Nutrition and Feeding of Fish

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Nutritional issues in aquaculture

- **Sustainability**
  - Finding alternatives to fishmeal and fish oil
  - Increasing diet efficiency while reducing nutrient levels in farm effluents
- **Fish health**
  - GI tract health, immune function, general wellness
- **Consumer issues**
  - Safety and quality (omega-3 levels) of farmed fish
- **Conservation issues**
  - Post-release survival of hatchery smolts
- **Economics of production**

Goals of production dictate feeds and feeding

- For commercial aquaculture, the goal is to rear fish for consumption and make a profit
  - Rapid and economical growth
  - Low economic feed conversion ratios
    - Cost of feed to rear a kg of fish (different than the cost of feed)
    - Most of the cost is in the grow-out phase
    - Therefore, feed formulations must avoid expensive ingredients unless the cost to include such an ingredient is justified by increased performance
- For fisheries enhancement, the goal is to produce fish that survive after release and return to the fishery
  - For salmon, this means healthy and robust smolts
    - This is difficult to assess
  - Cost of feed is secondary to smolt quality

Nutritional considerations

- Feeds: must be nutritionally complete, easy to manufacture, ship and store, and palatable to the fish
- Feeding practices: must match the goal of production
  - Economical growth for food fish
  - Targeted final weight for hatchery smolts
  - Can be ad libitum, percent per day, programmed, etc.
- The best or most expensive feed in the world is not going to be effective if it is not fed properly
- There is always pressure in agencies to purchase less expensive feed (coming from budget analysts)

Salmon farm feeding system (programmed)

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Trout farm feeding systems

Demand feeders in Idaho trout farm

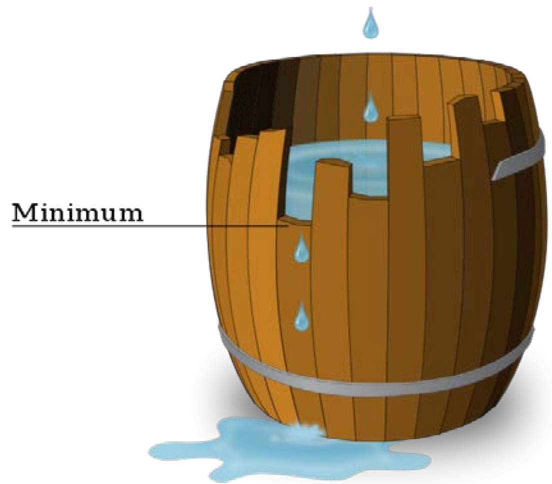
Programmed feeding system at Clear Springs Foods, Idaho

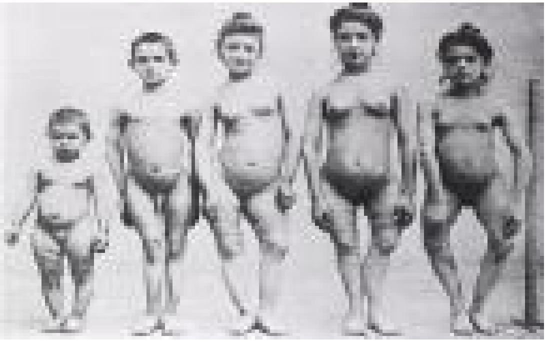
Historical perspective in nutrition

For 100-150 years, how we approach nutrition has been influenced by two concepts

1. Law of the minimum

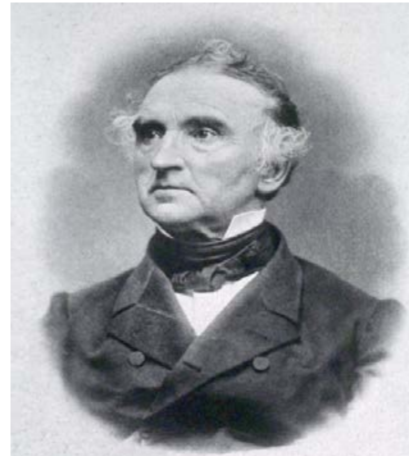
2. One nutrient = one disease (example is rickets)



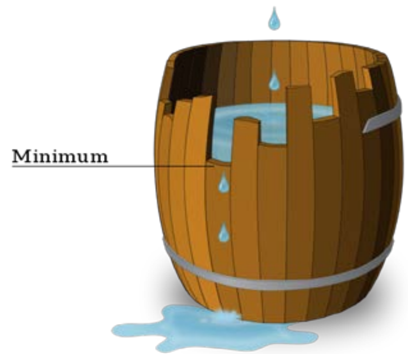


Law of the minimum – Leibig's barrel

- **Justus Von Leibig, prominent 19th century German chemist, discovered that plant growth rates are determined by the minimum level of an essential nutrient**



- **He used the height of staves on a barrel to illustrate this concept**
  - Each stave represents level of an essential nutrient



- **This led to the nutritional concepts of a minimum dietary requirement (humans) and first limiting nutrient, such as lysine, in an animal feed**
  - Level of limiting nutrient determines growth rate

Law of the minimum and fish nutrition

- Fishmeal has an ideal balance of amino acids for fish
- Plant proteins have amino acid profiles that match less well with fish amino acid requirements
  - Maize (corn) protein is low in lysine
  - Soy protein low in methionine
- Amino acid deficiencies in fish feeds are corrected by adding crystalline amino acids
- Nobody worries about levels of other amino acids that are present above minimum required levels

Increase methionine to here

One nutrient – one disease: Rickets

- Rickets (osteomalacia) is a disease associated with soft bones causing deformities of the femur, head, etc.
- It was described by Galen in ancient Rome, prevalent throughout history but known as the 'English disease' from the mid-17th century onward
- Major cause of childhood mortality
- Treatments included reducing miasma (malignant properties of air)
  - Cleanliness, good ventilation, outdoor air and <u>sunlight</u> (Florence Nightingale)

Rickets and short latency, nutritional diseases

- Cod liver oil was demonstrated to prevent/cure rickets in humans early in 20th century
- Vitamin D was shown using animal models to be the active constituent in cod liver oil (1920s)
- Key point is the concept of 'single nutrient – single disease' which depended on…
  - Short latency to develop clinical signs of deficiency
  - Single disease or clinical condition associated with dietary deficiency
  - Clinical condition could be cured at a certain dietary intake level
- This level became the dietary requirement
- Rickets model reinforced with other nutrients, such as niacin (pellagra), thiamin (beriberi), and ascorbic acid (scurvy, scoliosis)

These nutritional concepts ruled

- **Minimum dietary intake should be at the dietary level needed to prevent clinical deficiency signs**
  - Combining Leibig's barrel with the single nutrient – single disease model
- **New response variables were developed but they were simply refinements of absence of deficiency signs, weight gain or feed efficiency metrics, for example**
  - Enzyme activity when a specific vitamin or mineral was an essential co-factor
  - Tissue nutrient levels above minimum threshold associated with clinical deficiency, such as whole body or vertebral phosphorus level

Considerations for feeding fish

- Species and natural diet
  – Carnivores compared to omnivores
- Production system
  – Raceways, tanks, pond, marine cages
- Purpose
  – Food fish
  – Fish for restocking or fisheries enhancement
- Economics

Salmon or trout anatomy diagram

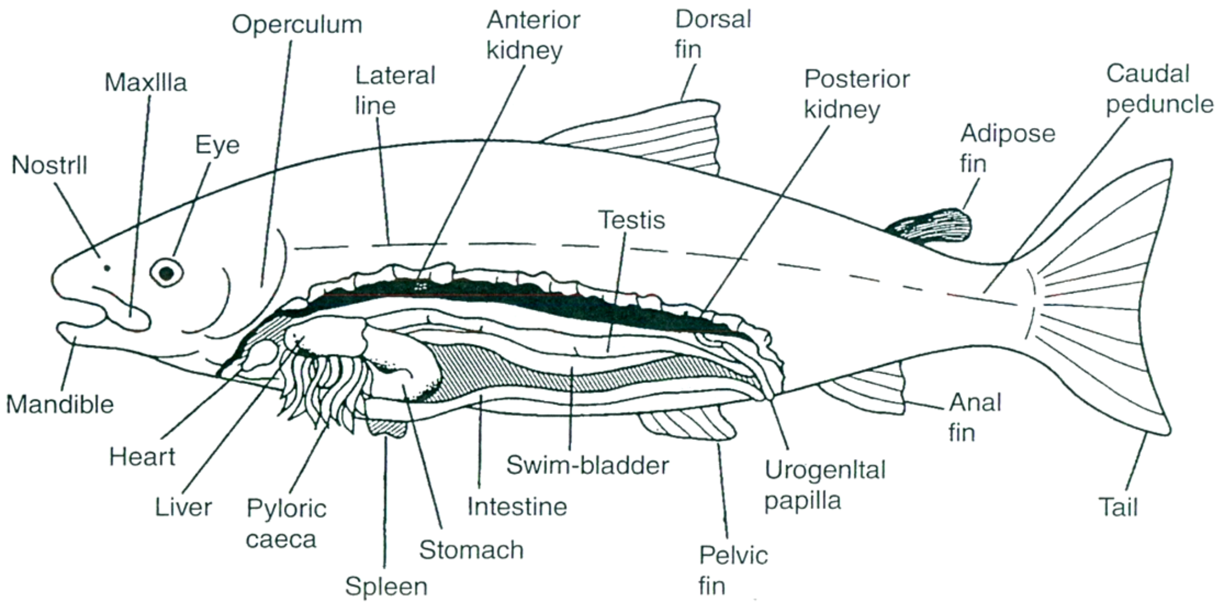


Figure 2.7 Diagram of the basic anatomy of a salmonid fish.

GI tract of salmon, a carnivore

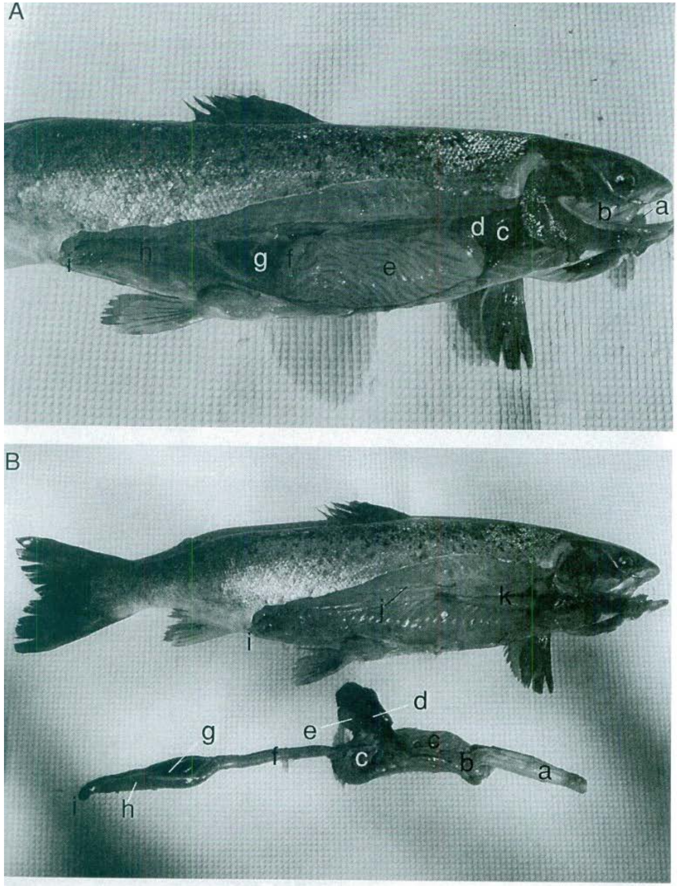


FIG. 7.4

(A) Dissection of an Atlantic salmon. (a) Oral cavity, (b) pharynx, (c) liver, (d) gallbladder, (e) pyloric ceca, (f) upper or small intestine, (g) spleen, (h) lower or large intestine, and (i) anus. (B) Digestive tract removed. (a) Esophagus, (b) stomach near the esophagal sphincter, (c) pyloric ceca, (d) liver, (e) gallbladder; (f) upper or small intestine, (g) spleen, (h) lower or large intestine, (i) anus, (j) kidney, and (k) ovaries. Photographs by Michael Rust.

GI tract of common carp, a stomach-less fish

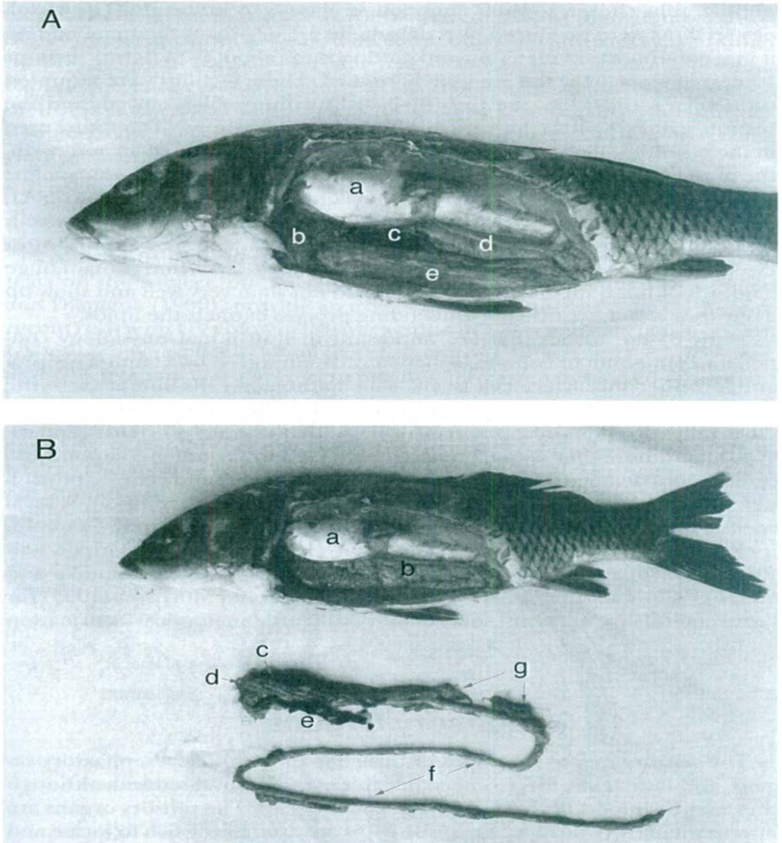


FIG. 7.8

(A) Dissection of the common carp. (a) Swimbladder, (b) liver, (c) spleen, (d) ovary, and (e) coiled gut. (B) Digestive tract removed. (a) Swimbladder, (b) ovary, (c) liver (under), (d) esophagus, (e) spleen, (f) intestine, and (g) pancreatic tissue (around the gut in several locations). Photographs by Michael Rust.

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GI tract of tilapia, an omnivore

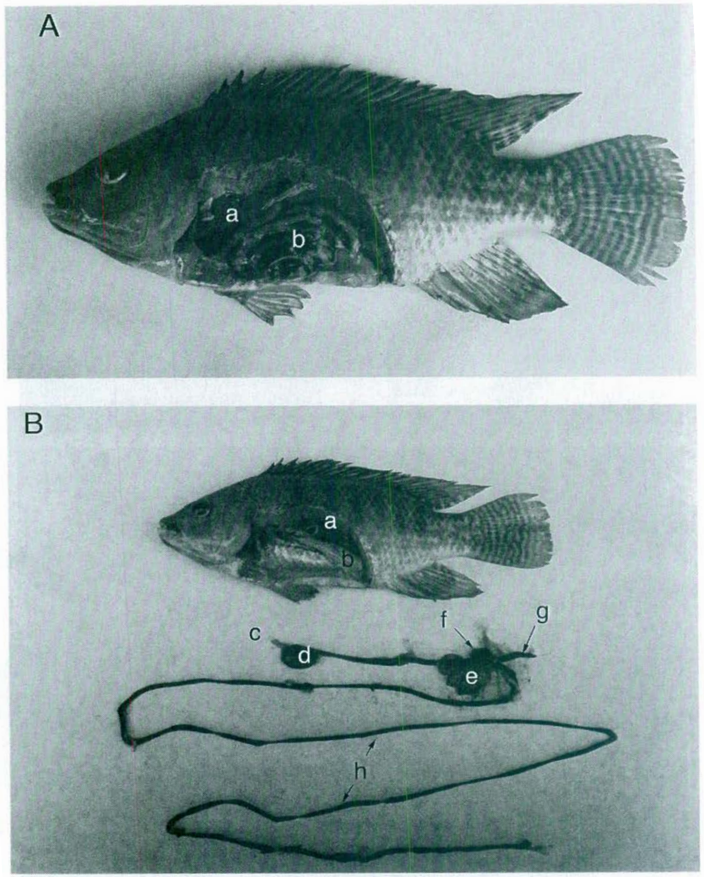

Fig. 7.7

(A) Dissection of a Nile tilapia with the intestinal tract in place. (a) Liver and (b) coiled gut. (B) Dissection of Nile tilapia with the intestinal tract removed. (a) Kidney, (b) ovary, (c) esophagus, (d) stomach, (e) liver, (f) gallbladder, (g) spleen, and (h) intestine. Photographs by Michael Rust.

Aquaculture production systems where fish are fed

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Major aquaculture species in NA

- **Channel catfish (*Ictalurus punctatus*)**
- **Rainbow trout/steelhead (*Oncorhynchus mykiss*)**
- **Pacific salmon**
  - **chinook (*O. tschawytscha)***
  - **coho salmon *(O. kisutch)***
- **Atlantic salmon (*Salmo salar*)**
- **Hybrid striped bass, walleye, yellow perch, Arctic char, tilapia, cobia and others**

Best nutritional data is on salmon and trout

- Salmon and trout nutrition have the longest history of study among fish (farming, stock enhancement)
- Salmon and trout are excellent models for other carnivorous fish species
- Fry and fingerlings of many omnivorous fish species are carnivorous (carp, catfish, tilapia)
- Advances in salmon and trout nutrition have greatly improved production and thus good models for other species

Historical background – Trout

In 1924, Embody & Gordon, from Cornell University got funded to go on a fishing trip from NY to MN to examine the stomach contents of trout

Proximate composition of natural diet of trout

Component	Percent in diet (dry weight basis)
Crude protein	49 (no adjustment for chitin)
Fat	15-16
Crude fiber	8
Ash	10

Pioneering fish nutrition research

- Development of semi-purified diet (1953)
- Establishment of quantitative dietary requirements of vitamins & amino acids (1960s)
  - USFWS Western Fish Nutrition Laboratory
    - John Halver & colleagues
    - Pacific salmon were focus, hatchery support
    - all work was conducted with fry & fingerlings

Semi-purified diet for salmonids

Ingredient	Percent in diet
Vitamin-free casein	40.0
Gelatin	8.0
Dextrin	10.0
Wheat starch	10.0
Carboxymethylcellulose	1.3
Alpha-cellulose	6.0
Mineral mixture	4.0
Vitamin mixture	3.0
Amino acid mixture	2.0
Choline chloride (70% liquid)	0.3
Herring oil	17.0

Proximate category	Percent
Moisture	28-30
Crude protein	34
Fat	17
Ash	5

Determining nutrient requirements in fish

- Feed semi-purified diet, adding back graded levels of single essential nutrient
- measure response variables
  - growth, feed conversion ratio, survival (1950's)
  - tissue nutrient levels, assuming that they plateau at requirement level (1950's through today)
  - measure activity of enzymes that require essential nutrient as co-factor (same assumption, 1980's)
  - measure excretion of nutrient or metabolites (1990's)
  - Nutrigenomics (study of effects of nutrients on gene expression and single gene products in tissues)

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Nutrient requirements of salmonids

- **Protein** — **Ten essential amino acids**
- **Lipids** — **Omega-3 fatty acids (1% of diet)**
- **Energy** — **Supplied mainly from lipids and protein**
- **Vitamins** — **15 essential vitamins**
- **Minerals** — **10 minerals shown to be essential**
- **Carotenoid pigments** — **Needed for viable eggs**
- **NOTE: Other minerals are probably essential but can be obtained from rearing water**

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Protein levels in salmon feeds

Stage of feeding	Dietary protein (%)
Fry	48-52
Juvenile (to smolt)	44-48
Post-juvenile (grow-out)	38-44
Maturing salmon	45

Freshwater Fish Requirements, AA

	Atlantic Salmon	Common Carp	Rohu	Tilapia	Channel Catfish	Rainbow Trout	Pacific Salmon
Arginine	1.8	1.7	1.7	1.2	1.2	1.5	2.2
Histidine	0.8	0.5	0.9	1.0	0.6	0.8	0.7
Isoleucine	1.1	1.0	1.0	1.0	0.8	1.1	1.0
Leucine	1.5	1.4	1.5	1.9	1.3	1.5	1.6
Lysine	2.4	2.2	2.3	1.6	1.6	2.4	2.2
Methionine	0.7	0.7	0.7	0.7	0.6	0.7	0.7
Methionine+ cystine	1.1	1.0	1.0	1.0	0.9	1.1	1.1
Phenylalanine	0.9	1.3	0.9	1.1	0.7	0.9	0.9
Threonine	1.1	1.5	1.6	1.6	1.6	1.8	1.8
Tryptophan	0.3	0.3	0.4	0.3	0.2	0.3	0.3
Valine	1.2	1.4	1.5	1.5	0.8	1.2	1.2

The chart is complete but some of the values are based on studies conducted 50 years ago and most are with fingerling fish. The values shown are recommendations for normal growth under normal conditions and assume 100% availability.

Marine Fish Requirements, AA

	Yellowtail	Red Drum	European Sea Bass	Japanese Flounder	Grouper	Asian Sea Bass	Cobia
Arginine	1.6	1.8	1.8	2.0		1.8	
Histidine							
Isoleucine							
Leucine							
Lysine	1.9	1.7	2.2	2.6	2.8	2.1	2.3
Methionine	0.8	0.8		0.9		0.8	0.8
Methionine+ cystine	1.2	1.2	1.0			1.2	1.1
Phenylalanine							
Threonine		0.8	1.2				
Tryptophan			0.3				
Valine							
Taurine	R	R	0.2	R	R	R	R

The chart shows many voids, but does include levels for most of the critical limiting amino acids. The values shown are recommendations for normal growth under normal conditions and assume 100% availability

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Essential fatty acid requirements

- Pioneering research in 1970s at Oregon State University by Castell and Sinnhuber
  - trout needed 1% of diet as omega-3 fatty acids
  - these could be EPA (20:5) or DHA (22:6)
- Reported clinical signs of essential fatty acid deficiency
  - heart pathology, fainting, poor growth
  - time of onset depended upon previous dietary history
  - requirement confirmed with Pacific salmon

Omega-3 fatty acids in various fish oils (%)

Fatty acid	Herring oil	Menhaden oil	Pollock oil
C20:5n-3	5.5	10.2	13.1
C:22:6n-3	3.9	12.8	6.8
Total n-3	12.4	25.8	25.4
Amount needed in diet to supply 1% omega-3:			
	8%	4%	4%

Primary functions of vitamins in fish

Vitamin	Primary function
Vitamin A	Normal vision
Vitamin D	Calcium metabolism, bone formation
Vitamin E	Cell membrane maintenance
Vitamin K	Blood clotting
Thiamin	Carbohydrate metabolism
Pyridoxine	Amino acid metabolism
Riboflavin, niacin	
Pantothenic acid, biotin	Energy metabolism
Folic acid, Vitamin B12	Synthesis of nucleotides
Ascorbic acid	Collagen synthesis
Choline, inositol	Component of phospholipids

Primary vitamin deficiency signs in fish

Vitamin	Anorexia	Primary deficiency signs
Vitamin A	Yes	Vision problems
Vitamin D	Yes	Impaired bone calcification
Vitamin E	Yes	Anemia, ascites, membrane fragility
Vitamin K	No	Anemia, prolonged prothrombin time
Thiamin	Yes	Hyperirritability, convulsions
Riboflavin	Yes	Lens cataracts
Pyridoxine	Yes	Convulsions, erratic swimming
Pantothenic acid	Yes	Clubbed gills
Niacin	Yes	Skin lesions
Biotin	Yes	Muscle atrophy
Folic acid	Yes	Macrocytic anemia
Vitamin B12	No	Anemia
Inositol	Yes	
Choline	Yes	
Ascorbic acid	Yes	Lordosis, scoliosis, hemorrages

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Vitamin requirements of salmon and trout

(IU or mg/kg dry diet)

Vitamin	Salmon	Trout
Vitamin A	2500	2500
Vitamin D	2400	2400
Vitamin E	50	50
Vitamin K	unknown	unknown
Thiamin	1	unknown
Riboflavin	7	4
Pyridoxine	6	3
Pantothenic acid	20	20
Niacin	unknown	10
Biotin	unknown	0.15
Folic acid	2	1
Vitamin B_{12}	0.01	0.01
Ascorbic acid	50	50
Choline	800	1000
myo-Inositol	300	300

Marine Fish Requirements, ws Vitamins

	Yellowtail	Red Drum	European Sea Bass	Japanese Flounder	Grouper	Asian Sea Bass	Cobia
mg/kg							
Thiamin	11						
Riboflavin	11						
Pyridoxine (B6)	12						
Pantothenic acid	36						
Niacin	12						
Biotin	0.67						
Cyanocobalomine (B12)	0.05						
Folate	1.2						
Choline	1000	600					700
Myoinositol	420				350		
Ascorbic acid (C)	43-52	15	20		18	30	45-54

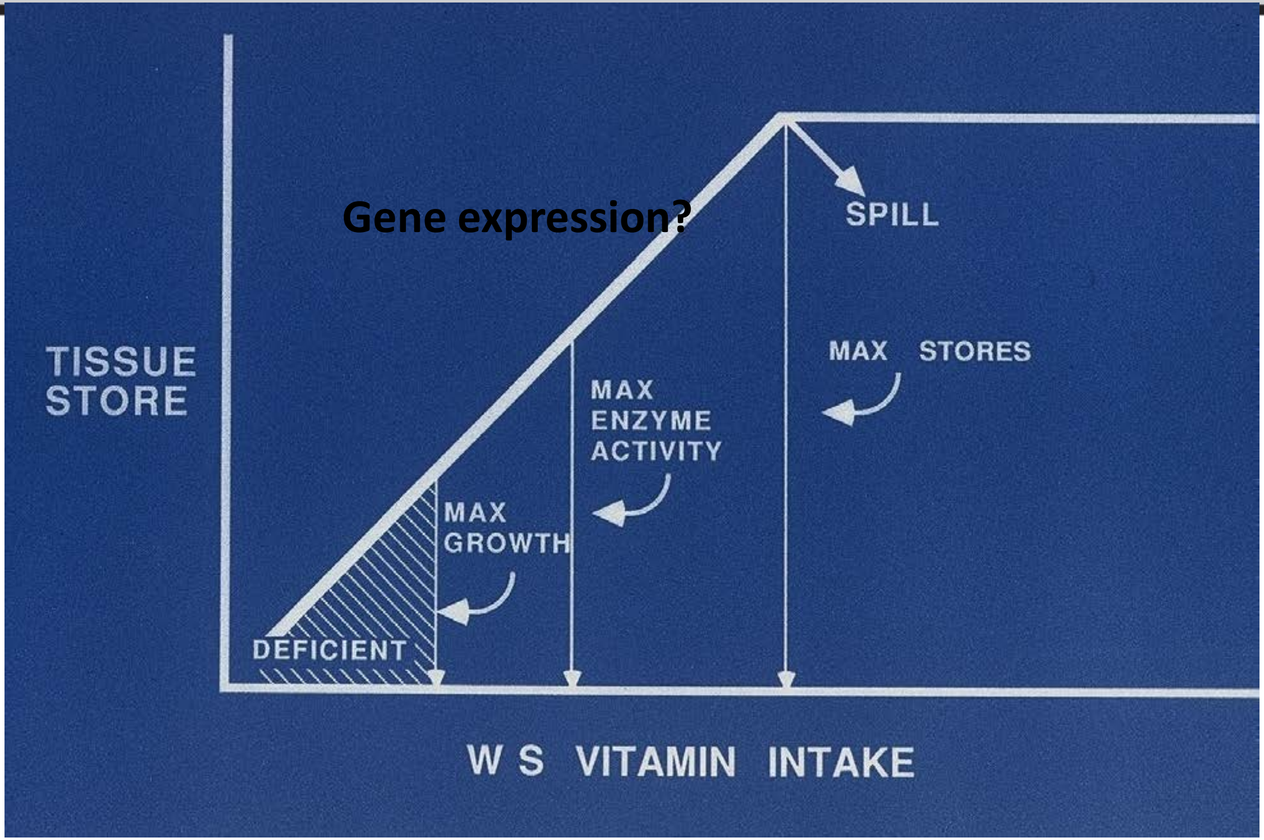
Water soluble vitamin requirements were only available for the yellowtail. Again, vast areas for new research needs are apparent. Values are for fish reared in laboratory settings, not in commercial aquaculture.

Marine Fish Requirements, FS Vitamins

	Yellowtail	Red Drum	European Sea Bass	Japanese Flounder	Grouper	Asian Sea Bass	Cobia
Fat Soluble Vitamins							
A (*IU/kg* diet)	5.6		31	2.7	0.9		
D (*IU/kg* diet)							
E (*mg/kg* diet)	119	31			115		
Lipids							
n-3 PUFA %	2.0-3.9	0.5-1.0	1.0	1.4	1.0		
Phospholipids %			2.0-3.0	7.0			
18:3n-3 %							

Fat-soluble vitamin requirements have been listed for most of these species, and general lipid requirements for the polyunsaturated fatty acids have been studied during the past 10 years

Water soluble vitamins



The apparent requirement can be calculated from maximum growth, maximum enzyme activity, or at maximum tissue stores

Ascorbic acid requirements of salmonids

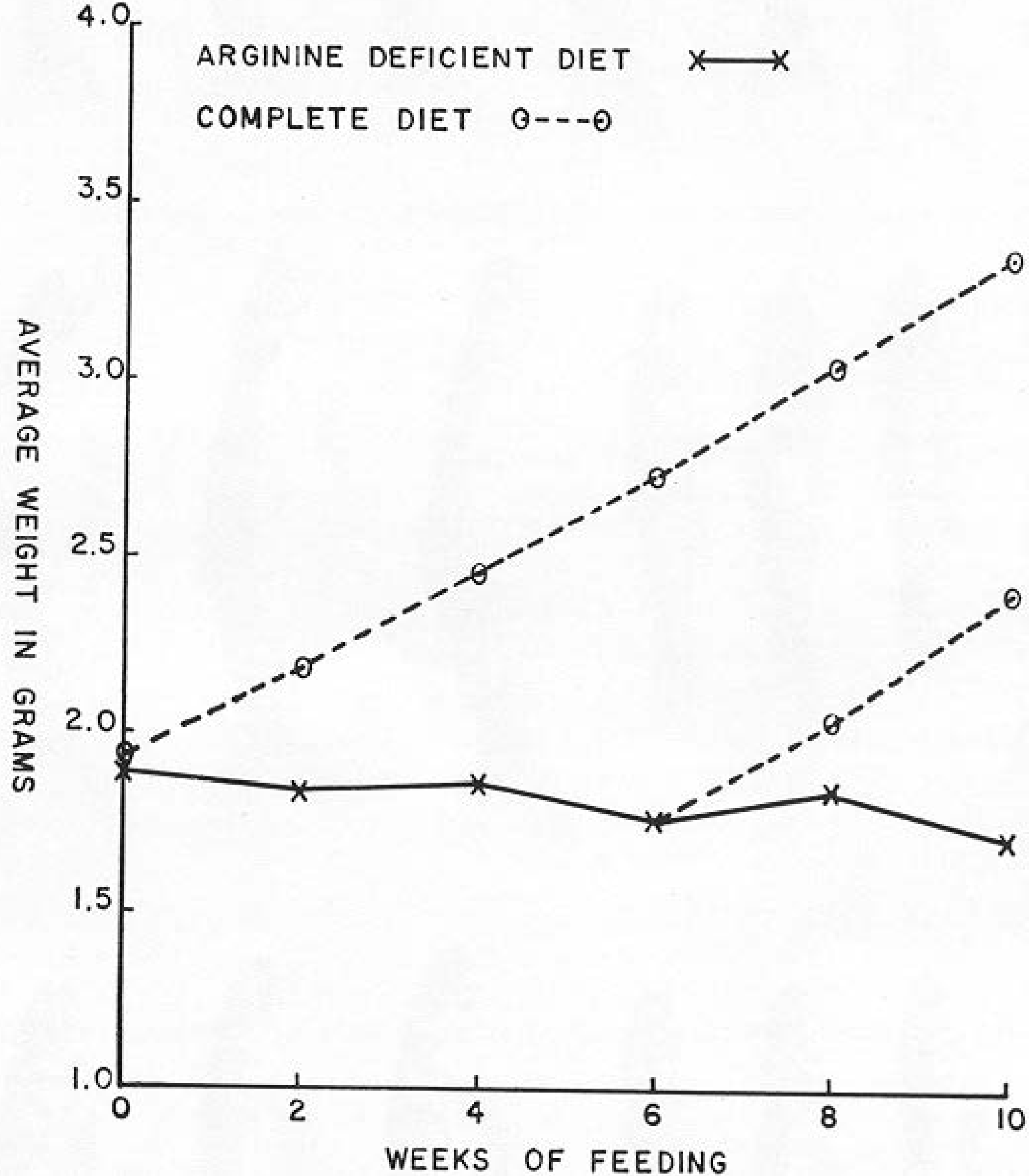
Requirement*	Comments
15-20 ppm	Prevents deficiency signs
250-500 ppm	Supports maximum wound healing activity
1000-2500 ppm	Supports maximum disease resistance in laboratory challenges
>2500 ppm	Maximum tissue storage levels and max. immune response

* When included in purified diet, with ideal conditions and no oxidation of vitamin C

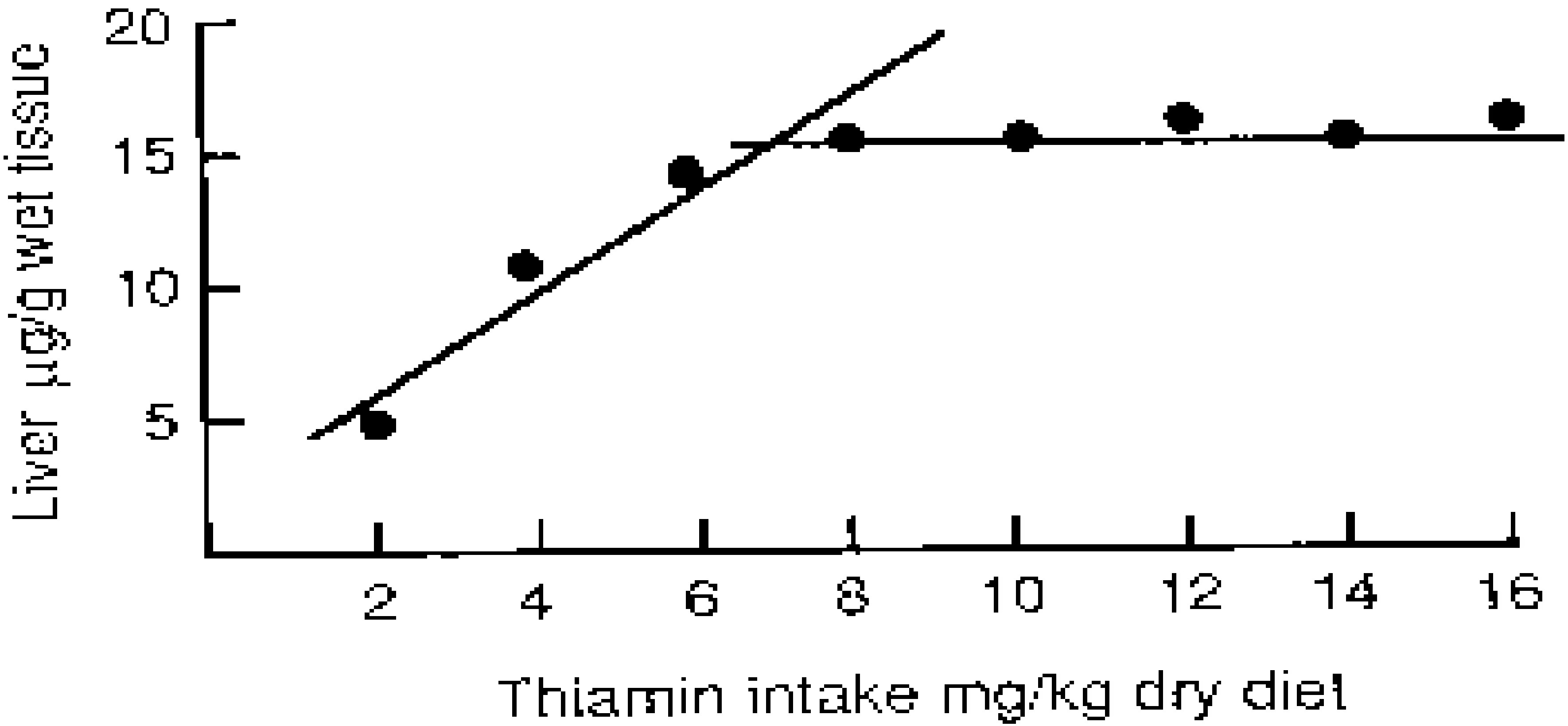
Criteria or method used affects vitamin requirement

- Response variable
  - absence of deficiency sign (minimum level)
  - tissue saturation or plasma level (not very useful)
  - enzyme activity (good for some micro-nutrients)
- Statistical evaluation
  - broken-line (Almquist plot)
  - curve-fitting and models
    - fit curves but are they biologically relevant?
    - do we chose 95% or 100% response as requirement?
- Real-world environmental conditions
  - crowding, water quality, pathogen load etc.

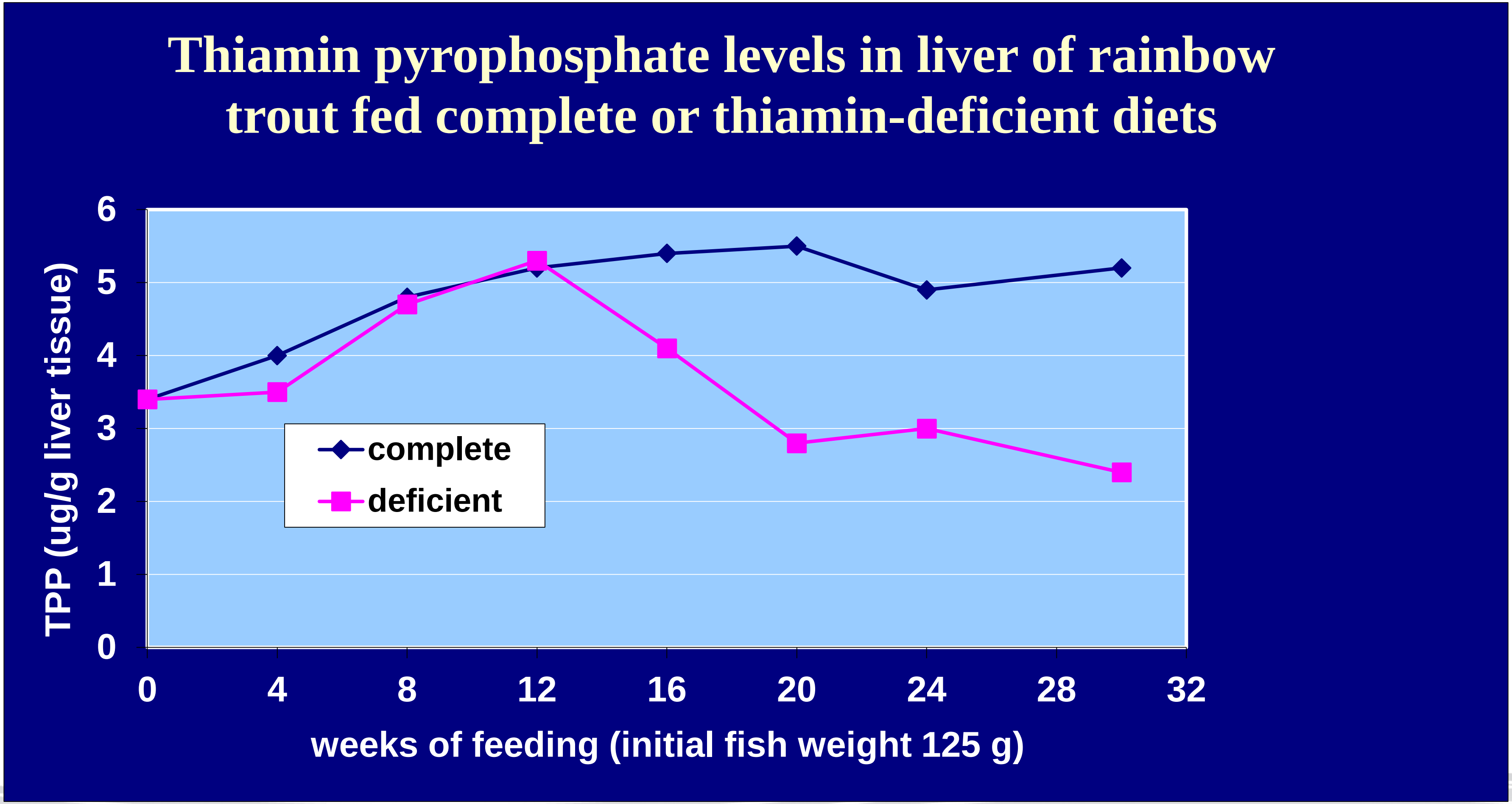
Qualitative dietary arginine requirement (Halver et al., 1960)



Relationship between thiamin intake and liver thiamin concentration



Detecting sub-clinical deficiencies - liver



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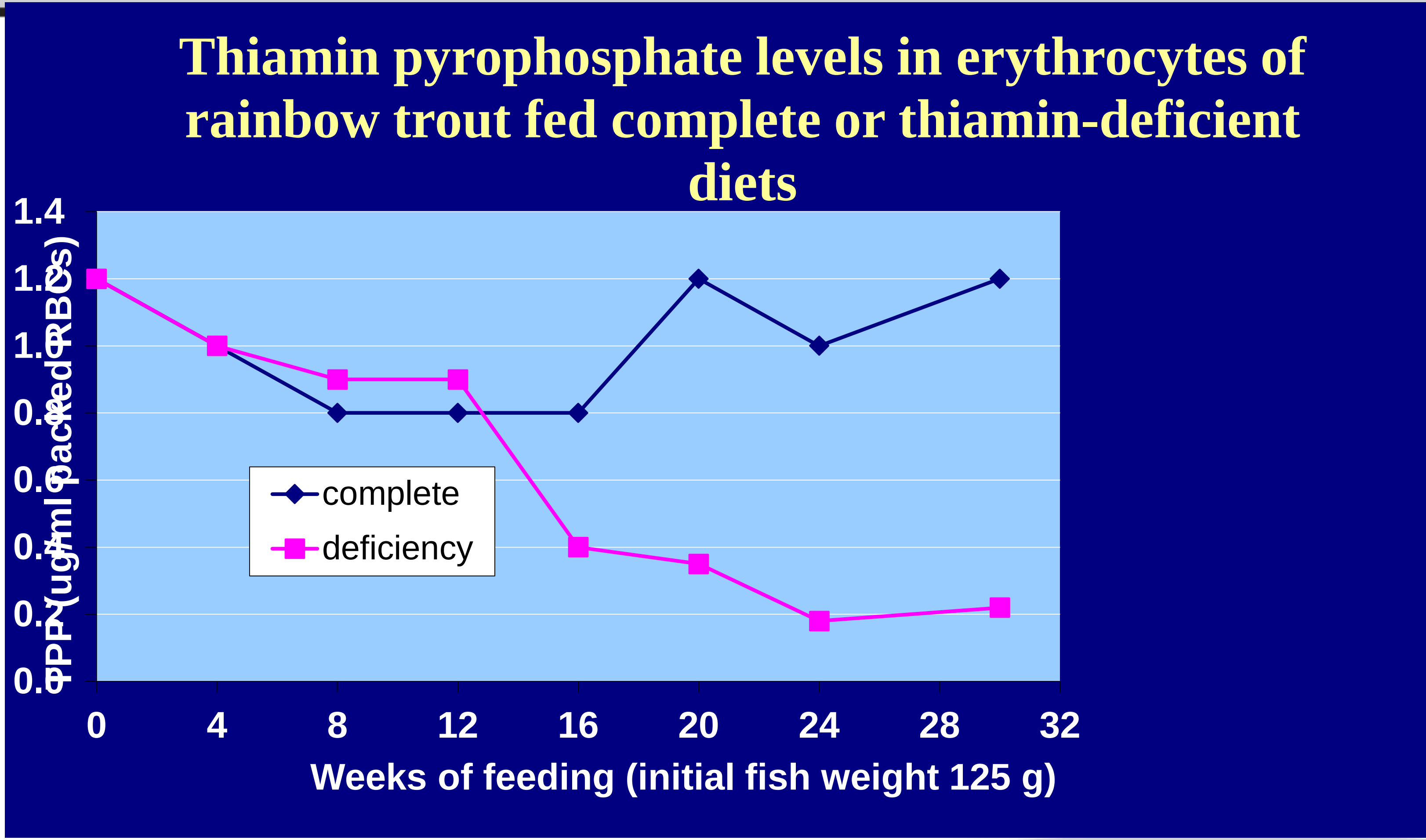
Detecting sub-clinical deficiencies - blood



Minerals essential for life

Macrominerals (g/kg diet)	Microminerals (mg/kg diet) (trace elements)
Calcium	Iron
Phosphorus*	Manganese*
Sodium	Copper
Potassium*	Zinc*
Chlorine	Cobalt
Magnesium*	Selenium*
Sulfur	Iodine*
	Molybdenum

* Required in the diet, but not always supplemented in practical feeds

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Functions of essential minerals

Calcium	Bone, scale, skin, muscle function
Phosphorus	Bone, scale, skin, phospholipids
Magnesium	Bone, scale, skin, muscle function
Sodium & Potassium	Ionic balance (with Cl^-)
Iron	Hemoglobin
Manganese	Cofactor for enzyme activity
Selenium	co-factor for glutathione peroxidase (protection against free radicals)
Zinc	Cofactor for enzyme activity

North American feed manufacturing technology

- **1920's through 1940's**
  - salmon & trout feeds were made at the hatchery
  - locally-available materials were used
  - wet feeds & wet/dry mixtures were used
  - mixtures were formed into clumps or noodles
  - feed made from animal organs, old horses, carp & suckers, plus dry blends (dried milk, yeast, wheat)

Feed manufacturing : Trout in 1950's

- mid 50's: compressed pellets for trout were first produced by Clark Co. in New Mexico, then by Murray Elevators & Rangen
- trout formulations were based upon Cortland formulations: first formulations were 42% CP and 6% fat
- by 1961, all trout farms used compressed pellets; over next 20 years, trout production increased 15x (2 to 30 million lbs.)
- emphasis was on 'cheap' feed

Cortland trout diet formulation

Dry mixture No. 6, 1953

Ingredient	Percent in diet
White fish meal	40.0
Cottonseed meal	15.0
Wheat mids	25.0
Brewers dried yeast	10.0
Dried skim milk	7.0
Fish oil	3.0

Proximate category	Percent
Moisture	9
Crude protein	42
Fat	6
Ash	8

Feed manufacturing : Trout in 1960's

- early 1960's: extruded pellets for trout were first commercially produced by Ralston Purina
- formulations contained nearly 40% wheat by-products (based on catfish diets-high CHO)
- cooking-extrusion increased availability of starch from <10% to 25-26% of diet
- high incidence of fatty liver syndrome, mortality
- trout industry blamed extrusion, for decades refused to use extruded pellets

Feed manufacturing : Trout in 1990's

- Low-polluting feeds become in demand
- Cooking-extrusion pelleting became accepted
  - Fewer fines
  - Lower CHO
  - Higher fat diets (20-24%)
- Trout feed sales:
  - extrusion > pelleting > expansion

Early salmon feed research

- Conducted by USFWS, state fisheries agencies and universities
- Focus was exclusively on fry and fingerlings grown in hatcheries for release (at 4-25 g weight)
- Key issue was fish survival and return to fishery, not economical fish production
- In feed formulations, emphasis was on avoiding deficiencies, even if it meant over fortification of essential nutrients

Feed manufacturing: Salmon in 1960's

- Early 1960's: Oregon Moist Pellet replaced hatchery-made feeds in Pacific states
- OMP eliminated transmission of fish tuberculosis to salmon fry via the feed
- OMP demand led to creation of several new feed companies (Bio-Oregon and Moore-Clark Co.)
- OMP's success led to expansion of salmon enhancement hatchery system to >55 hatcheries

Oregon Moist Pellet formulation

Ingredient	Percent in diet
Fish meal	28.0
Wheat germ meal	remainder
Cottonseed meal	7.0
Poultry byproduct meal	8.0
Dried whey	5.0
Corn distillers dried solubles	4.0
Trace mineral premix	0.1
Vitamin premix	1.5
Choline chloride (70% liquid)	0.5
Ascorbic acid (polyphosphate)	0.5
Fish oil	6.5
Wet fish hydrolysate	30.0

Proximate category	Percent
Moisture	28-30
Crude protein	37
Fat	13
Ash	7

Feed manufacturing: Salmon in 1970's

- Early 1970's: commercial marine net-pen farming of coho salmon to 'pan-size' began
- OMP used to rear the fish
- Logistics (frozen storage) and cost made OMP uneconomical
- Diet formulations based upon USFWS Abernathy diet replaced OMP

Abernathy diet formulation

Ingredient	Percent in diet
herring meal	50
blood meal	10
wheat germ meal	5
poultry BP meal	1.5
whey	5
condensed fish solubles	3
wheat mids	12.3
lignin sulfonate	2
vitamin/mineral premixes	2.2
fish oil	9

Proximate category	Percent
Moisture	8
Crude protein	52
Fat	16
Ash	12

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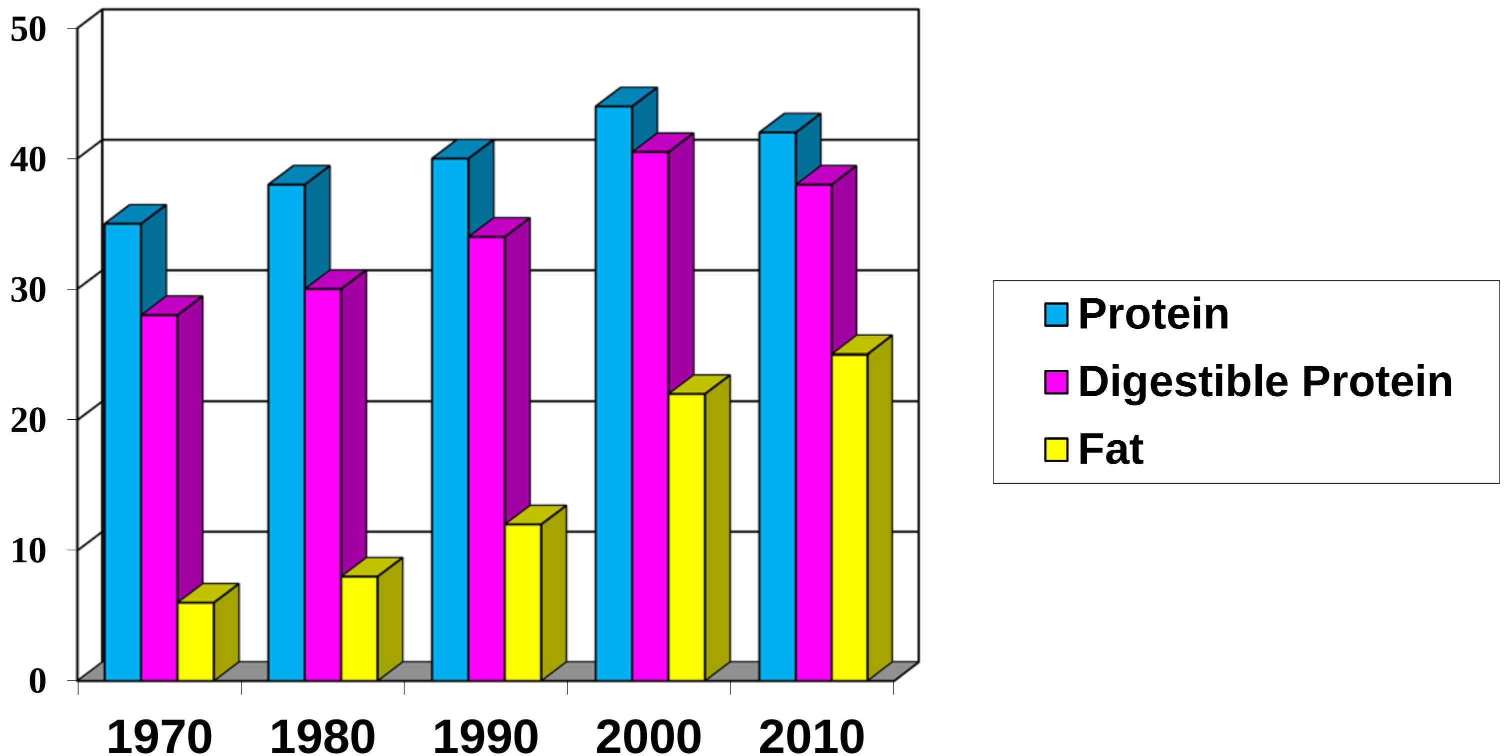
Feed manufacturing: Salmon in 1980's

- **Norwegian & Scottish companies invest in US & Canada**
  - **they demanded extruded pellets in farming operations**
- **Extruded pelleting technology was 're-imported'**
- **Benefits of extrusion pelleting**
  - **Pellet buoyancy can be controlled, so less feed waste from feed falling through nets**
  - **harder pellets, less fines than compressed pellets**
  - **more fish oil can be added by top-dressing**

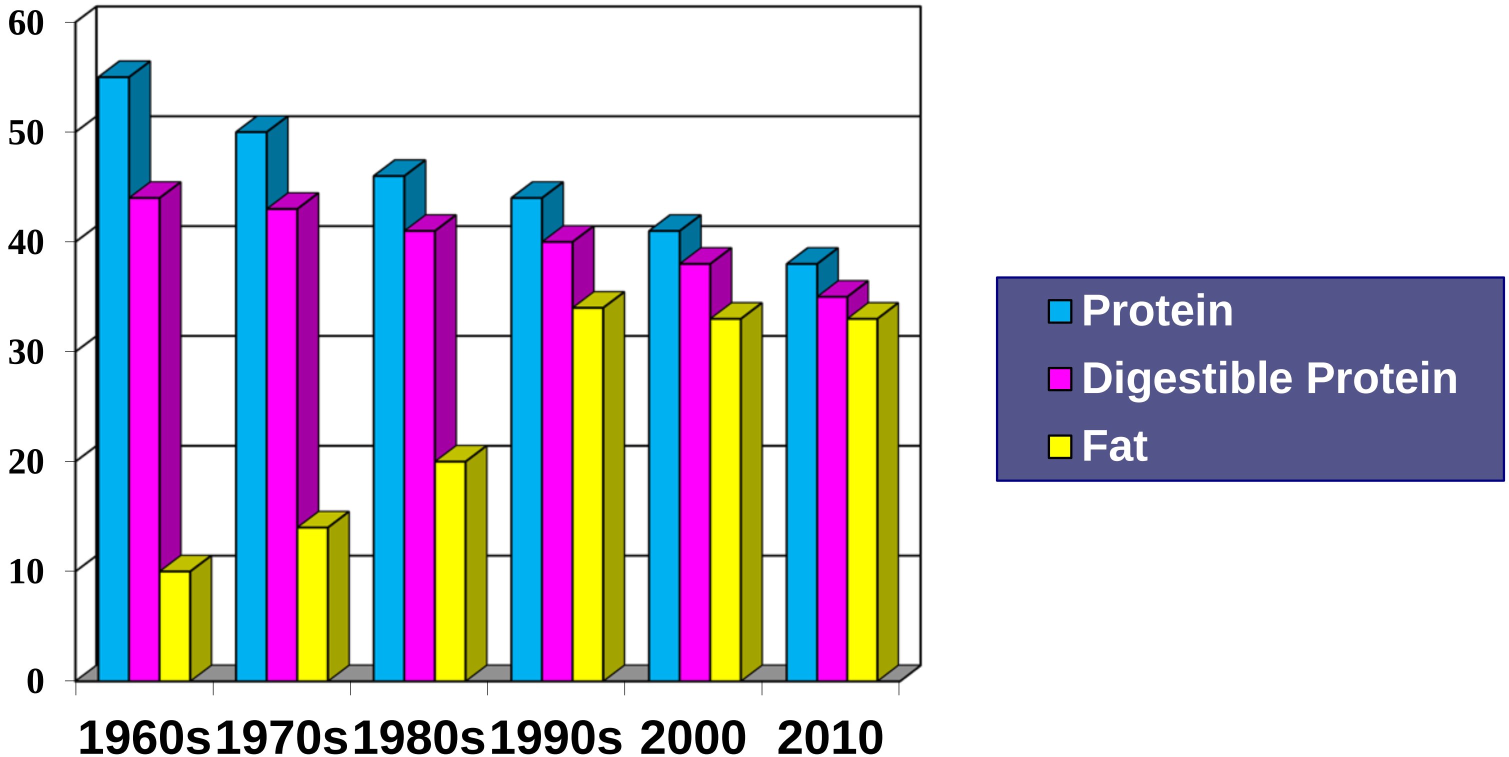
Salmon & trout grow-out feeds

Ingredient (%)	Salmon	Trout
Fish meal	25	15
Soybean products	20	12
Animal by-product meals	0	15
Cereal products	10	25
Gluten products	15	15
Vitamins/ minerals	3	2
Fish/plant oil	25	15
Others	2	1
Crude protein (%)	*44*	*44*
Crude lipid (%)	*35*	*20*

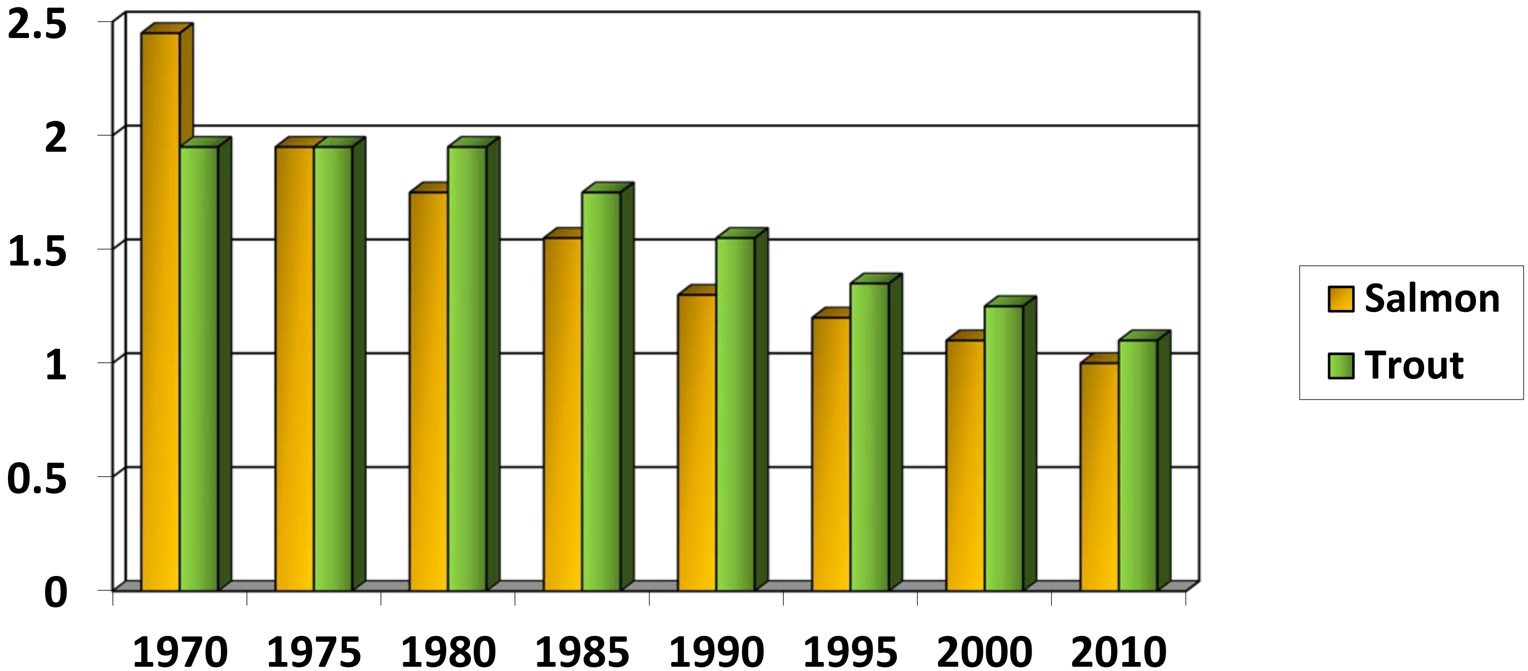
Changes in protein and fat levels in trout feeds



Changes in protein and fat levels in salmon feeds



Changes in FCR for grow-out salmon and trout



Feeds used in aquaculture

- Starter feeds – high protein, highly palatable
- Grower feeds – medium protein, high fat for salmon; lower protein and medium fat for trout
- Broodstock feeds – somatic vs gonadal growth, post-spawning recovery in rainbow trout and Atlantic salmon
- Special feeds
  - Larval fish feeds
  - Product quality (pigmentation, omega-3 fatty acids, frozen storage stability)
  - Transition feeds (used for transitioning wild-sourced fingerlings)

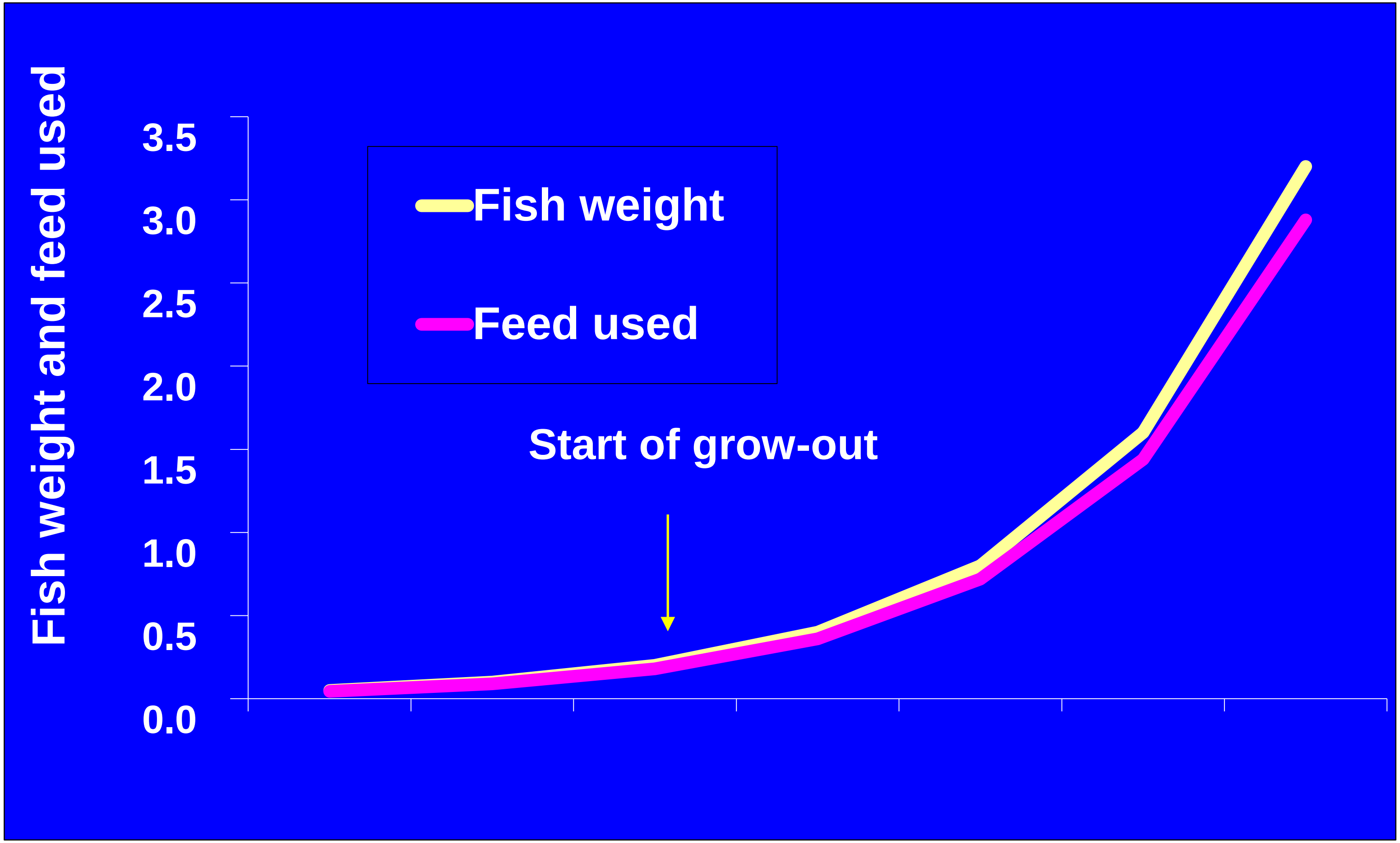
Development of grow-out feeds

- The need for grow-out feeds only emerged in the last 32 years when aquaculture became the dominant form of fish rearing
- Early fish nutrition based upon needs of salmon and trout enhancement hatcheries. In the past 32 years, food fish production has changed the type of research being conducted by fish nutritionists, and placed economics of production at the top
- Hatchery feeds are now made by companies in the commercial aquaculture feed business

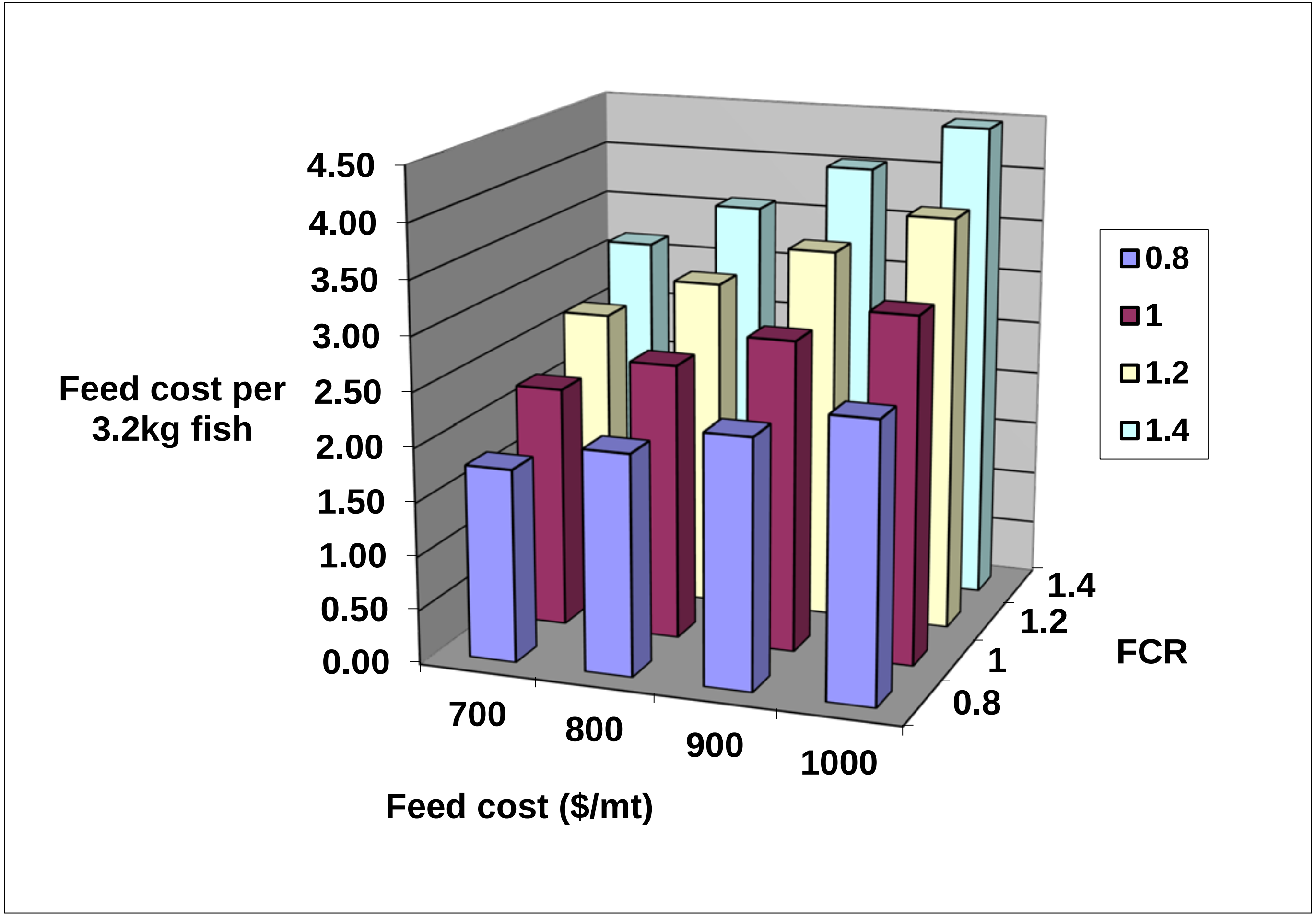
Why are grow-out feeds important?

- At least 90%of the feed used to raise fish to harvest are fed during the grow-out stage
- Feed represents >50% of ex-farm gate cost of producing salmon
- Grow-out feeds have major impact on farm profits and environmental compliance

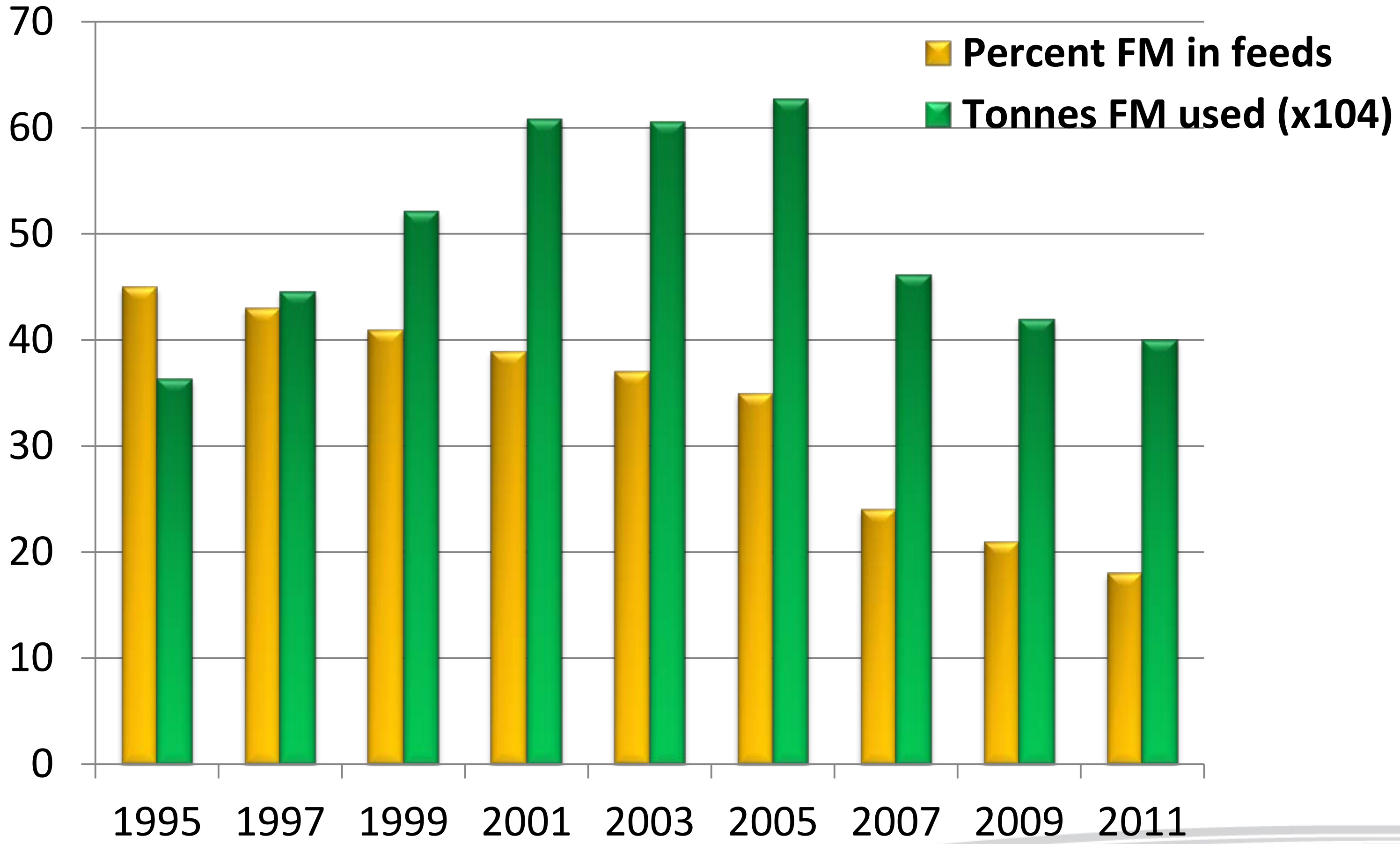
Cumulative feed consumption during production of 3.2 kg salmon at FCR of 1.2



Effects of FCR and feed price on feed costs to grow a 3.2 kg salmon



Fishmeal in salmon feeds



Feed changes are driven by unexpected developments

- **Fishmeal prices departed from the 30-year trading range in 2006-2007**
  - From \$400-\$800 mt^{-1} to \$1600 mt^{-1}
  - This drove a 50% reduction in use levels in fish feeds
- **Replacing 50% of fishmeal protein with plant protein concentrates is not difficult**
- **Long-term trend is to lower fishmeal levels further but this is difficult and doing so has consequences**
- **What will be the next 'unexpected' development that affects fish nutrition and physiology?**

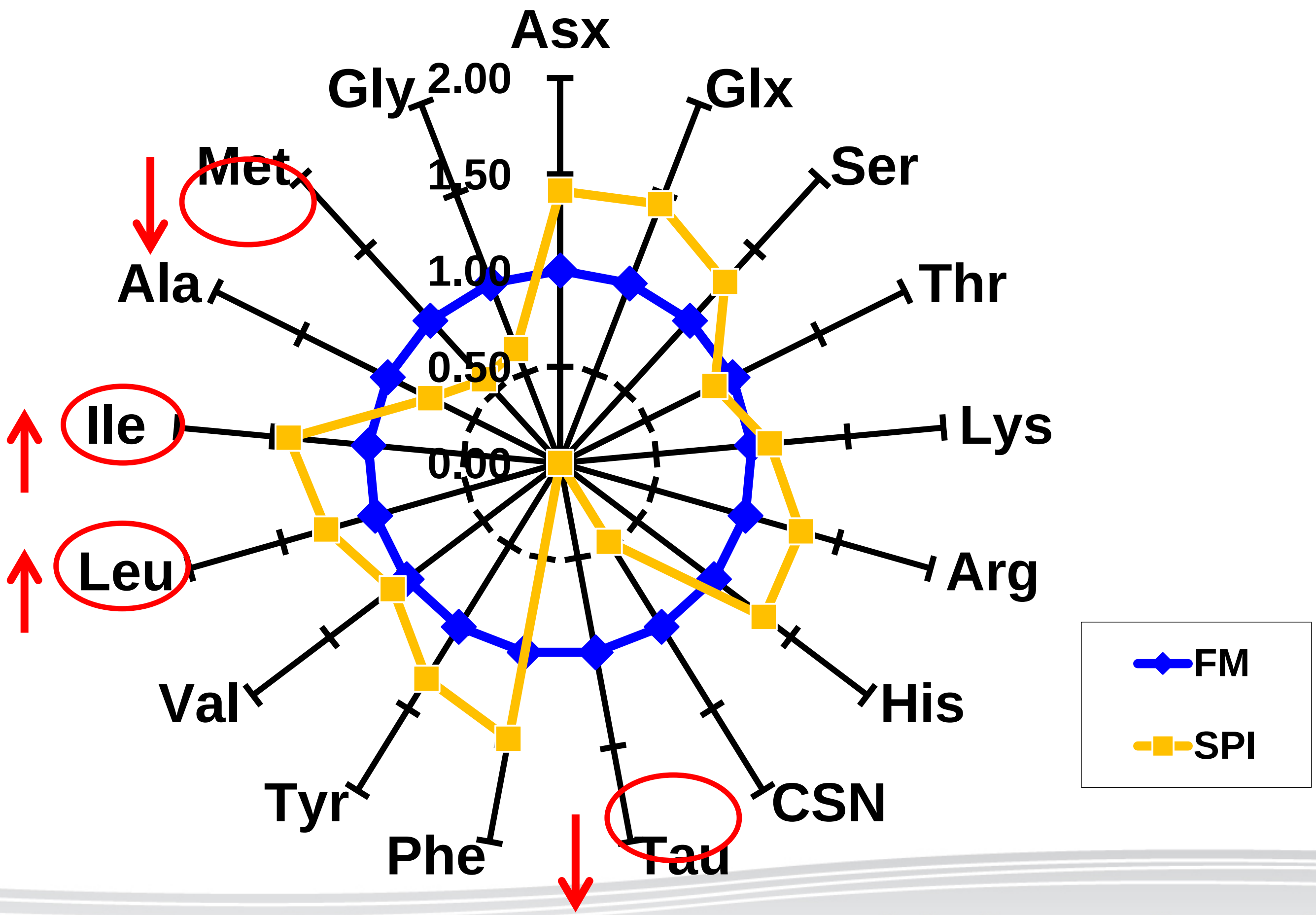
Genomics has changed the research landscape

- **Gene expression studies show that…**
  - Nutrients affect multiple cellular mechanisms
  - All cells require all essential nutrients, not just cells associated with short-latency diseases
- **Low dietary intakes cause conditions or disorders distinct from the short-latency clinical disease first linked to specific nutrients**
  - Other disorders may not be clinically evident or specific, but nevertheless serious
- **This altered the single nutrient – single disease mind set and also the Leibig's barrel concept**

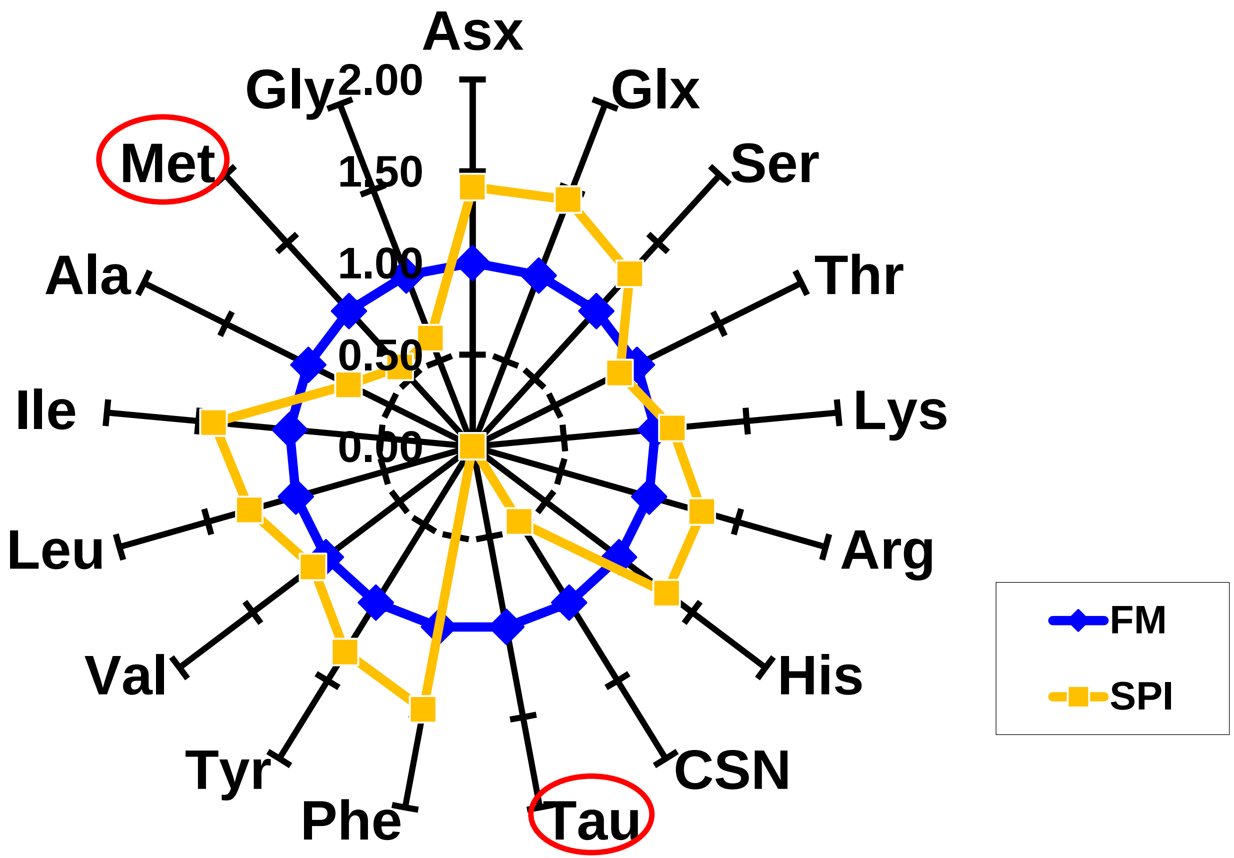
Replacing fishmeal with plant proteins

- Amino acid profiles of plant proteins differ from that of fish meal
- Using Leibig's barrel approach, we supplement limiting amino acids in plant-based feeds to better match levels required by fish
- Result – fish weight gain is improved
- But…feed efficiency is reduced
  - Higher protein synthesis
  - Even higher protein catabolism
  - Net: lower protein retention as growth

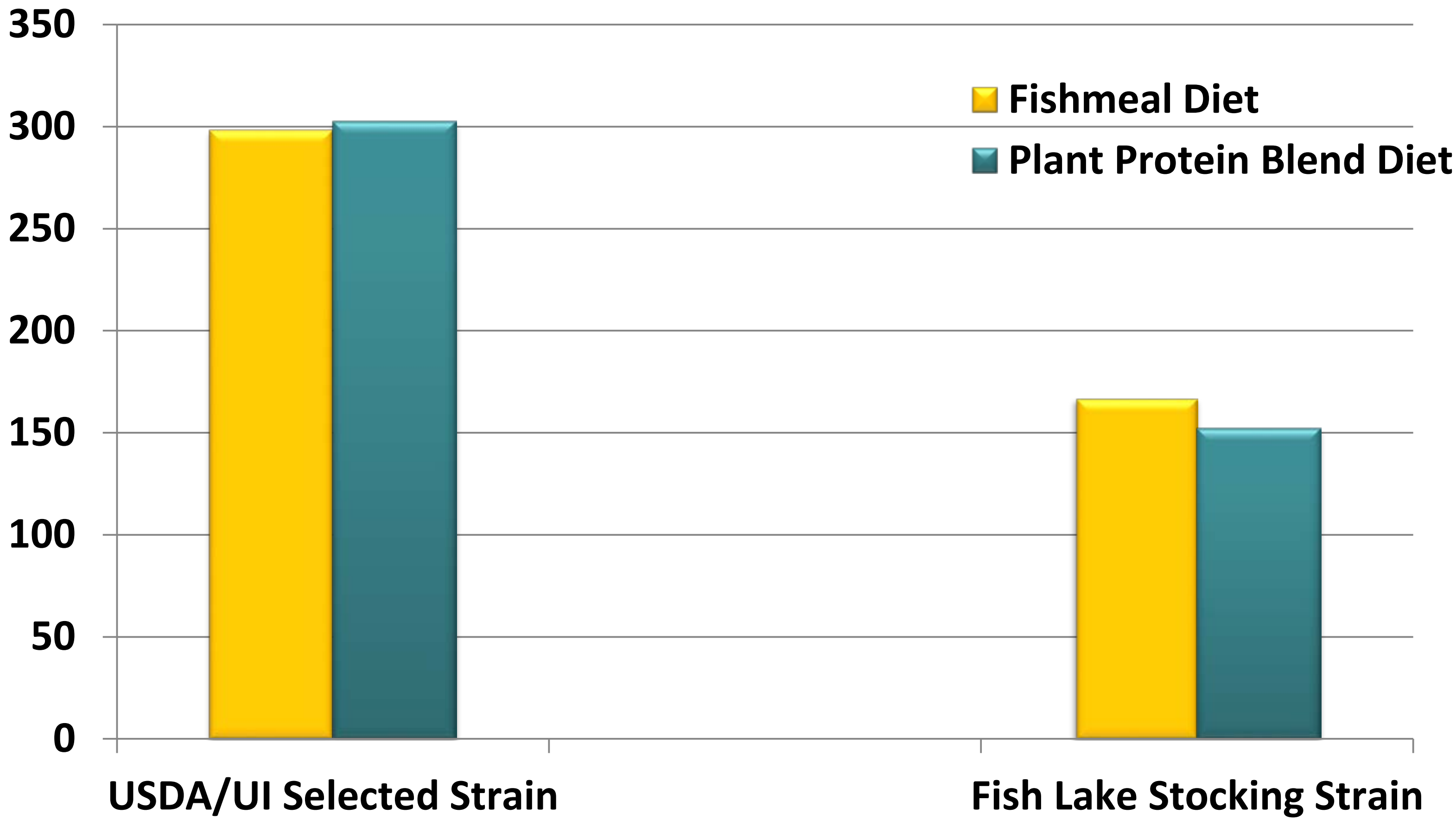
Amino acid profile soy protein and fishmeal



Add Met and Tau (limiting) to match FM levels

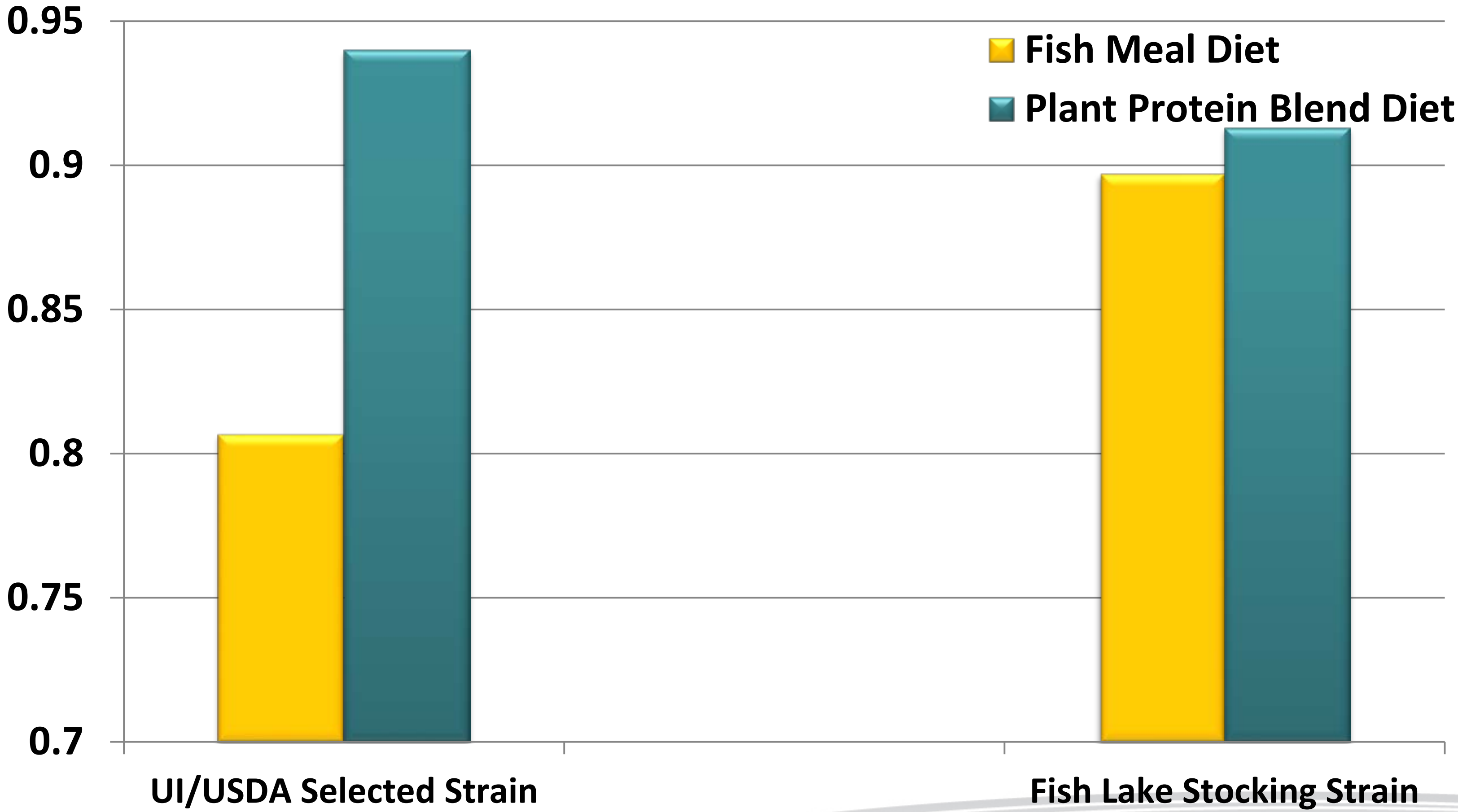


Weight of trout fed FM or PP diets



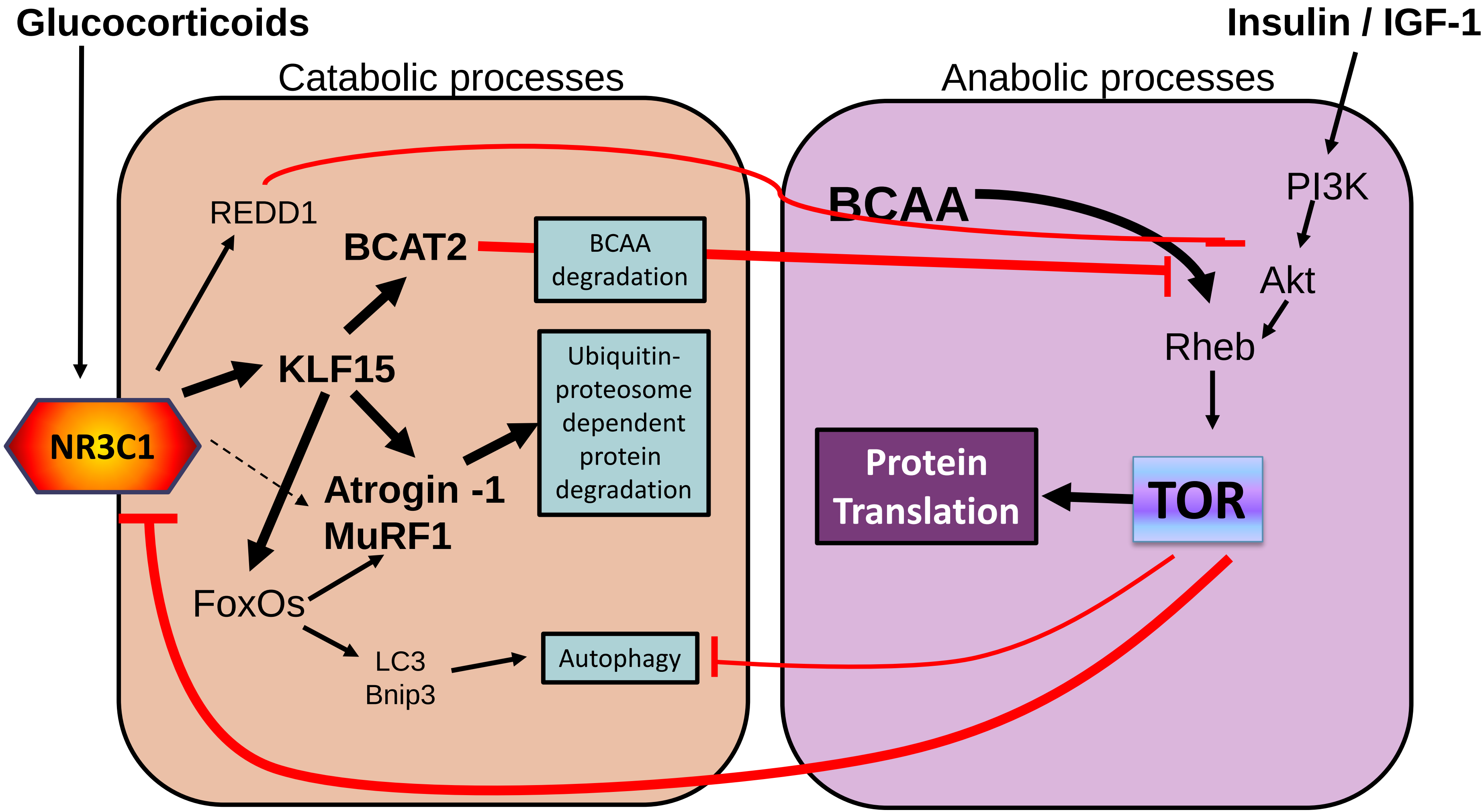
University of Idaho
A LEGACY OF LEADING

Feed conversion ratios in diet x strain study



What is going on?

- According to Leibig's barrel, we met the Law of the Minimum
  - Growth improved but… feed efficiency is lower
- We need to consider amino acid balance, not just minimum (limiting) levels
  - In the spider diagram, some AAs were higher than levels in fishmeal
- Possible issues with imbalanced dietary amino acid levels
  - Protein (amino acids) being used to supply metabolic energy to cells
  - Oxidative stress to cells
  - Signaling activity of amino acids that can enhance protein anabolism or protein catabolism, depending on the amino acid

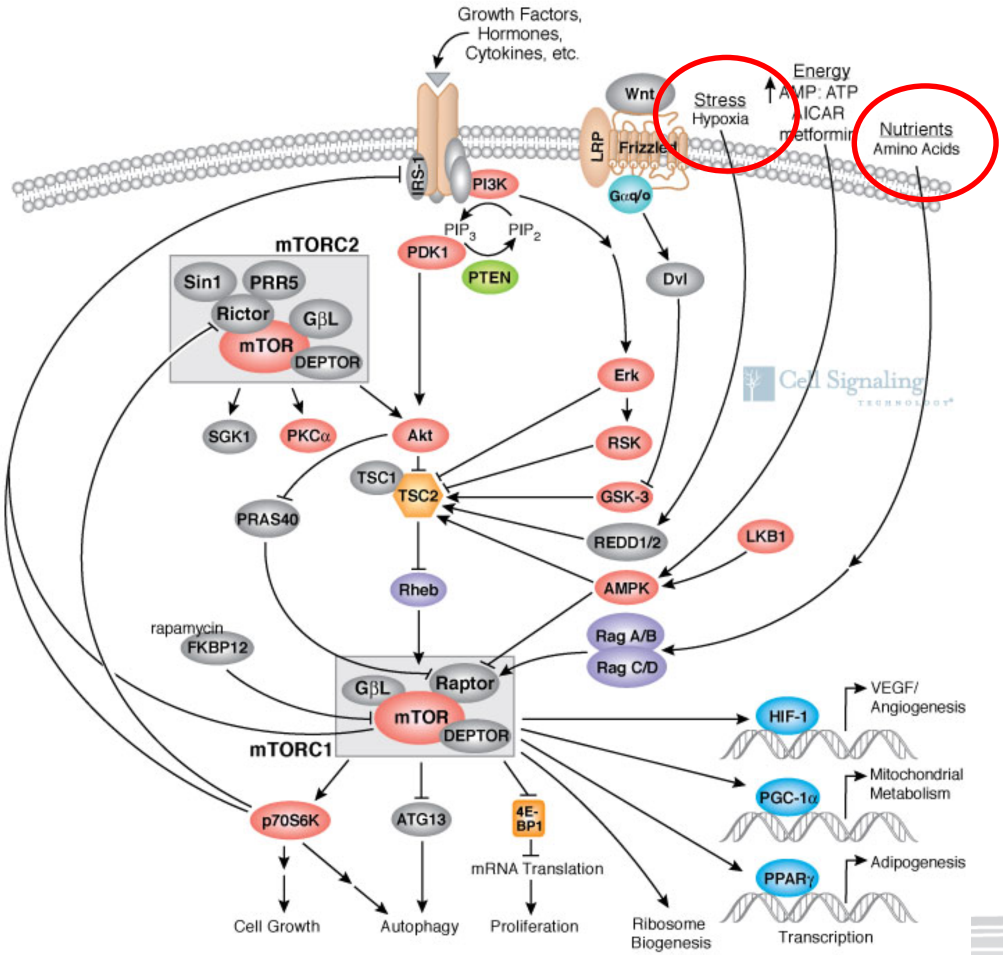


Balance between anabolism and catabolism in cells

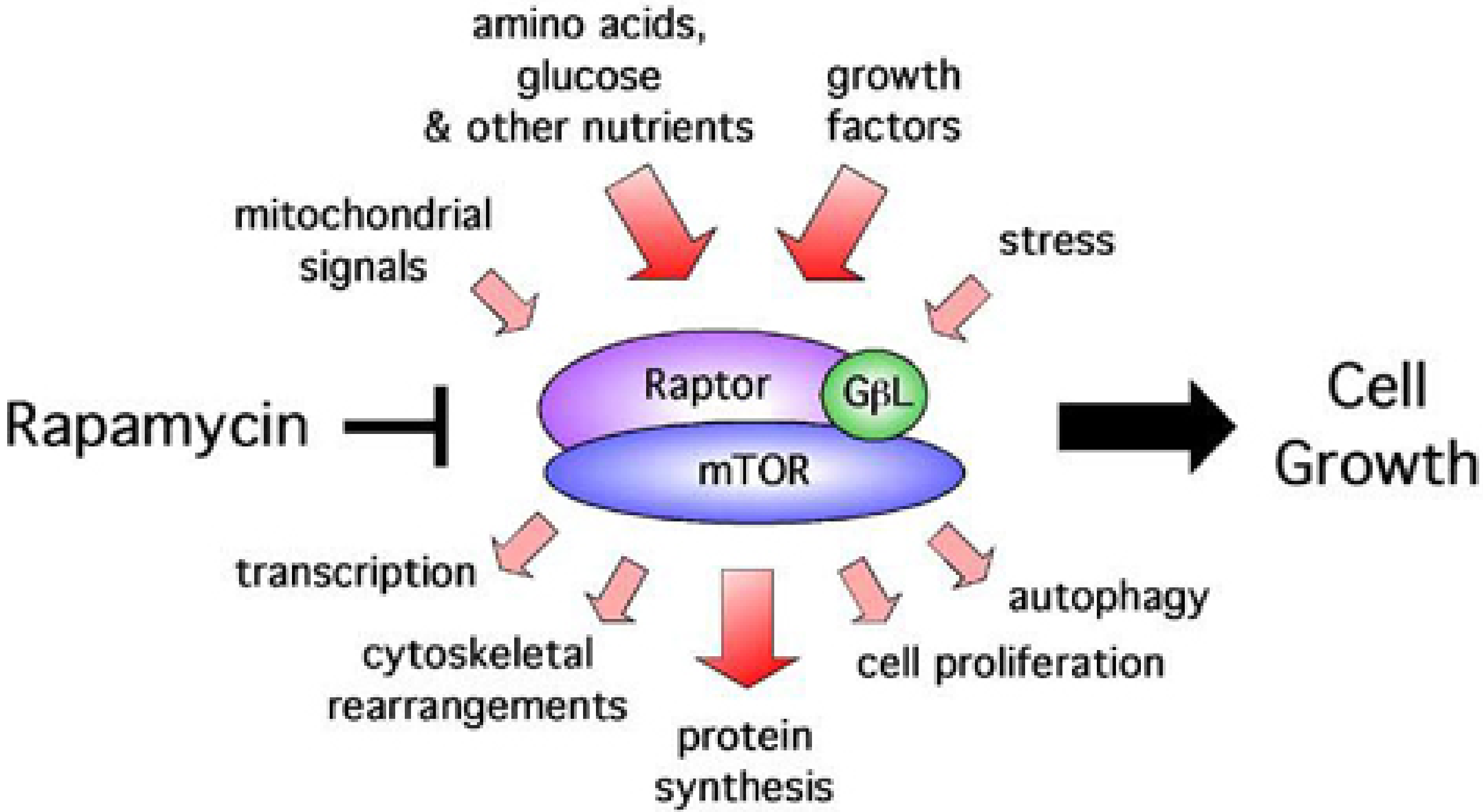
Conditions that increase catabolic processes

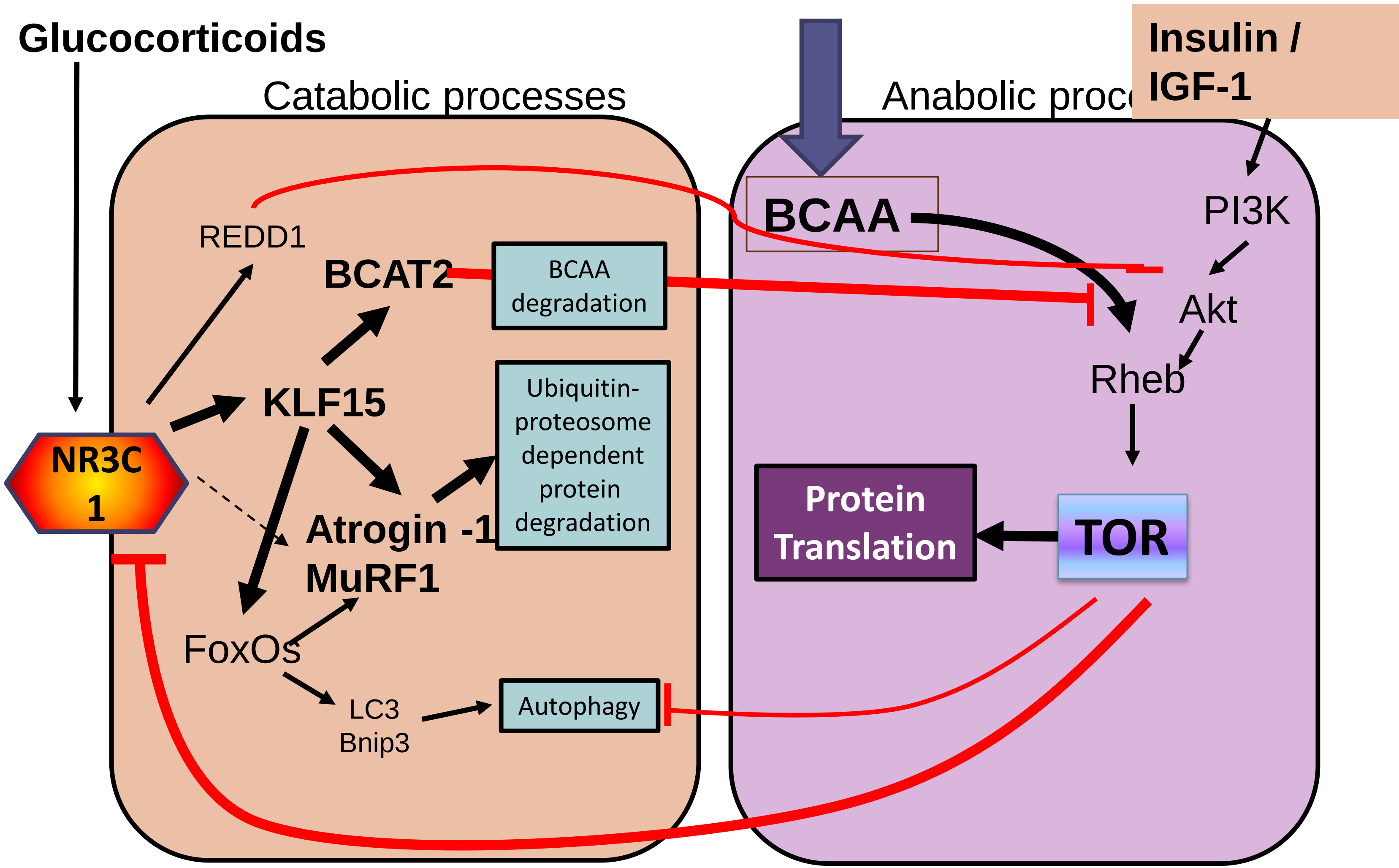
- Stress (glucocorticoids) inhibit TOR cascade
  - Up-regulate REDD1, KLF15 and BCAT2
  - This leads to higher rate of protein breakdown in cells
- Low levels of branched chain amino acids (BCAA) leading to protein breakdown to supply the cell
- Insufficient Redox activity

Cell signaling affecting the TOR cascade



Simplified TOR cascade





Glucocorticoids
Catabolic processes
Anabolic proc
Insulin /
IGF-1
BCAA
PI3K
REDD1
BCAT2
BCAA degradation
Akt
Rheb
KLF15
Ubiquitin-proteosome dependent protein degradation
NR3C1
Atrogin -1
MuRF1
Protein Translation
TOR
FoxOs
LC3
Bnip3
Autophagy

In vivo studies on BCAA levels in feeds

- Plant proteins contain higher levels of BCAA than does fishmeal
- Trout fed plant protein-based diets exhibit changes in multiple genes and pathways
- Leucine shown to alter TOR activity
- Premise was that increasing dietary levels of branched chain amino acids (leucine, isoleucine, phenylalanine) could modulate the TOR cascade

Our findings with BCAA in trout diets

- **Compared fishmeal and soy protein diets, both supplemented with several levels of BCAA**
  - **We found that dietary protein source modified *TOR* and *REDD-1* transcriptional activities , reducing anabolic processes**
  - **No detectable effects on *TOR* associated with BCAA levels in diets**
- **We also found changes in expression of genes involved in hepatic protein metabolism and Redox status associated with the plant protein diet**
- **Bottom Line: it didn't work**

Other considerations with plant proteins

- Transporters – gene expression and timing
- Synchronization of plasma levels of essential amino acids
  - Plasma levels vary after a single feeding of a fishmeal diet or plant protein diet
  - Evidence that amino acids from plants take longer to appear in plasma than amino acids from fishmeal
  - Crystalline amino acids are well known to be more rapidly absorbed than amino acids from intact proteins
- Dual approach involving physiology and gene expression needed to resolve these questions

Example of UI research on plant protein feeds

- **We force-fed trout a standardized feed quantity (0.5% BW) and measured plasma AA levels in the hepatic portal vein (HPV) and caudal vein (CV)**
  - We sampled fish at 3, 6, 12, 18 and 24 hours after single feeding
  - HPV data shows AA absorption in the intestine
  - CV data shows availability of AAs at peripheral tissues (muscle) and also indicates AA catabolism and protein turnover
  - We also measured gene expression levels of intestinal AA transporters
- **We tested plant protein blends with or w/o AA supplementation, plus six ingredients (5 plant proteins and fishmeal)**

Experimental Set-up

Selection Diet

Ingredient	Percent in diet
Soy protein concentrate	**25.63**
Soybean meal	**19.55**
Corn protein concentrate	**17.54**
Wheat gluten meal	**4.07**
Wheat starch	8.91
Fish oil	15.70
L-Lysine	**1.40**
DL-Methionine	**0.38**
Threonine	**0.20**
Taurine	0.50
Mono-dicalcium phosphate	3.33
Potassium chloride	0.56
Sodium chloride	0.28
Magnesium oxide	0.05
Stay-C	0.20
Choline chloride	0.60
Trace mineral premix	0.10
Astaxanthin	0.06
Vitamin premix 702	1.00

❖ **2 trout strains**:

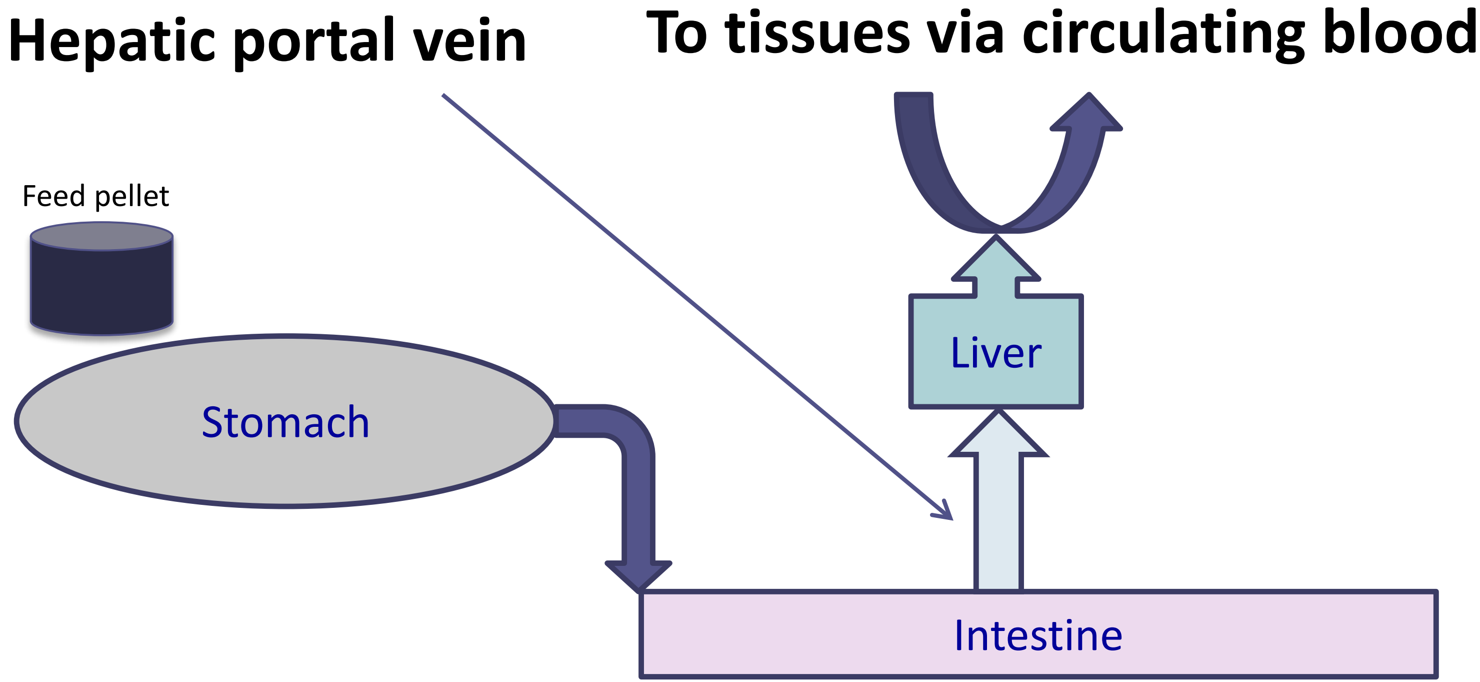
- Selected **(**6th Generation)
- Commercial
- 40 fish ~400g BW

❖ **Force feeding** (0.5%BW):

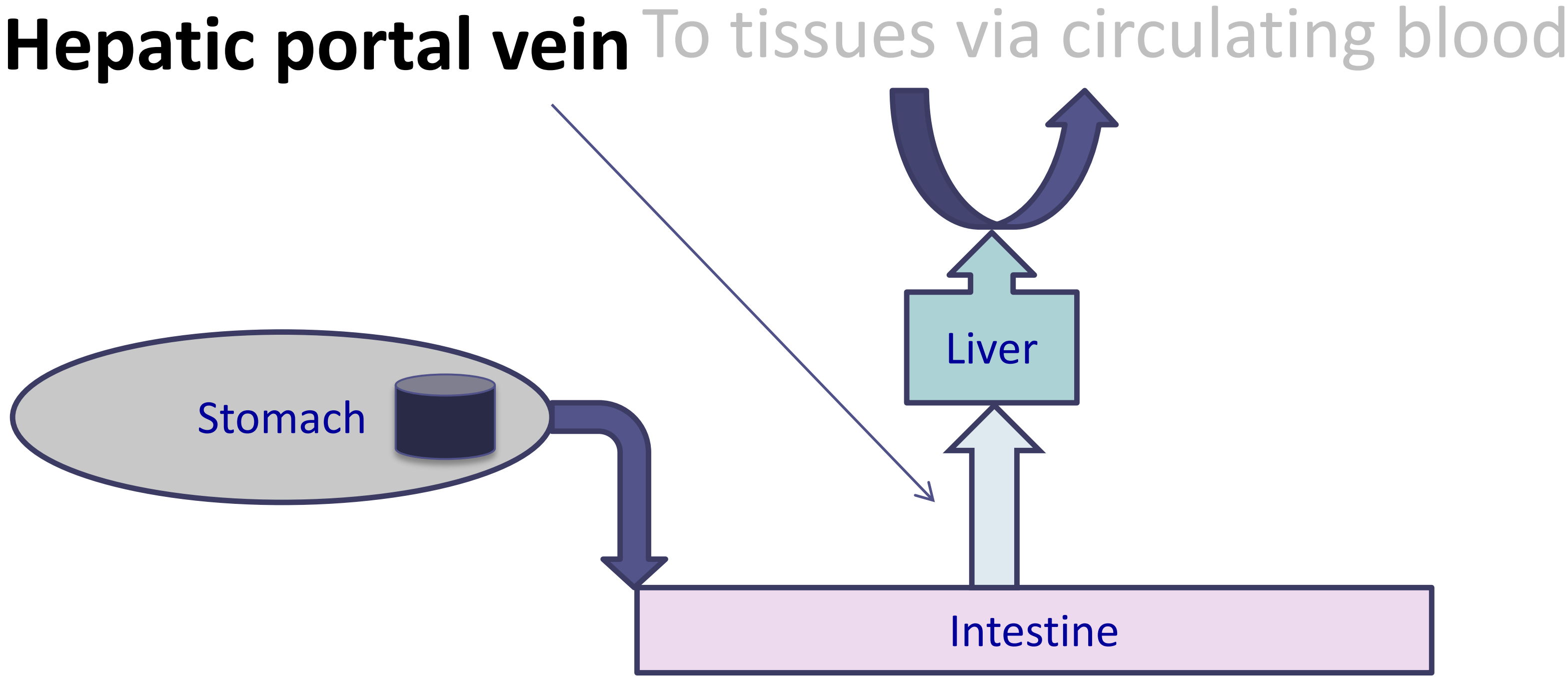
- Protein blend= **SPC, SBM, CPC, WG**
- Plant protein blend + **Lys, Met, Thr**

Force-feeding trout, 0.5% BW

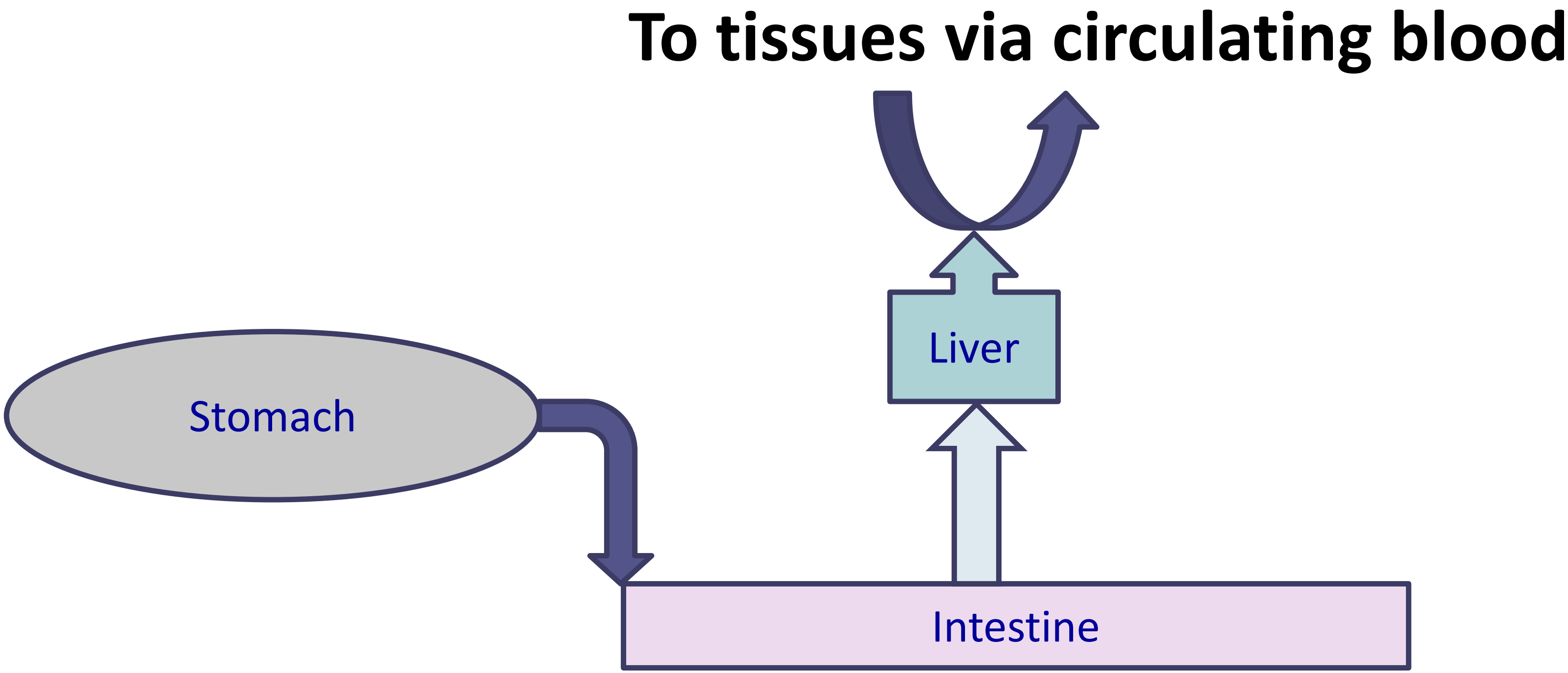
How dietary amino acids get to tissues



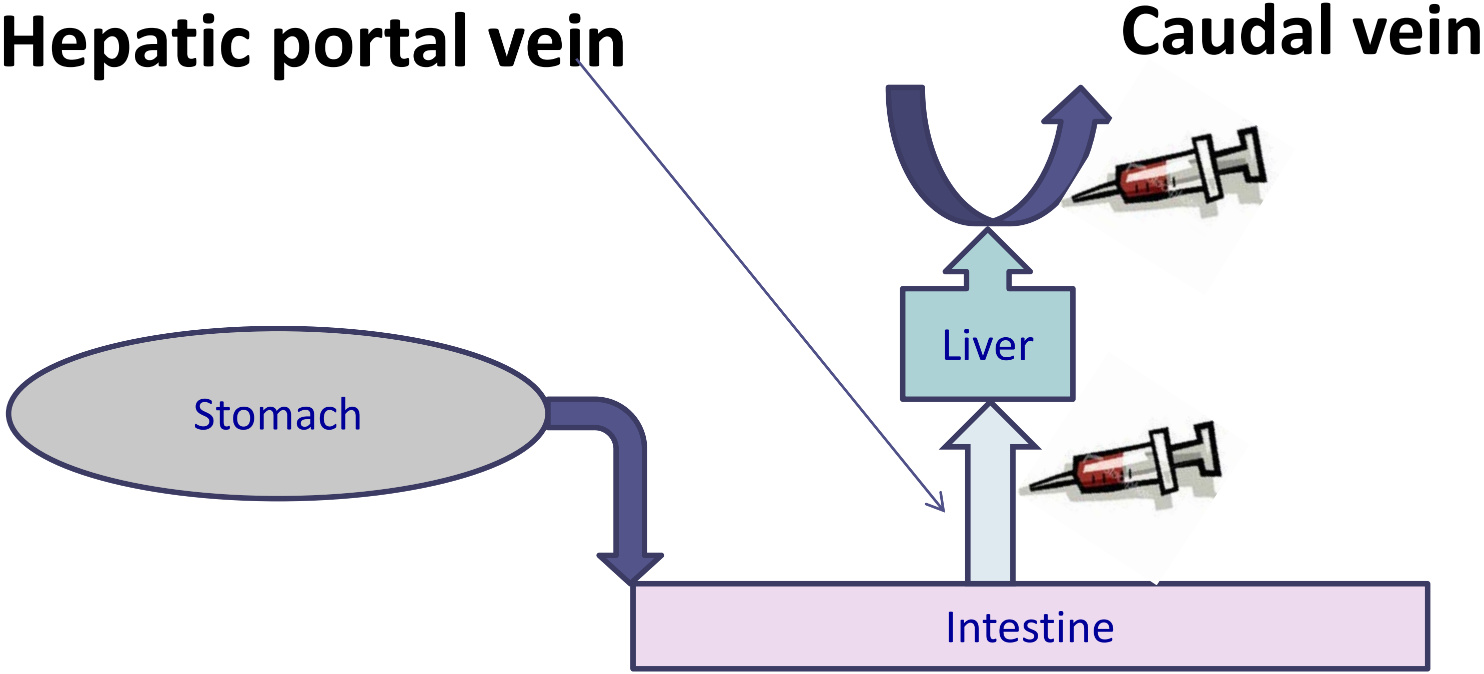
AA levels in HPV reflect digestion process



AA levels in circulating blood reveal AA fate

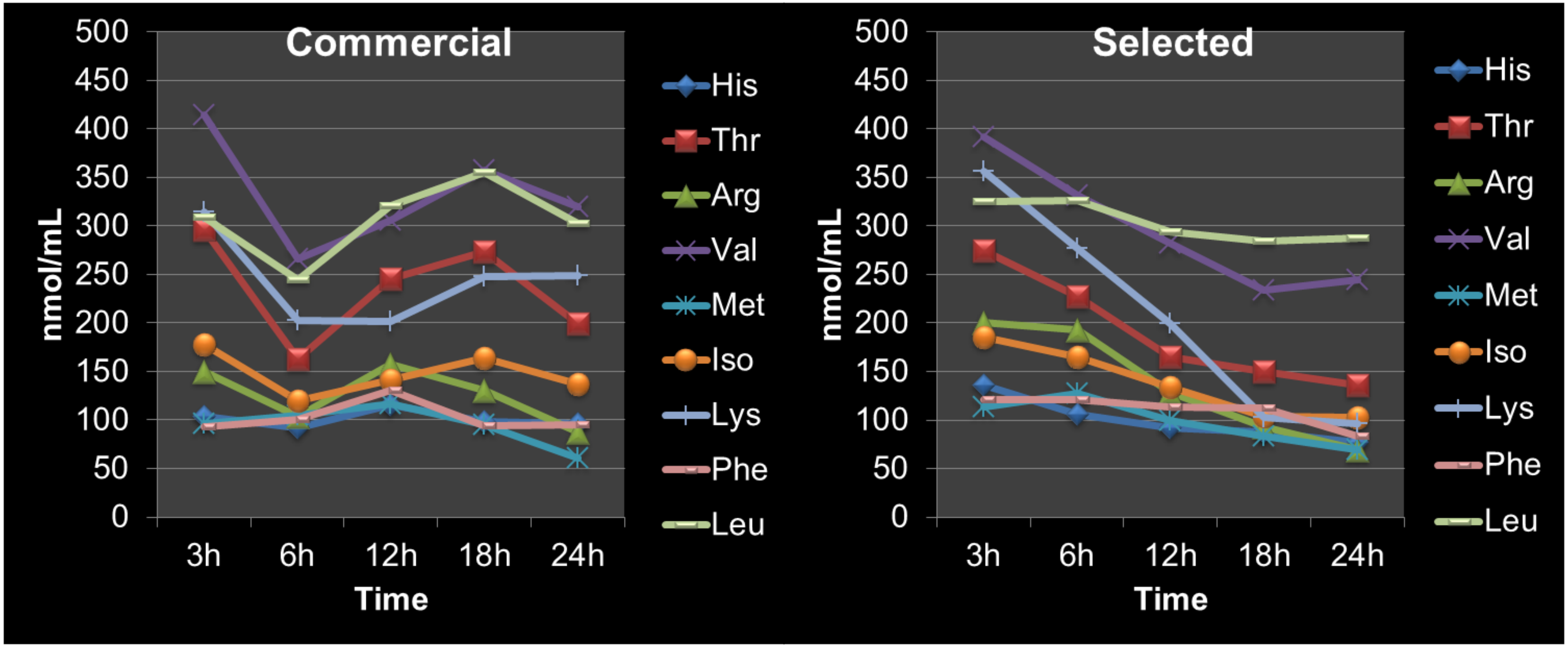


We sampled blood from two places



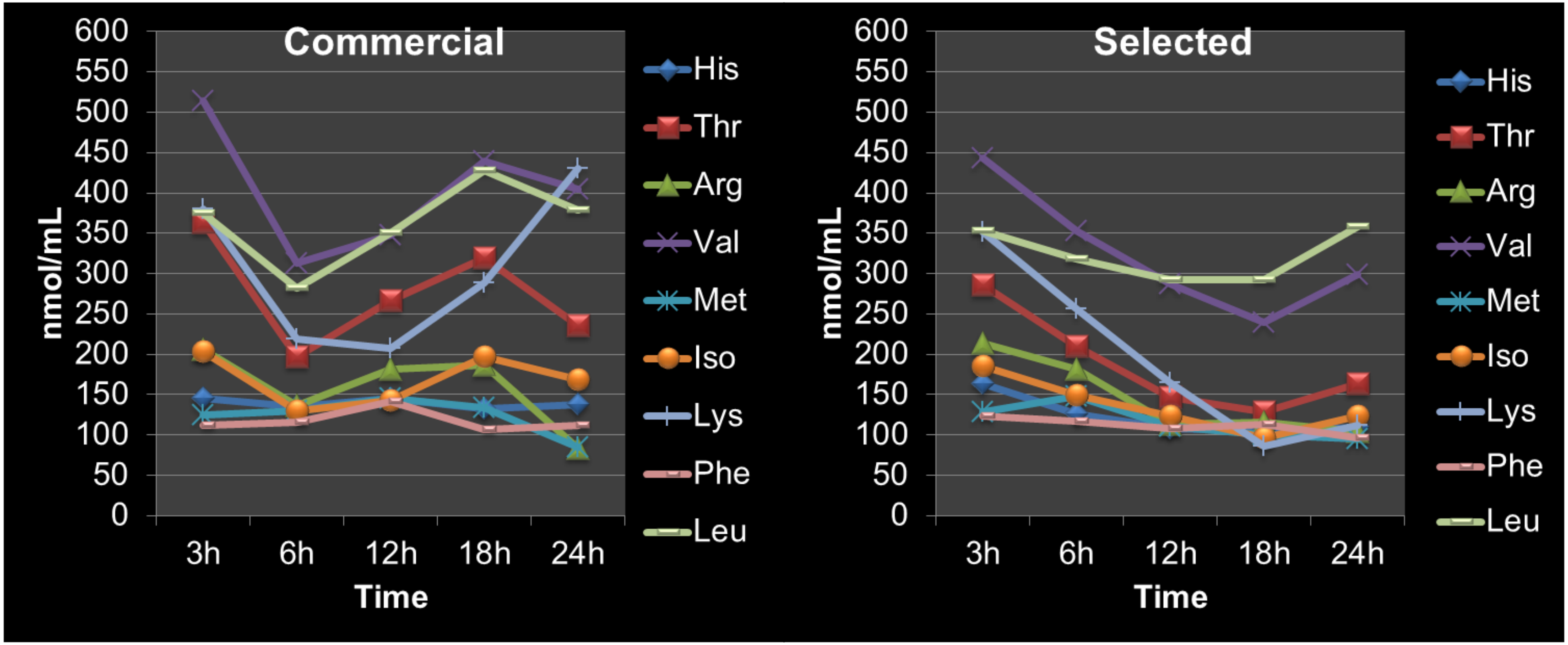
Results for plasma amino acids (AA) levels

Plasma AA levels Protein Blend (+AA)
Hepatic Portal Vein



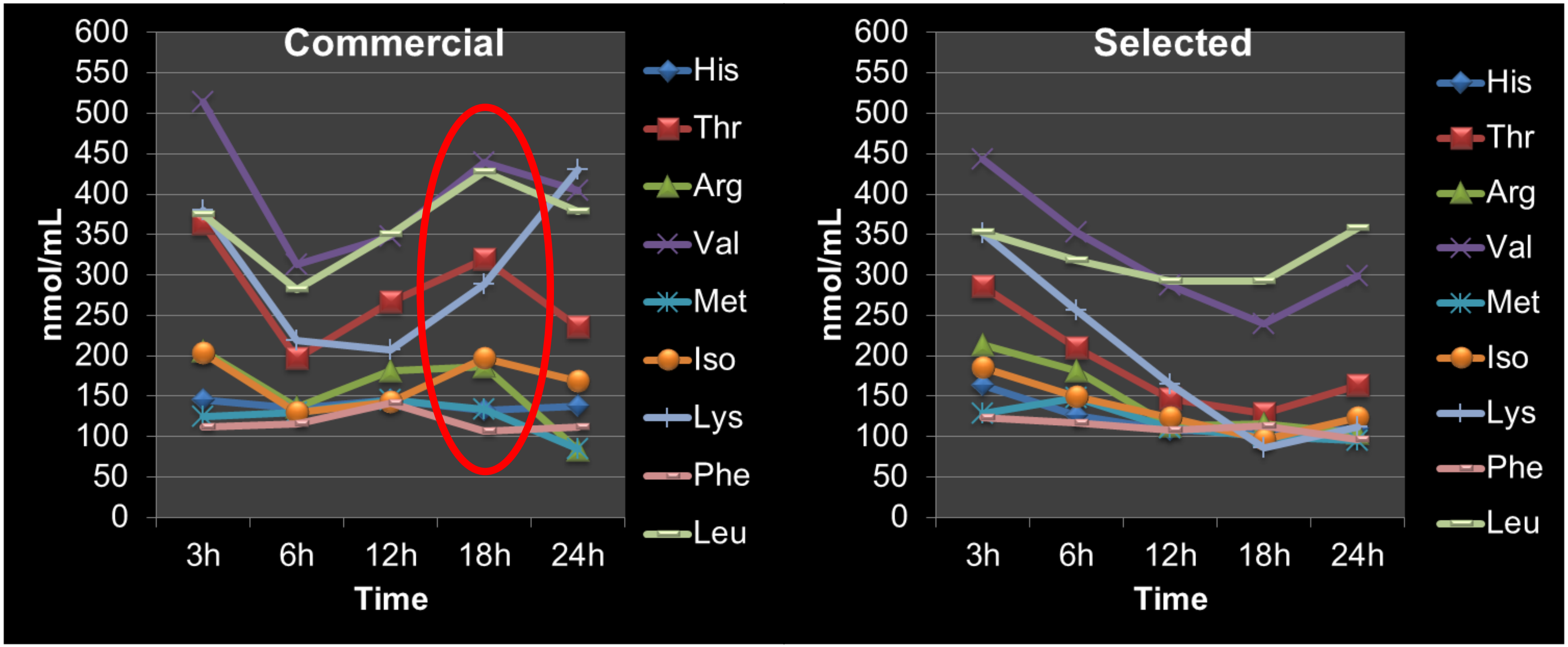
- **The commercial strain shows two major peaks**
- **The selected strain shows a controlled decreasing pattern**

Plasma AA levels Protein Blend (+AA)
Caudal Vein



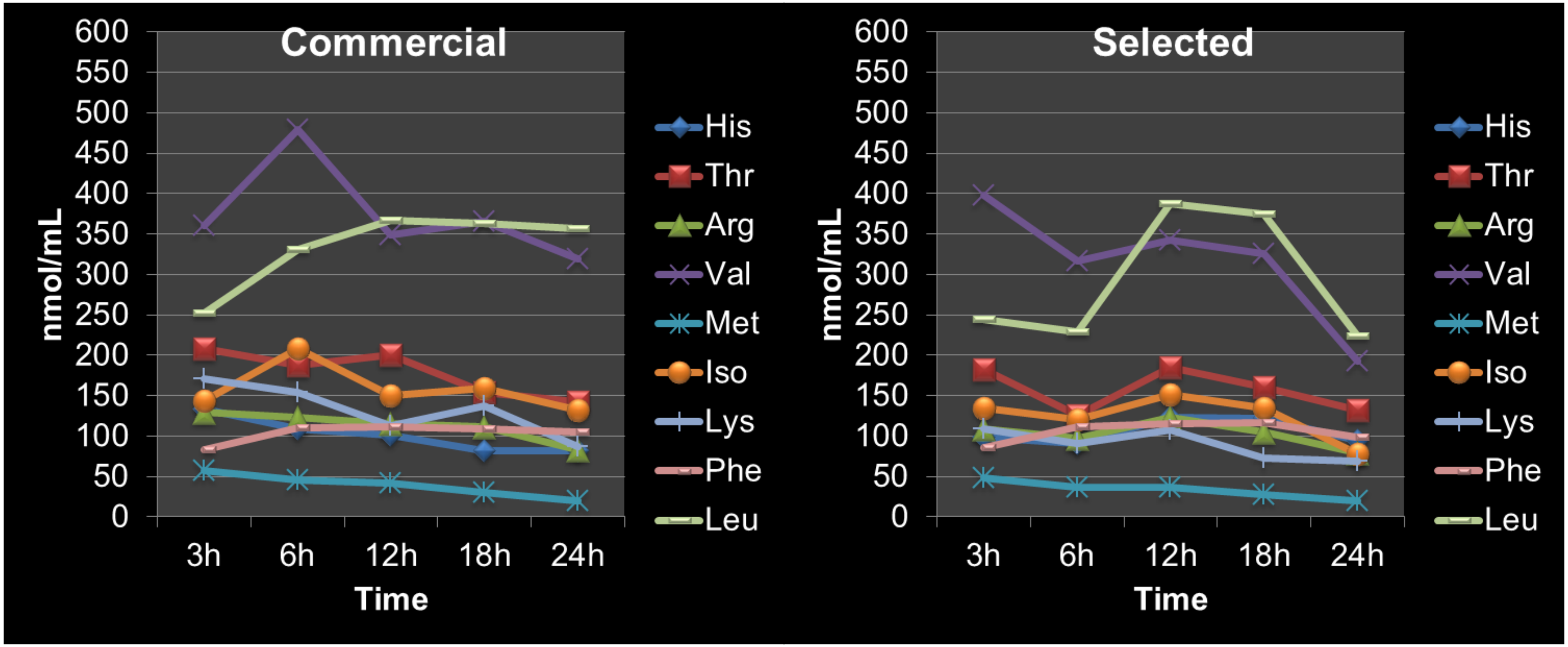
- Amino acid concentrations through time reflect tissue **<u>protein synthesis</u>** and **<u>protein turnover</u>**

Plasma AA levels Protein Blend (+AA)
Caudal Vein



- Amino acid concentrations through time reflect tissue **<u>protein synthesis</u>** and **<u>protein turnover</u>**

Plasma AA levels Protein Blend (-AA)
Caudal Vein



- **Strain effect is not so pronounced (masked by AA deficiency)**

Plasma AA results - Summary

- Digestion and absorption of AA differs between selected and non-selected trout
  - Differences between trout strains in timing and pattern of amino acids in hepatic portal vein
  - The selected strain showed a major peak at 3h followed by a similar decreasing pattern for all AAs
- The addition of crystalline AAs influenced the appearance of all AAs, shifting the timing of peaks (AA signaling)
- Plasma amino acid levels after 12 hours reflect protein retention and protein turnover
  - The pattern of lysine over time followed different patterns between strains
  - Increased lysine in CV samples after 18 hrs suggest higher protein turnover in non-selected strains

Conclusions

- **HPV plasma AA levels show that there is difference in the digestion patterns between the strains, and crystalline AA supplementation affects the digestion process**
- **CV plasma AA levels show the lower protein retention efficiency observed in commercial strains is due to asynchronous protein digestion and amino acid absorption**
- **The results show a need to measure plasma amino acids at multiple time points when new diet formulations are assessed**
- **Protein digestibility, even though a useful tool, does not predict protein retention when alternate protein ingredients are used**

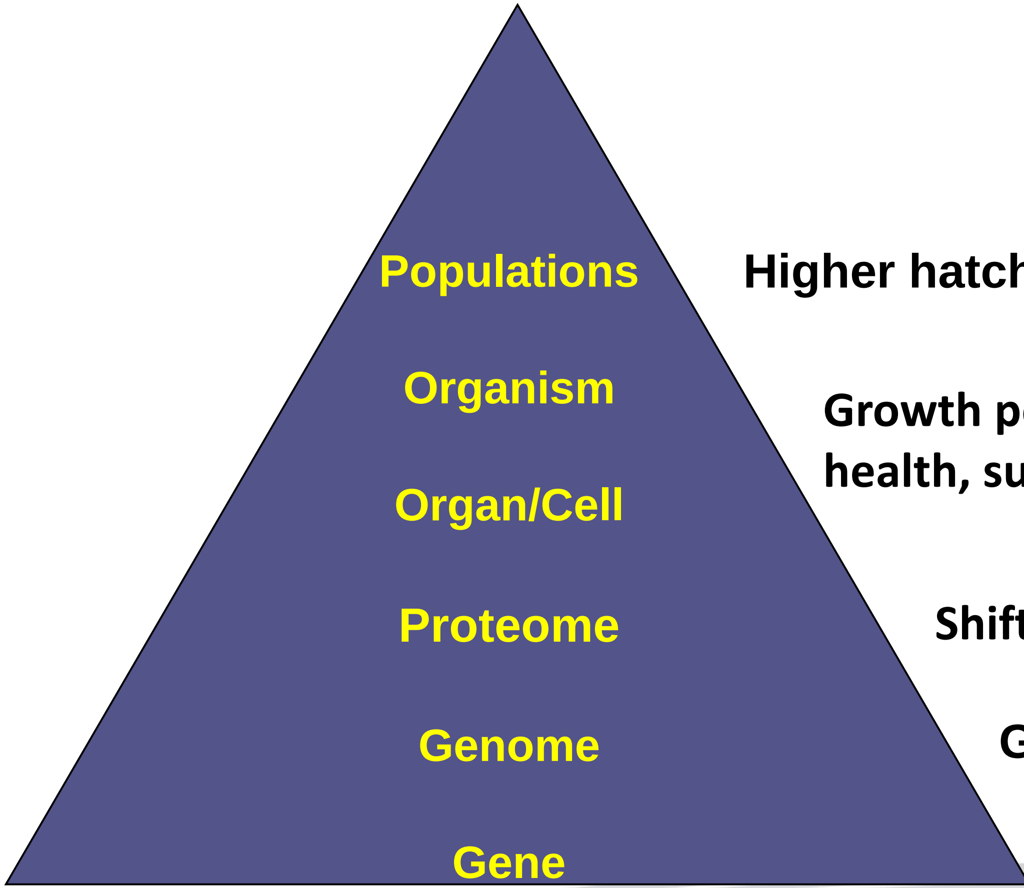
Impact of findings

- **Our findings are a game-changer as far as diet formulation and utilization of plant proteins are concerned**
  - Supplementing AAs to 'balance' the diet does not correct the problem with low protein retention in plant-based diets in commercial (non-selected) stains
  - But…the selected strain has overcome the problems that AA supplementation and plant (soy) protein digestion present
- **The selection diet and the selected fish are unique models to advance development of sustainable feeds**

Impacts of genomics on salmonid nutrition & physiology

- In a word – *transformational*
- In a decade genomics overturned nearly 100 years of conventional thinking regarding nutrient requirements
  - Law of the Minimum and one nutrient – one disease
- How best to capitalize on this new knowledge to address current issues?
  - Sustainable feeds for salmon
  - Product quality for consumers
  - Increased survival of hatchery releases for stock enhancement

Must keep going back up the pyramid



Higher hatchery returns

Growth performance, health, survival

Shifts in metabolism

Gene expression

Challenge for fish nutrition and physiology

- **Begin to move from complexity to key areas of gene regulation**
- **Integrate findings from cellular regulation with physiological data**
  - **Digestion/absorption rates of proteins**
- **Move to organism level**
  - **Feed composition**
  - **Early life history (epigenetics)**
  - **Supplements to increase survival and ability to resist pathogens**