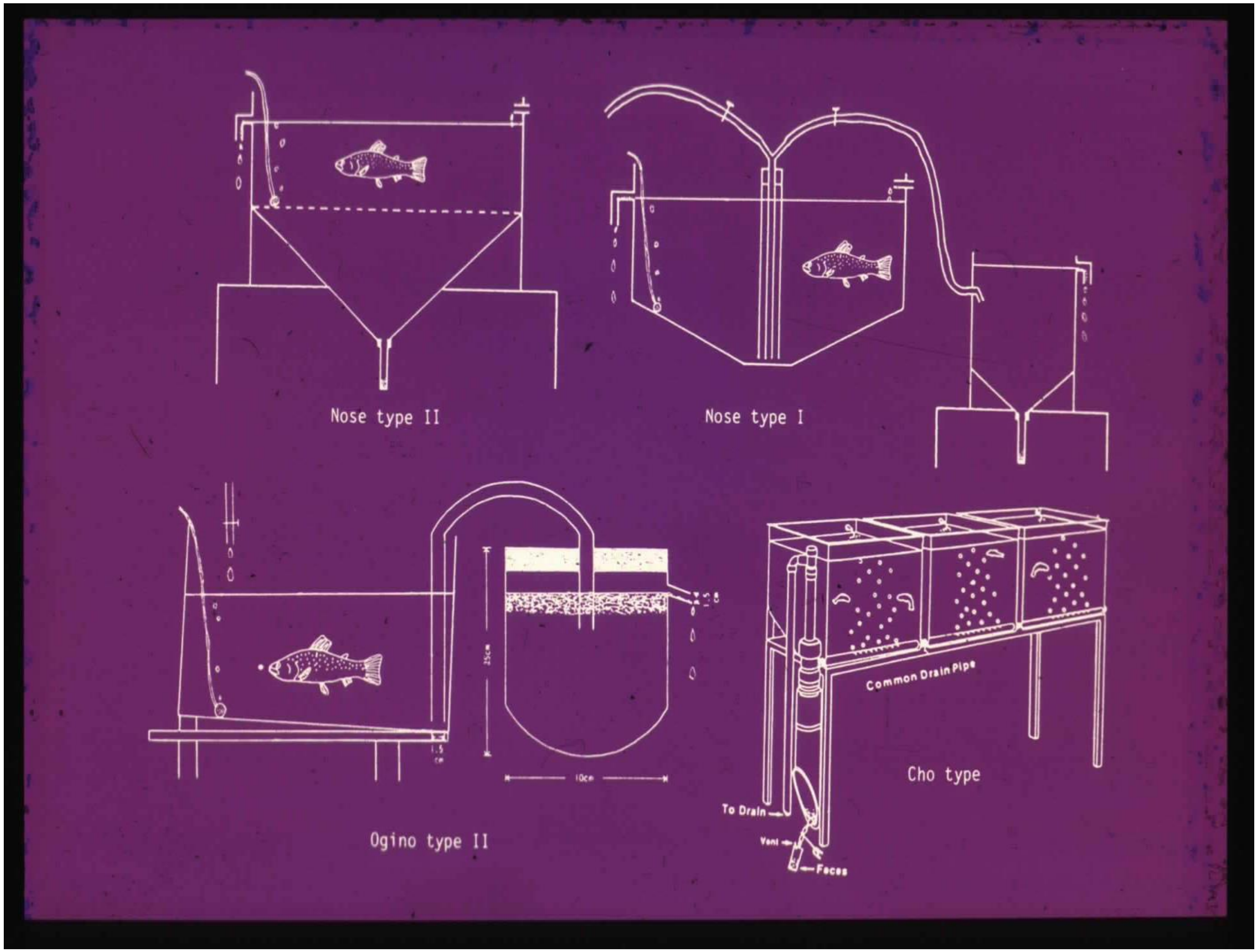
Measuring Digestibility in Fish

Several Methods:

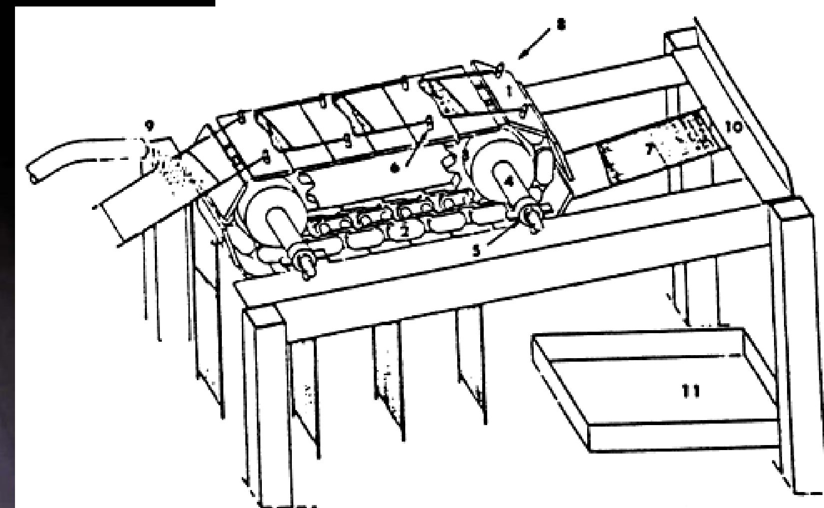
Stripping, dissection, siphoning

Three passive collection methods believed to be more reliable:

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

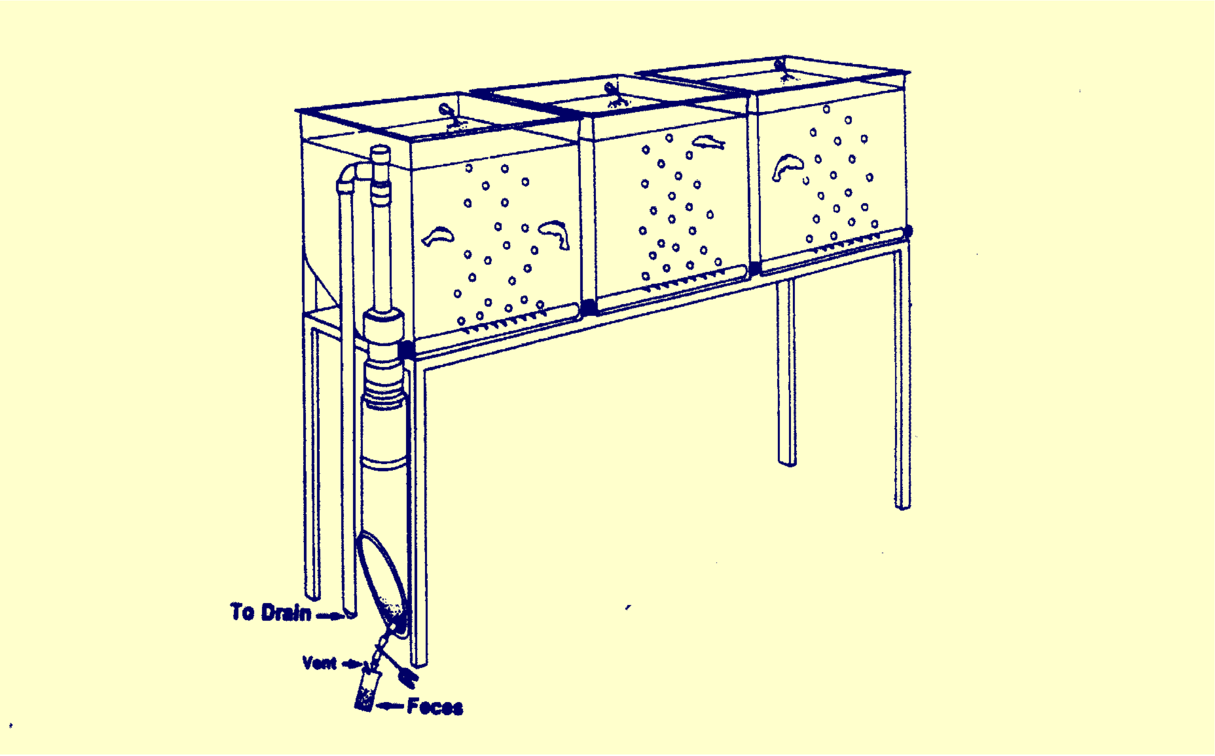

Nose type II
Nose type I
Ogino type II
Common Drain Pipe
Cho type
To Drain
Vent
Feces

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

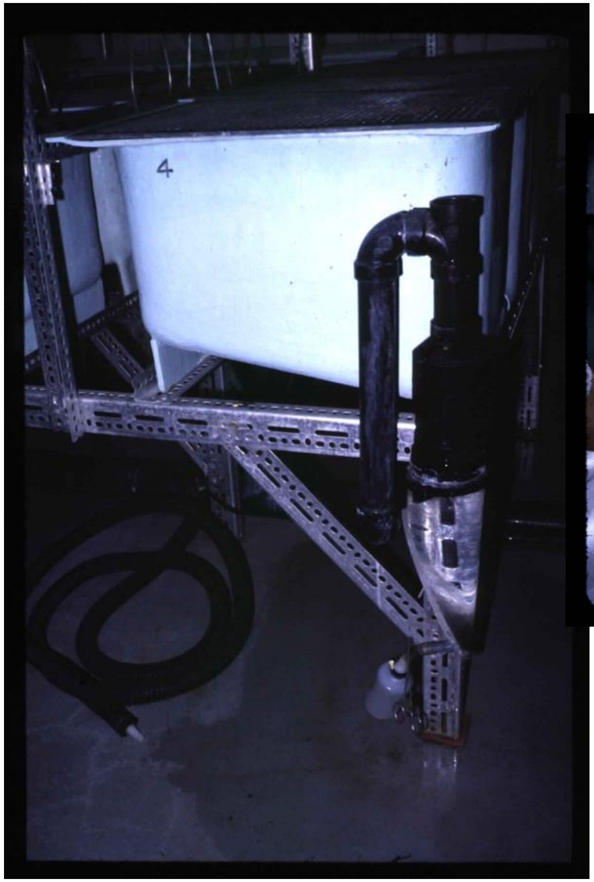


Choubert,G., de la Noue, J. and Luquet, P., 1982. Digestibility in fish: Improved device for the automatic collection of feces. Aquaculture, 29: 185-189.

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·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			
	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

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

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.

Apparent Digestibility of Feather Meals

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

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

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Aquaculture 261 (2006) 1314–1327

Aquaculture

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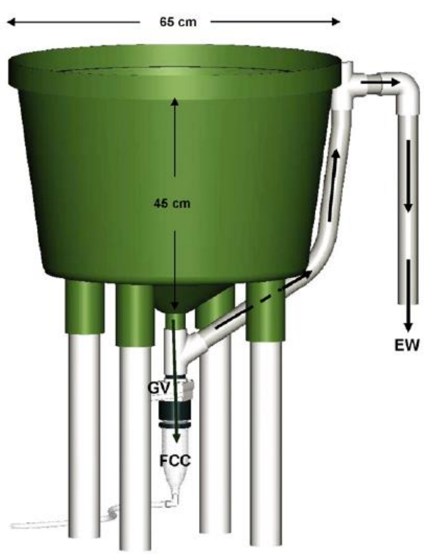
Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758)

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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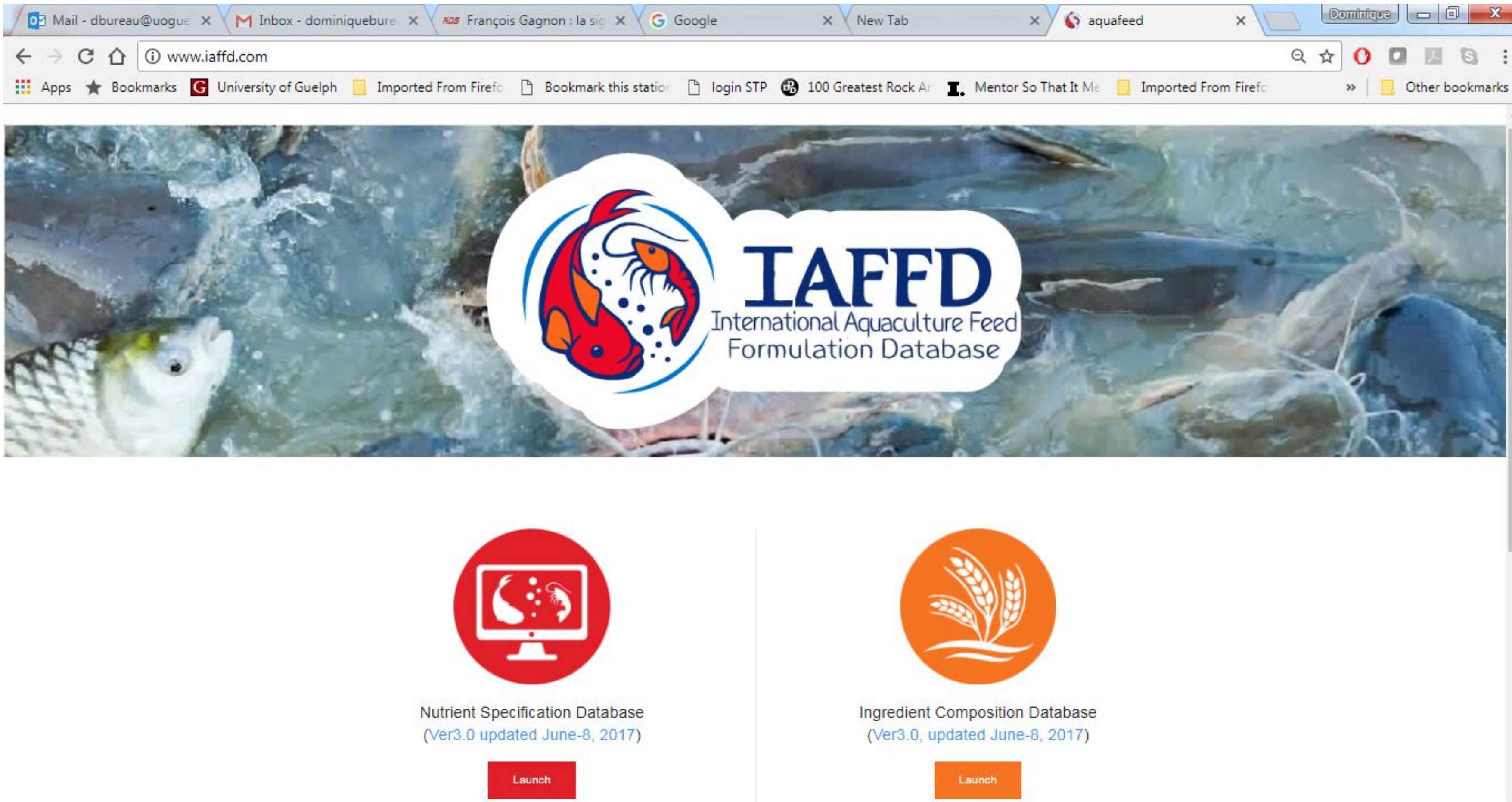
tranquocbinhaquaculture@yahoo.com.vn

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

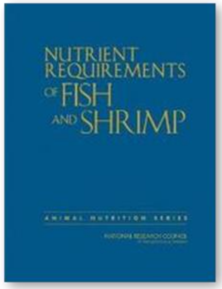
Estimates are available for Asian feed ingredients and aquaculture species
These are highly valuable to Asian aquaculture feed manufacturers



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
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)

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

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

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

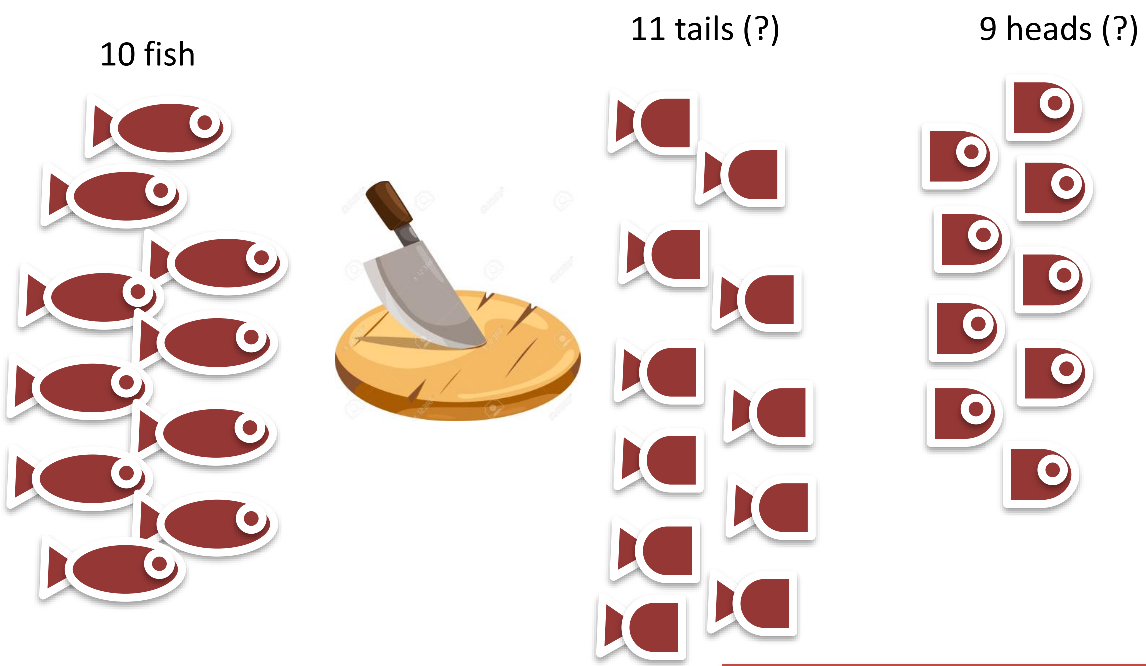
Ingredient	Organic matter ADC	Crude protein ADC	Lipid ADC	Gross energy ADC		Phosphorus availability
Select menhaden fish meal	93 9^{a} (4.9)	87.9^{ab} (1.4)	87.2^{a} (2.4)	95.0^{a} (2.7)	??	50.3^{ab} (6.7)
Regular menhaden fish meal	93.7^{a} (10.7)	76.9^{ab} (9.0)	67.6^{ab} (7.5)	92.1^{ab} (8.9)	??	47.9^{ab} (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)

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.

Apparent Digestibility of Processed Animal Proteins in the late 1990s

Ingredients	Apparent Digestibility Coefficients (%) DM	CP	GE
Trial #1			
Feather meal 1	82	81	80
Feather meal 2	80	81	78
Feather meal 3	79	81	76
Feather meal 4	84	87	80
Meat and bone meal 1	61	83	68
Meat and bone meal 2	72	87	73
Trial #2			
Meat and bone meal 3	72	88	82
Meat and bone meal 4	66	87	76
Meat and bone meal 5	70	88	82
Meat and bone meal 6	70	89	83
Trial #3			
Feather meal 5	86	88	84
Feather meal 6	83	86	81
Feather meal 7	83	88	83
Meat and bone meal 7	78	92	86
Meat and bone meal 8	72	89	81
Meat and bone meal 9	69	88	80

FNRL

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

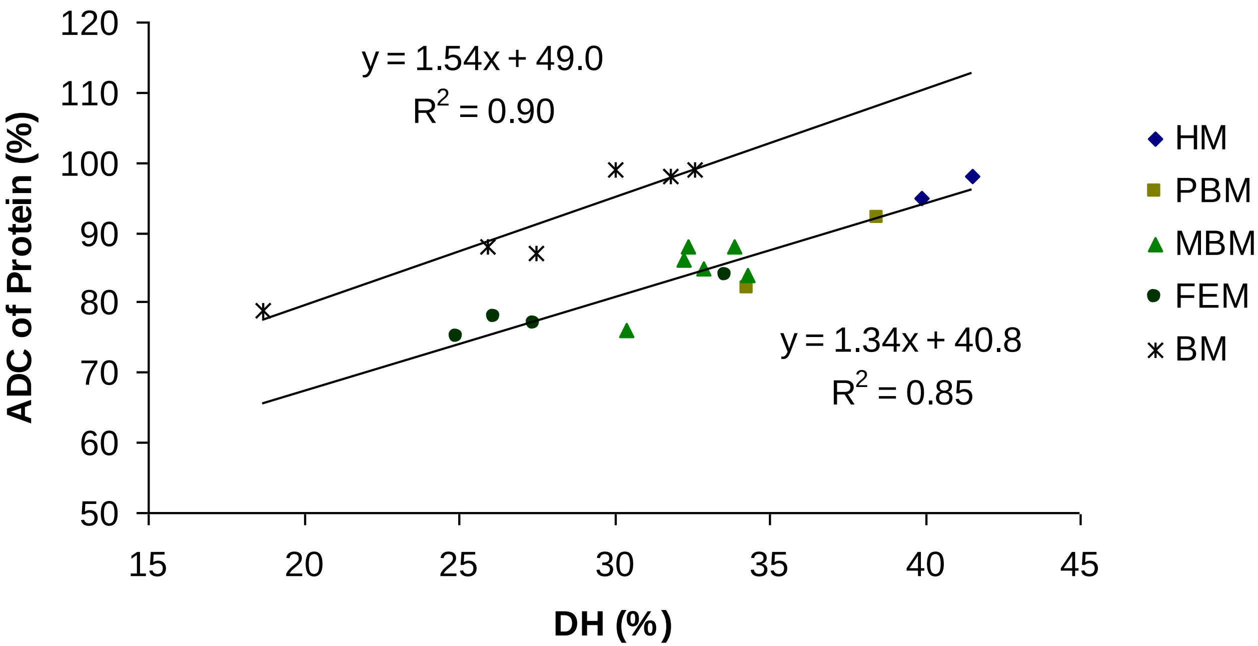
Collaboration with Dr. Adel El Mowafi, Shur-Gain AgResearch

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.

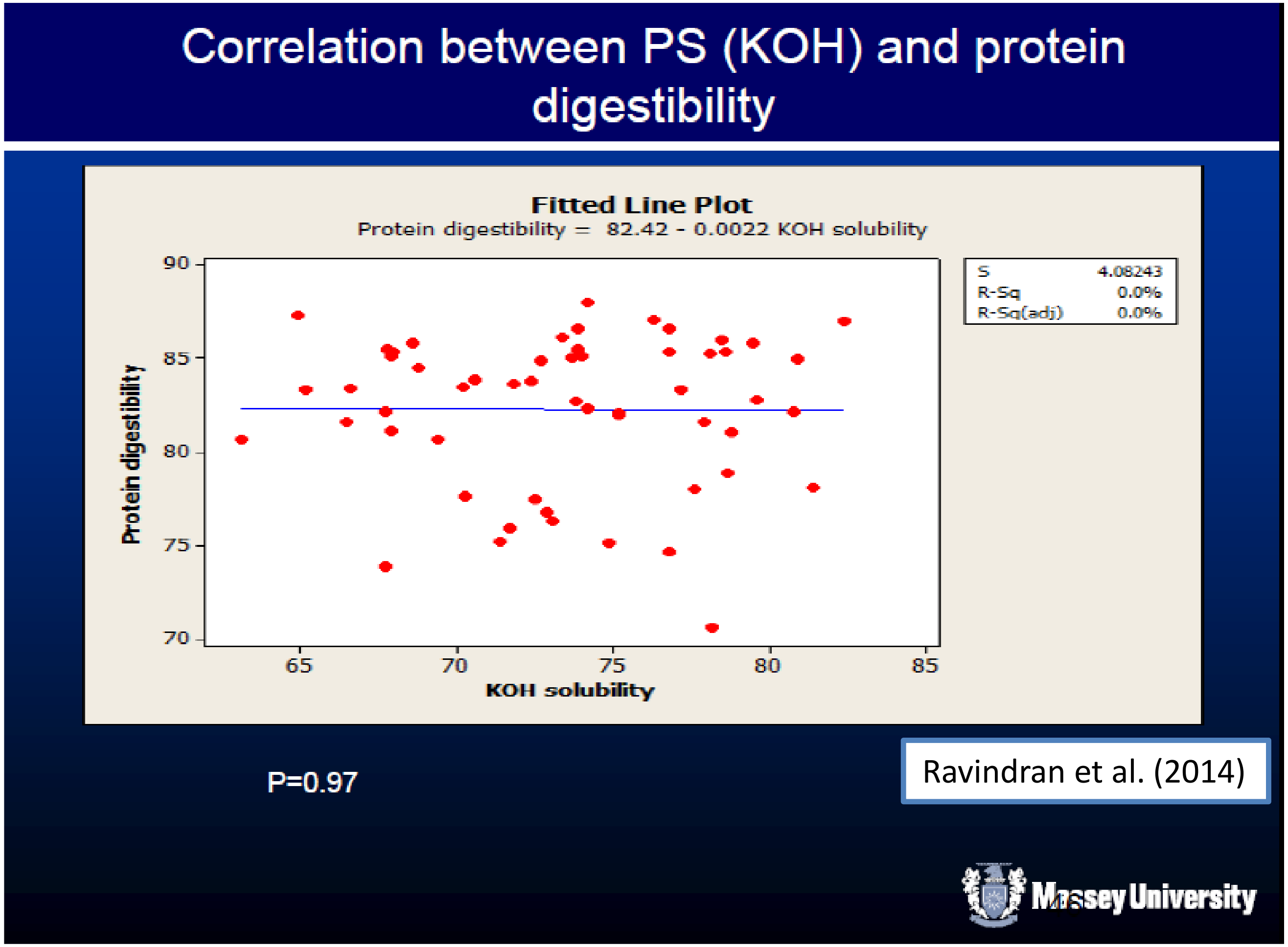


The results suggest that there is rational “chemical” bases to differences in apparent digestibility of proteins

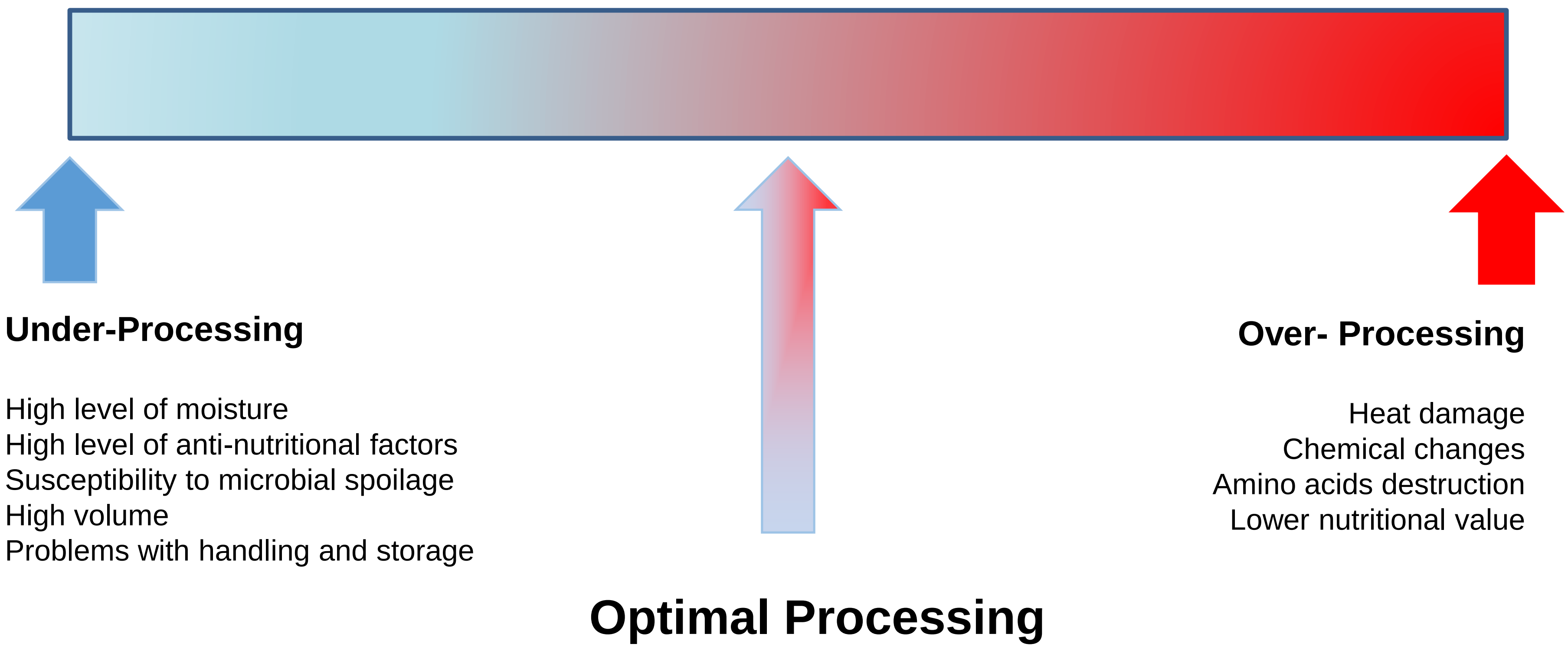
Legends: HM= herring meal, PBM= poultry by-products meal, MBM = meat and bone meal, FEM=feather meal, BM = blood meal

El Mowafi et al. 1999

High Variability in Protein Digestibility to Poultry of Commercial Soybean Meals from Various Origins



Thermal Processing of Protein Ingredients



Heat Treatment of Soybean Meal (SBM)

Control
(Not heated)

Autoclaved
SBM to 125°C
for 15 min

Autoclaved
SBM to
125°C for 30

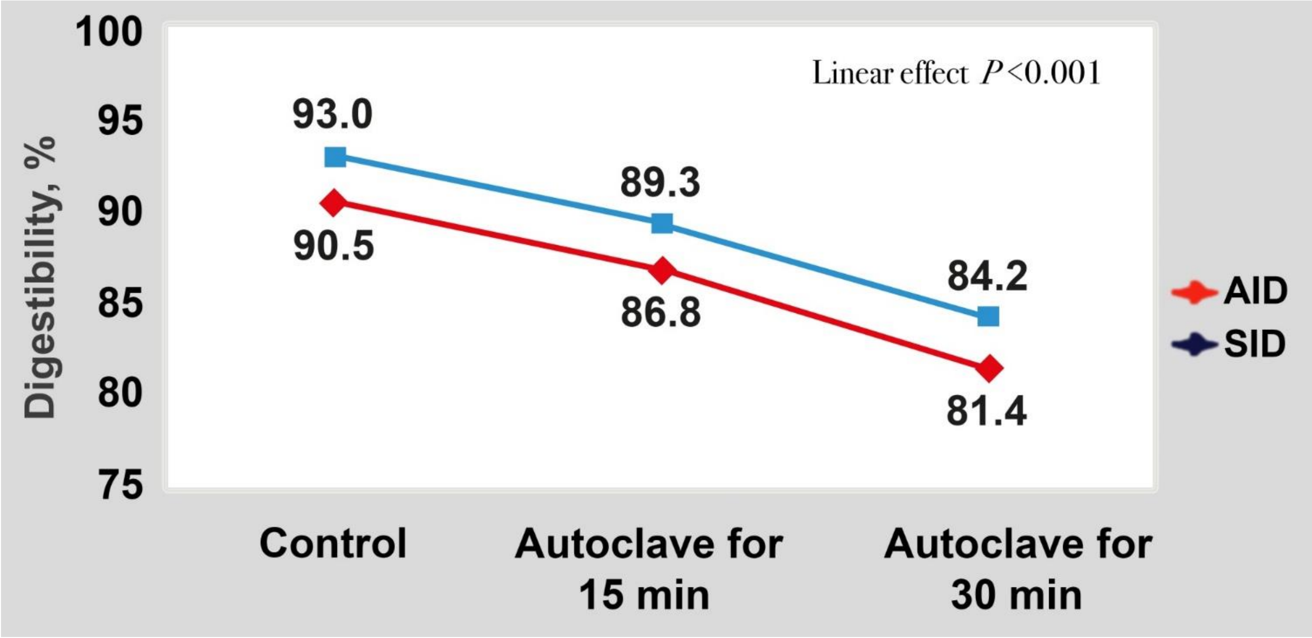
L*	76.7	**61.7**	**52.5**
a*	3.4	**10.0**	**12.5**

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

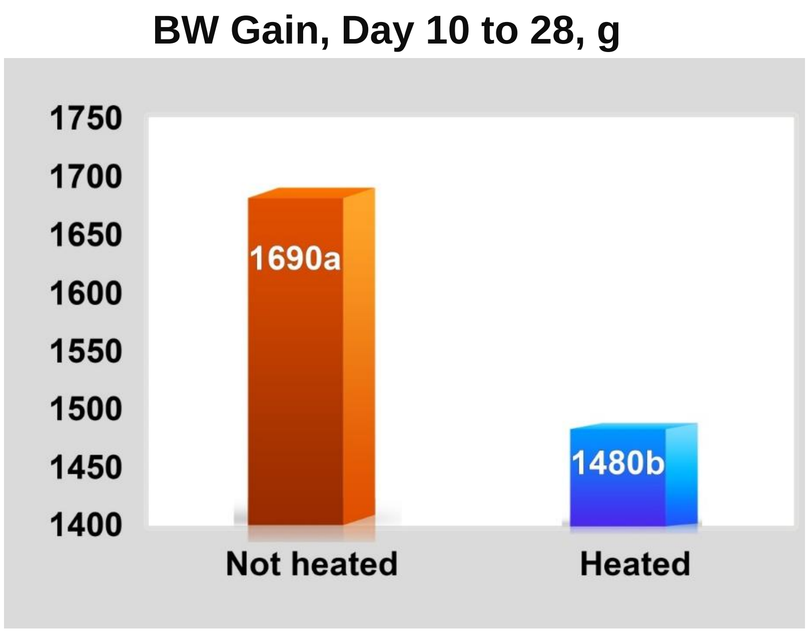
Effect of autoclaving time on apparent ileal digestibility (AID)
and standardized ileal digestibility (SID) of lysine
in pigs fed soybean treated products in their diets **(Temperature: 125 °C)**

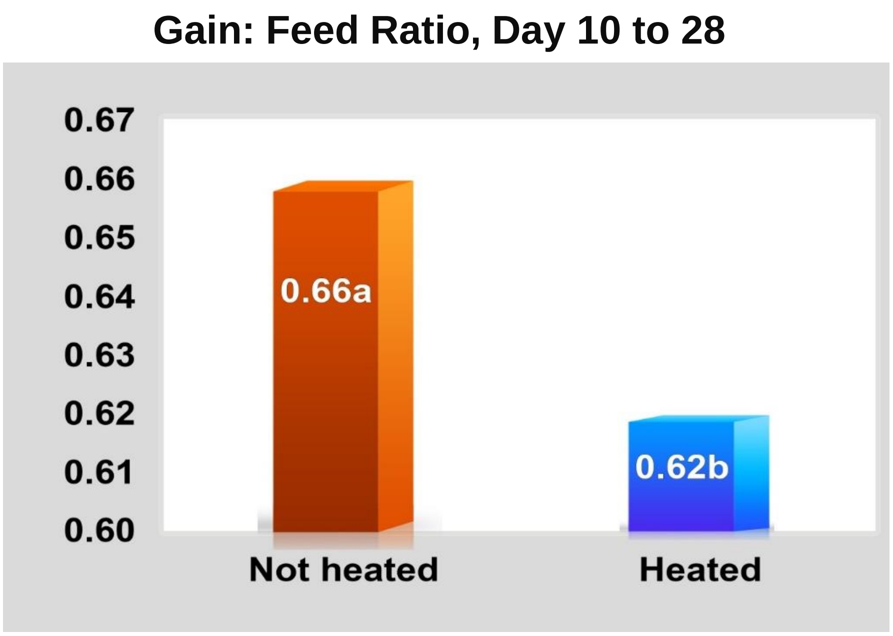


Gonzalez- Vega et al., 2011

Practical Impact of Heat Damage

Heat Damaged SBM fed to Broiler Chicks



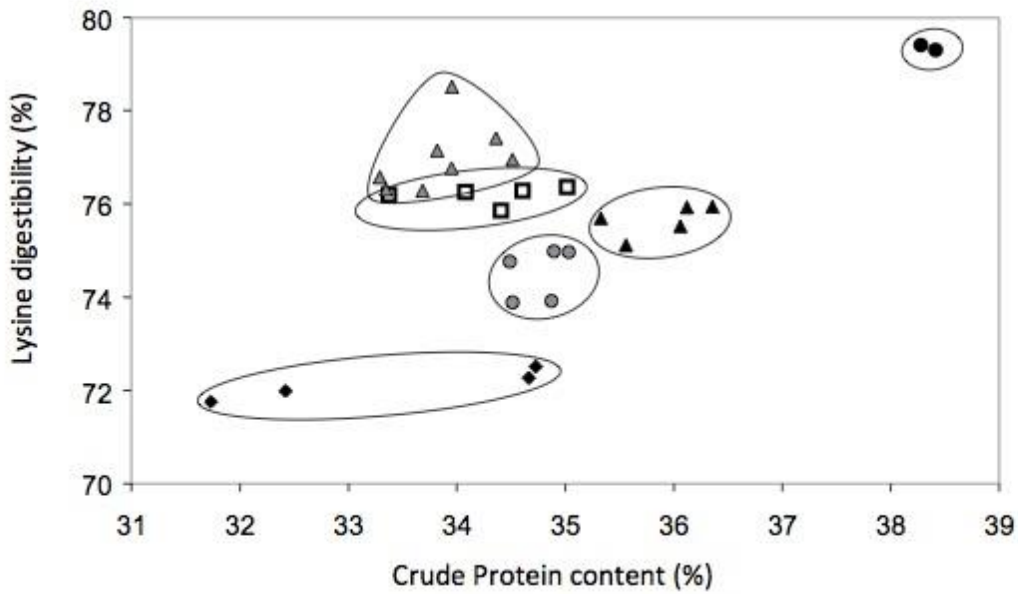


Heat Damaged Soybean Meal Through Autoclaving at 130°C for 60 minutes

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

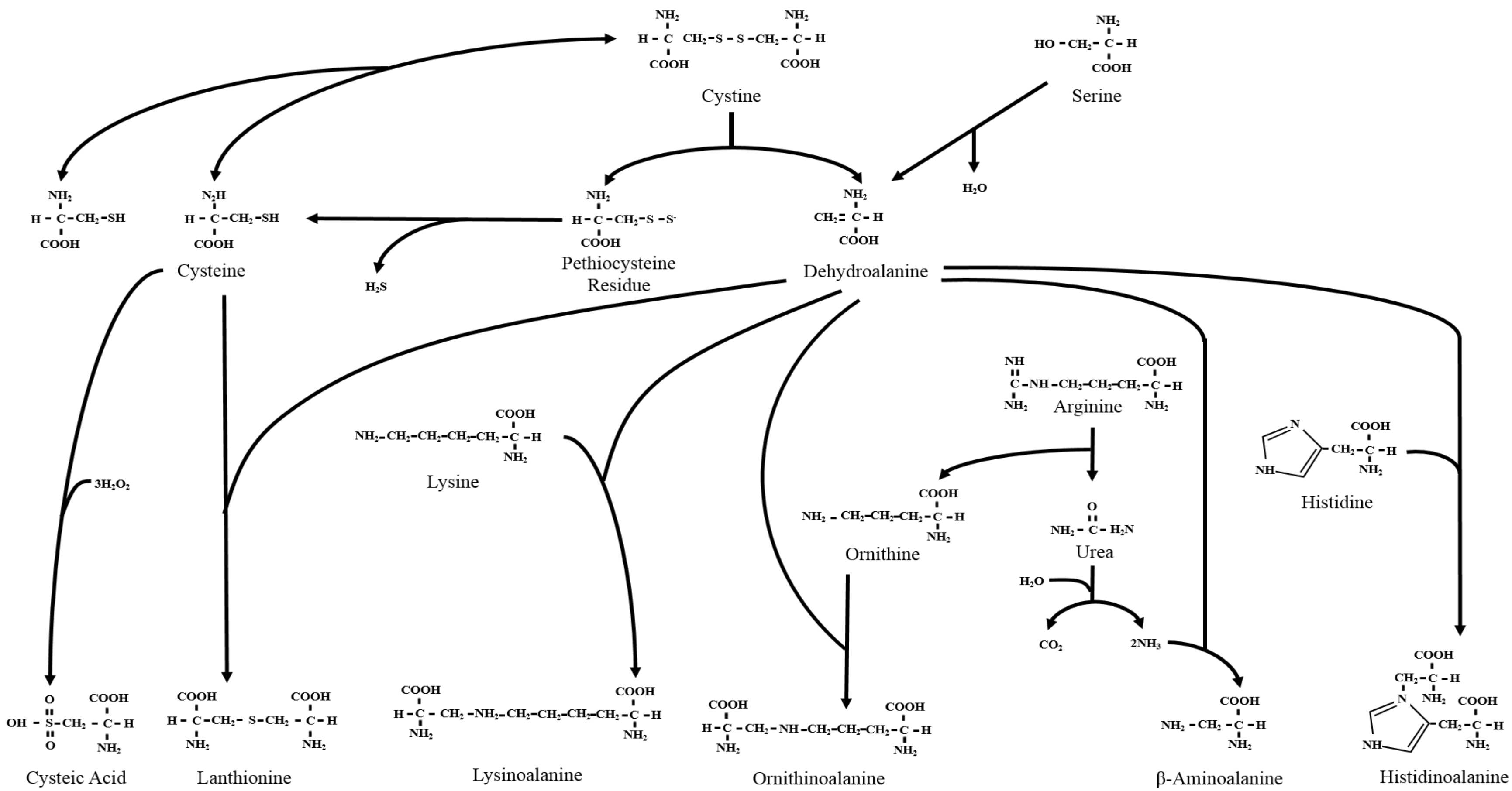
3. Racemization of amino acids

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

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

a) Disulfide bonds
b) Cross-linked amino acids

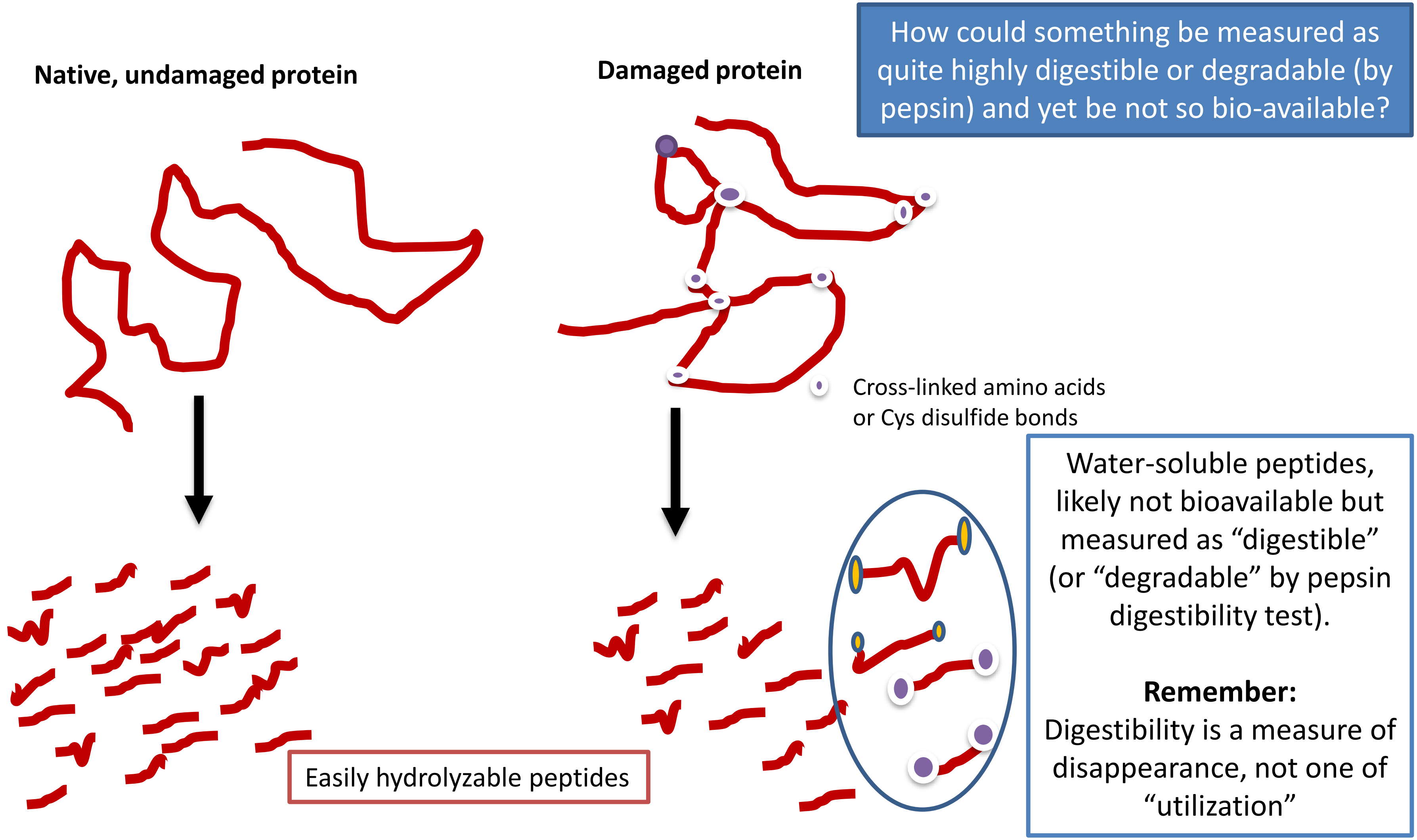
Heat Processing Promote the Formation of Cross-Linked Amino Acids



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



Native, undamaged protein
Damaged protein
How could something be measured as quite highly digestible or degradable (by pepsin) and yet be not so bio-available?
Cross-linked amino acids or Cys disulfide bonds
Easily hydrolyzable peptides
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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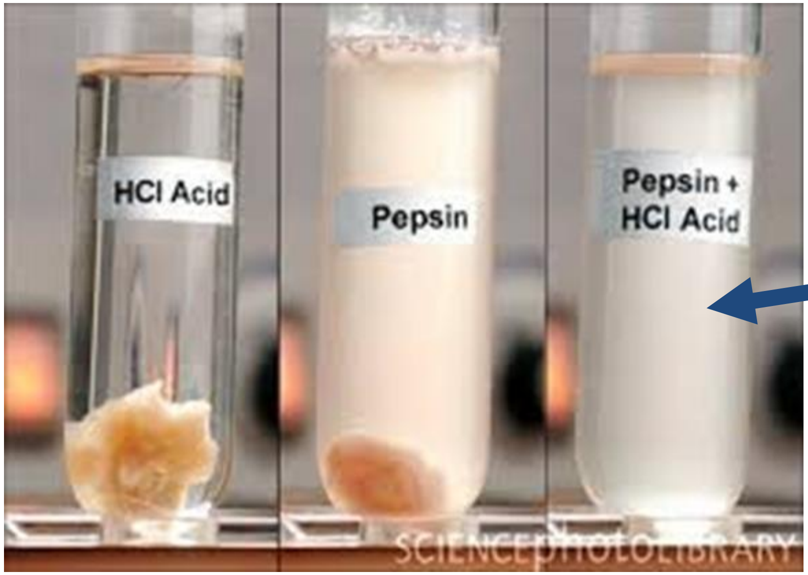
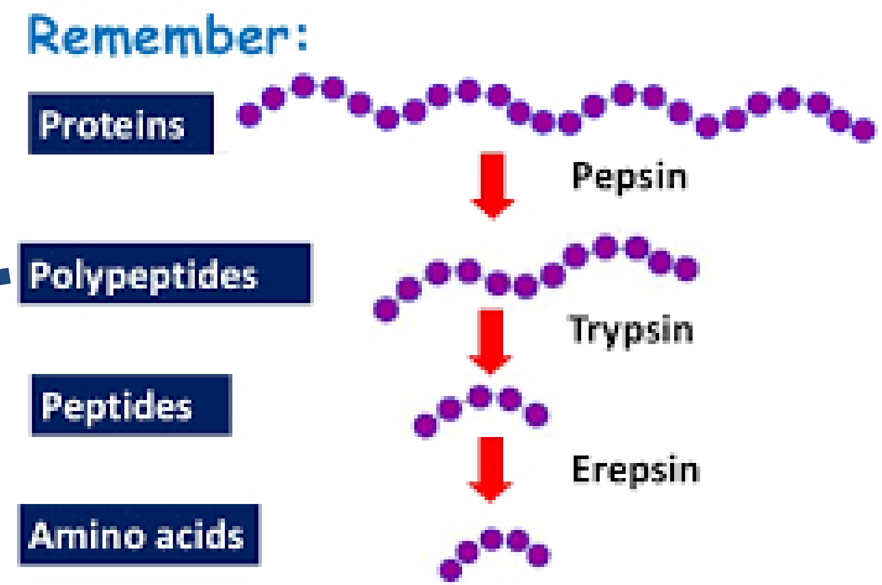
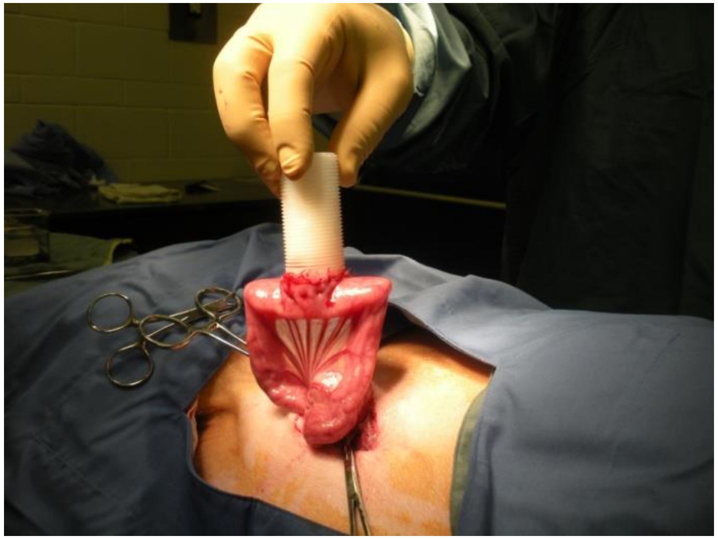
	345	89.2	88.1	79	4.00	2.18	.23

Increasing pepsin digestibility

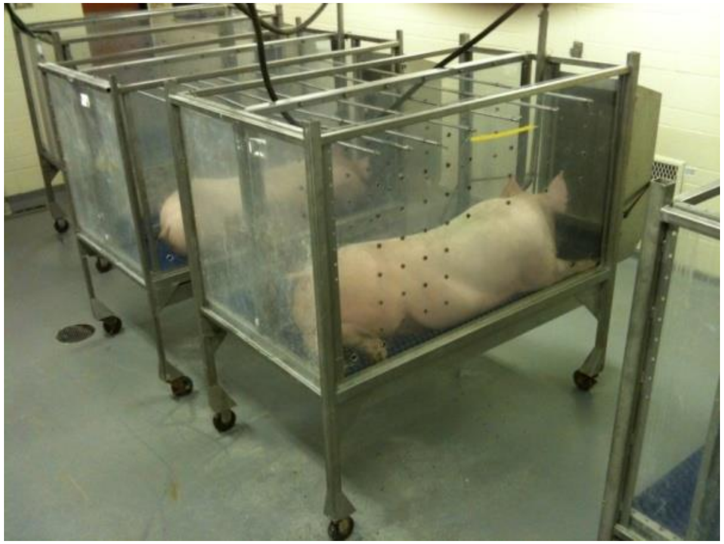
Increasing lanthionine


HCl Acid
Pepsin
Pepsin +
HCl Acid
SCIENCEPHOTOLIBRARY

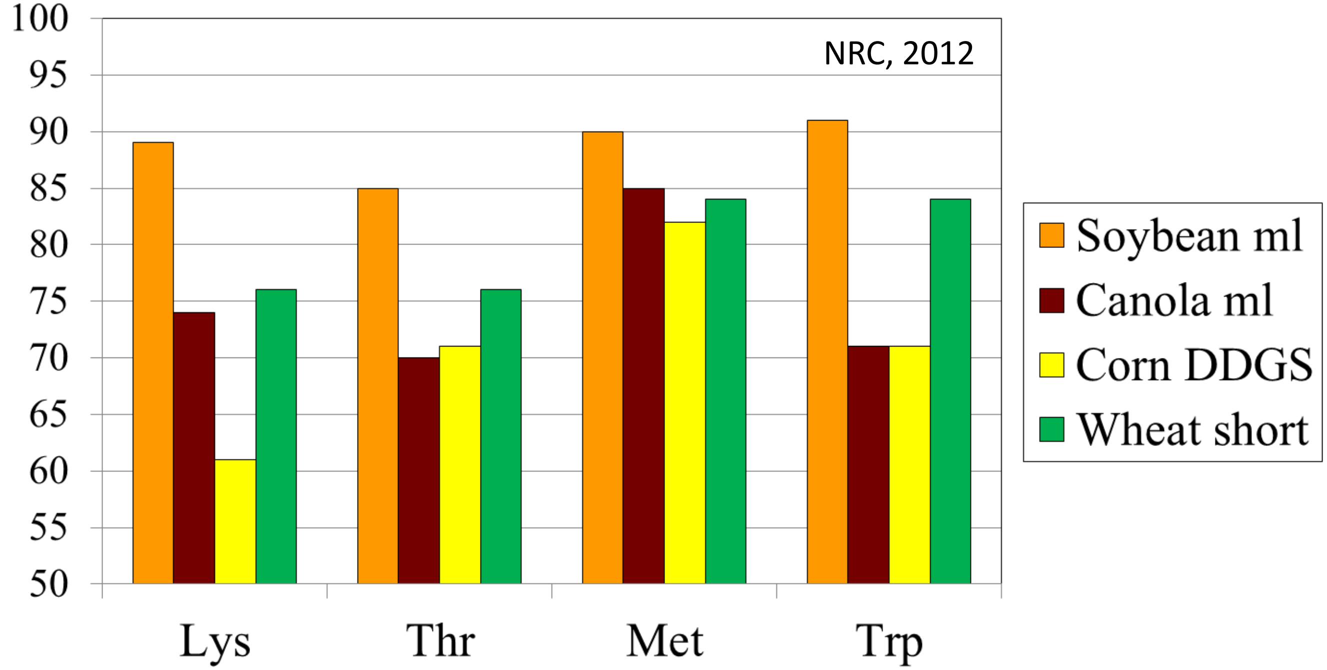

Remember:
Proteins
Pepsin
Polypeptides
Trypsin
Peptides
Erepsin
Amino acids



Univ. of Guelph Animal metabolism facilities



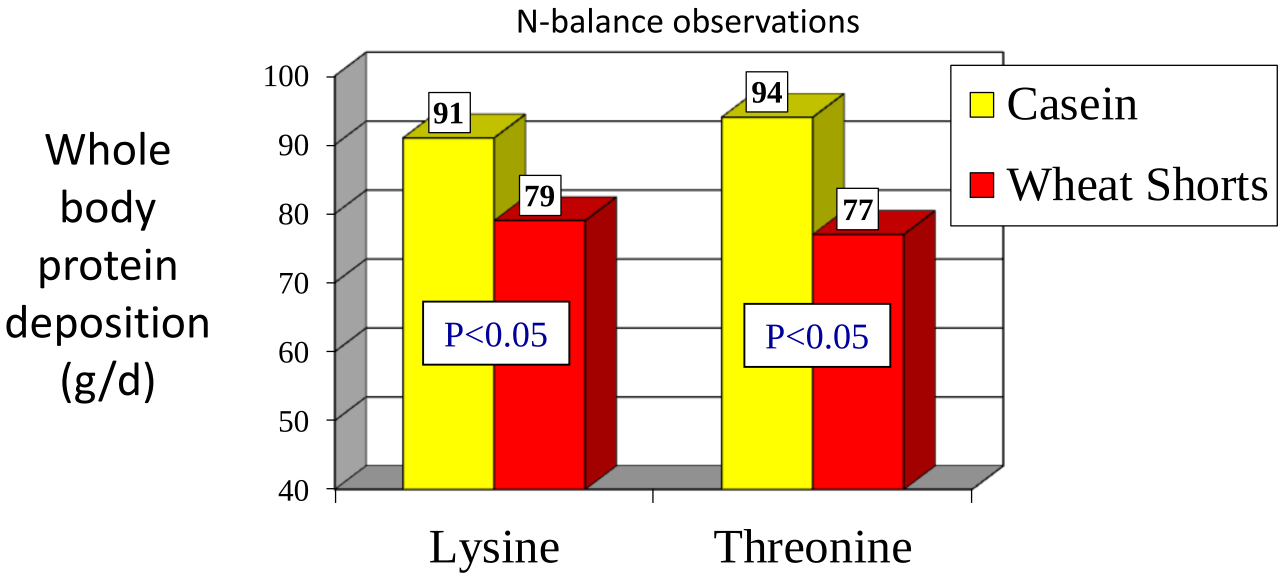
Standardized ileal digestibility (%) of key Amino Acids in Swine



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

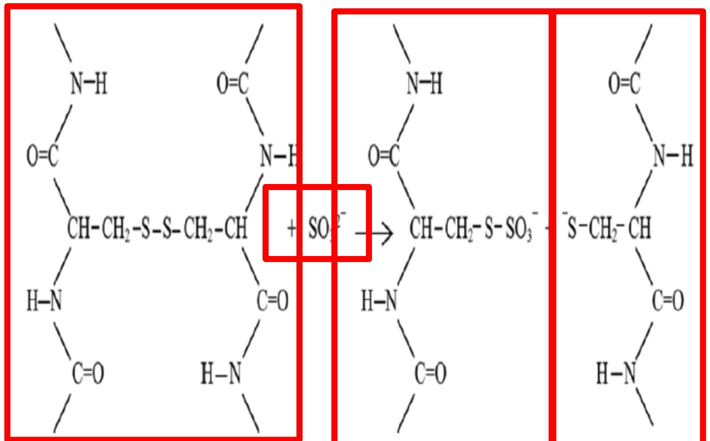
Take Home Message

- 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

Reducing Disulphide Bonds as an Approach to Improving the Digestibility and Bioavailability of Amino Acids in Commercial Feather Meals

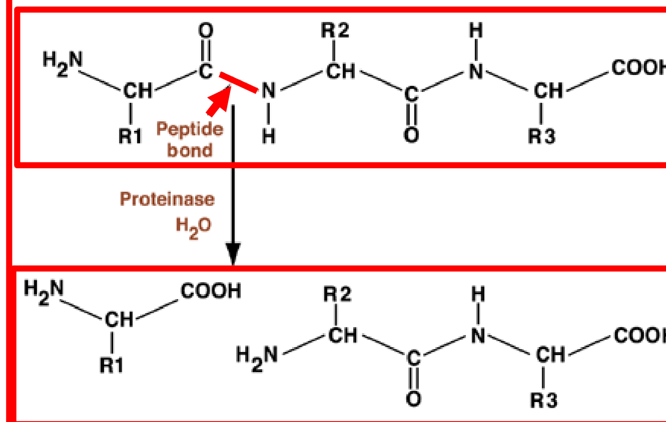
Pre-Treatment of Steam-hydrolyzed Feather Meals to Disrupt Residual Disulfide Bonds

1- Sulfitolysis using sodium sulfite (Na_2SO_3)



Cystine + Sulfite → Bunte Salt + Cysteine

2- Proteolysis using a commercial protease

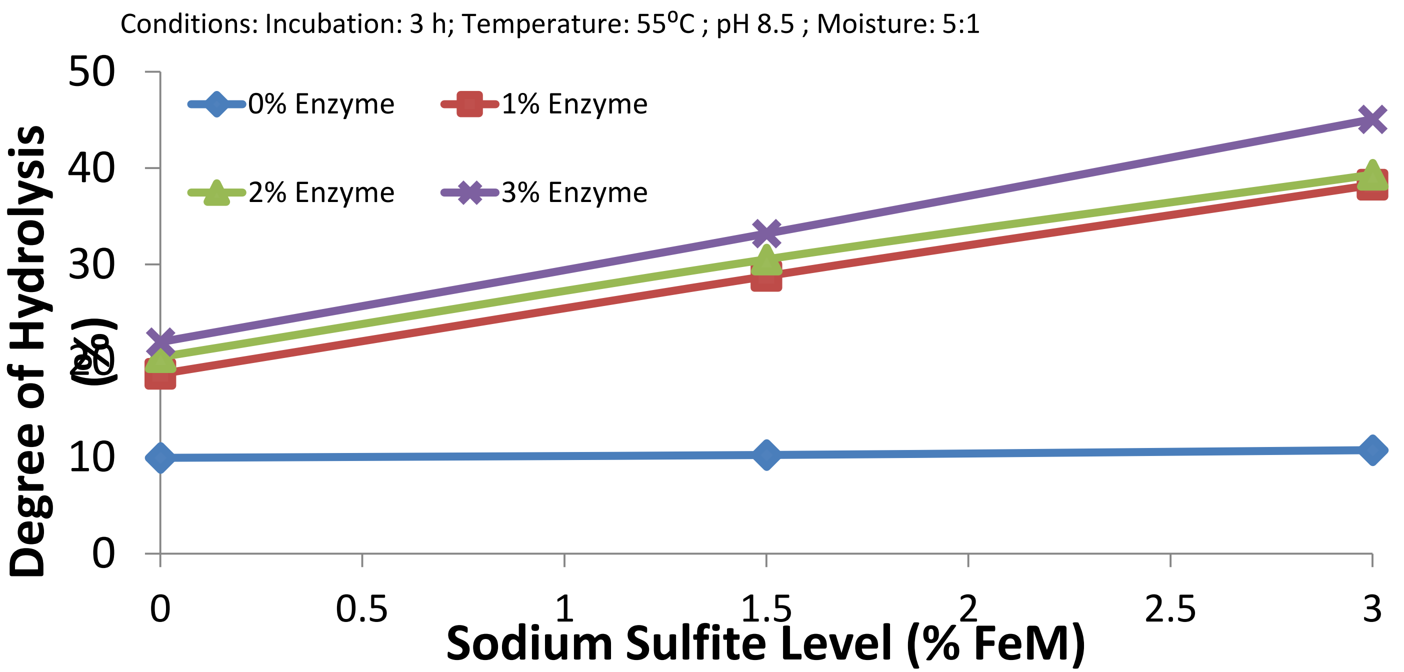


Development of a pre-treatment method for feather meal

Independent variables and their levels used in general factorial design

Independent Variables	Levels			
X1= Enzyme level (%FeM)	0	1	2	3
X2= Chemical Agent Level (%FeM)	0	1.5	3	-
X3= Water:FeM ratio	2:1	3.5:1	5:1	-

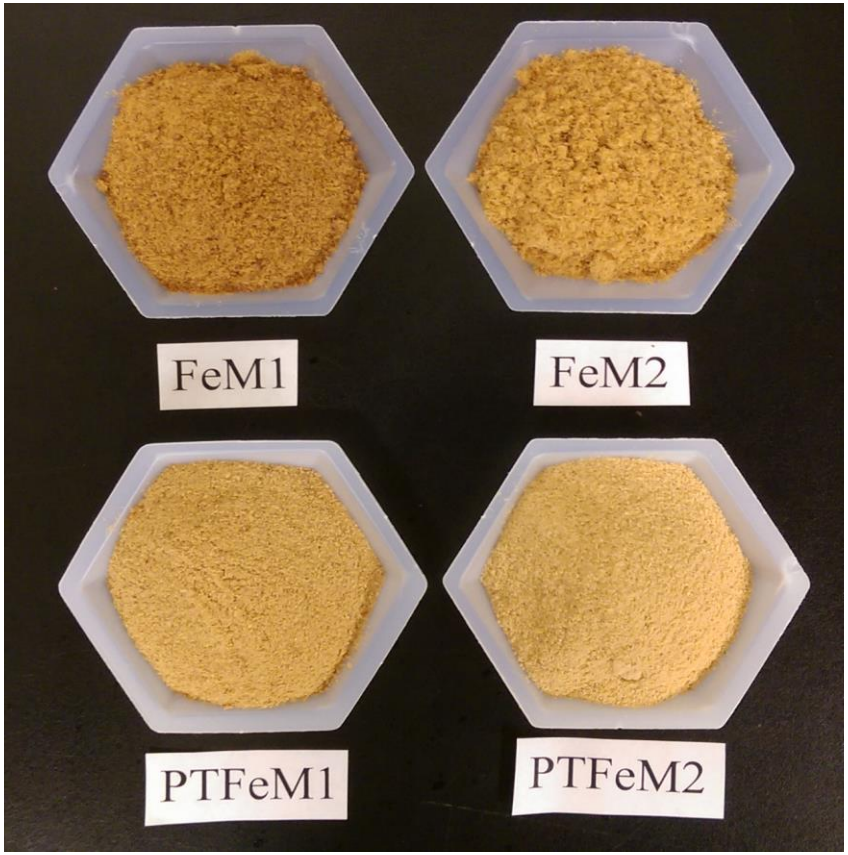
Effect of reducing agent and enzyme level on the degree of hydrolysis of feather meal



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

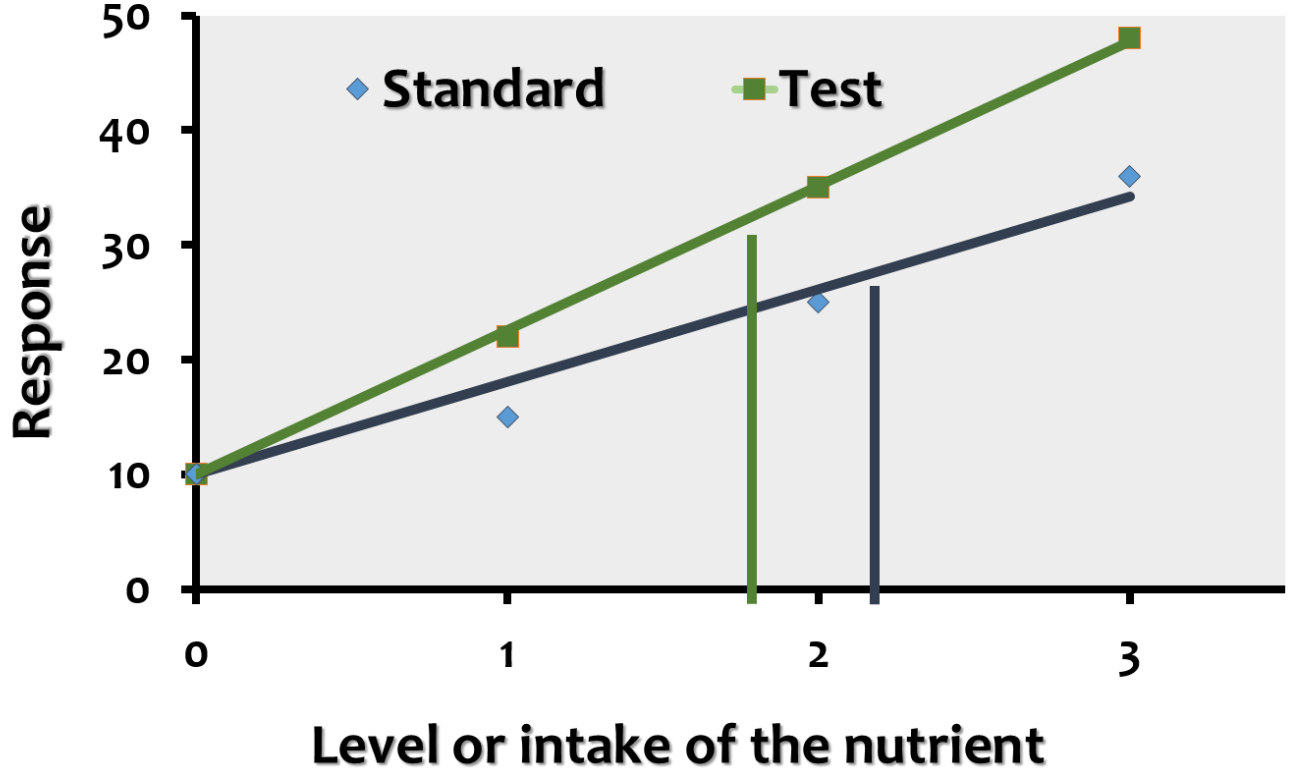


Treatment Significantly Improved Digestibility of Protein and Amino Acids

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}

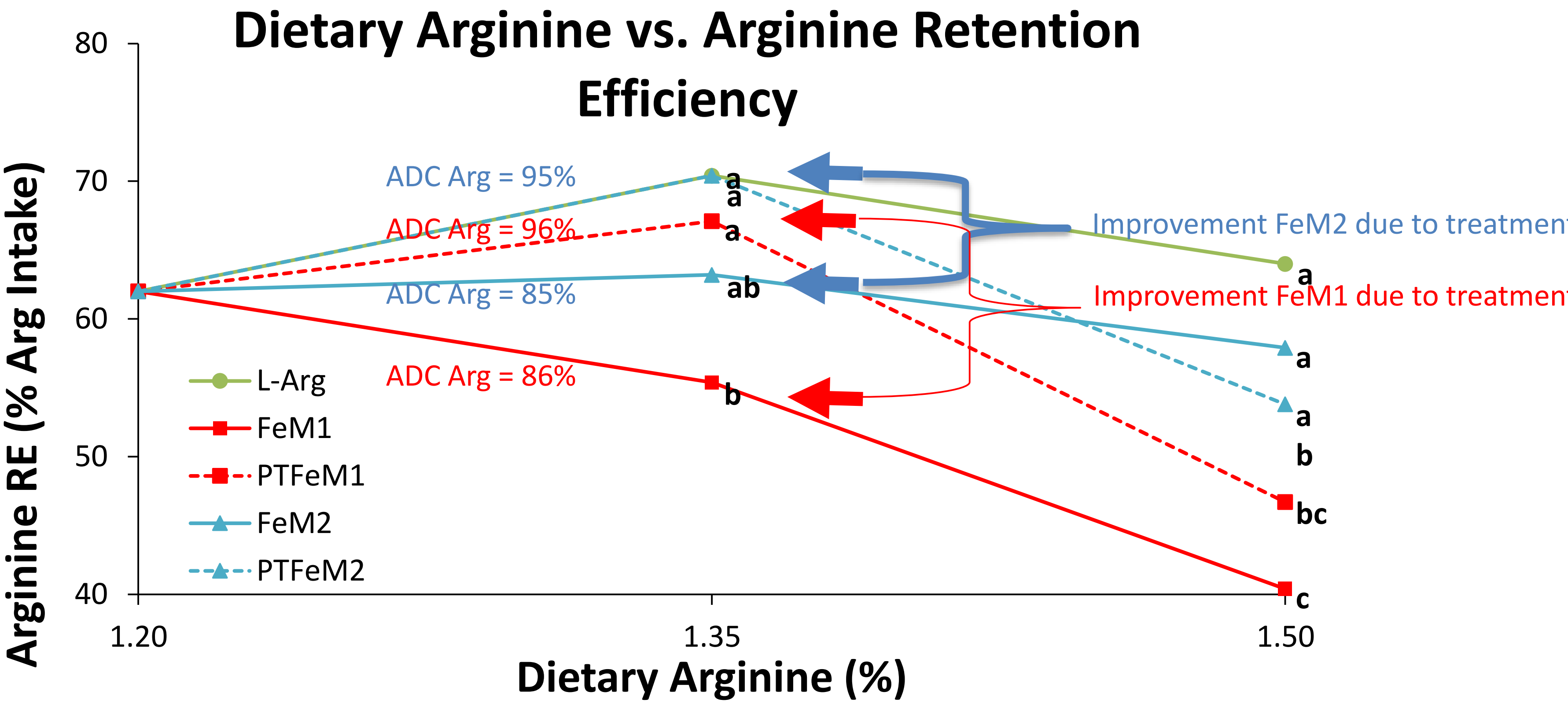
What About Bioavailability of Amino Acids?



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