Nutritional approaches to improving seafood quality

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Major seafood quality concerns are quality and safety

- Sensory attributes
  - Taste, odor, texture, color
- Stability during frozen storage
- Contaminants
  - Heavy metals, mainly methylmercury
  - Persistent organic pollutants (POPs)
  - Chemotherapeutics
  - Naturally-occurring toxins
- Parasites, worms
- Microbial spoilage

How do farmed and wild fish compare?

- Similarities
  - Effects of pre-processing handling
  - Effects of post-processing handling
  - Effects of life-history stage
  - Effects of season (photoperiod, water temperature)
  - Effect of food
- Differences
  - Harvesting and pre-processing handling – higher level of control in farmed fish
  - Seasonal food abundance or scarcity (temperate water fish)
  - Food vs. Feed
- Nutritional inputs are controlled in farmed fish, not so in wild fish
  - Feed is source of contaminants, so can control in farmed fish
  - Chemical/nutritional composition can be altered to some extent
  - Sensory attributes can be altered by diet

Very important distinction between farmed and wild fish

Quality of farmed fish

- Nutritional content
  - Protein and amino acids
  - Lipid level and fatty acid profile (omega-3s)
  - Vitamins
  - Minerals
  - Other compounds
- Sensory attributes
  - Color
  - Taste and odor
  - Texture
- Storage stability
  - Fresh
  - Frozen

Modifying nutrient content in farmed fish

- Protein and amino acids
  - Protein content of fish varies with life-history stage, but is not affected by diet except in starvation
  - Amino acid content of muscle is fixed due to dominance of muscle proteins, e.g., actin and myosin, so not affected by diet
- Lipid level and fatty acid profile (omega-3s)
  - Very easy to modify with diet
- Vitamins
  - Can modify fat-soluble vitamin content and ascorbic acid, but not B-vitamins
- Minerals
  - Homeostatically controlled, so little opportunity to alter
- Other compounds - astaxanthin

Modifying total lipid and fatty acid content in farmed fish fillets

- Tissue fatty acids are either polar or non-polar
  - Polar lipid are membrane phospholipids
    - Fatty acid profiles are relatively fixed, but change with water temperature
    - Constitute about 1-2% of wet body weight
  - Non-polar lipid are triglycerdies and present in storage lipids
    - Found in muscle, liver or in abdominal cavity surrounding GI tract
    - Fatty acid profiles respond quickly to dietary fatty acid profiles
    - Percent of body weight (or fillet weight) varies with species
      - Salmon fillets range from 5-12% lipid
      - Trout are about 5-9% lipid
      - Yellow-fin jack is about 10-12% (Kona Kampatche)
- Consumers expect farmed fish to be equivalent to wild fish as sources of omega-3 fatty acids

Modifying total lipid content of farmed fish fillets

- Tissue lipid levels vary with…
  - Life-history stage
  - Dietary lipid level and/or protein/lipid ratio
  - Feed intake
- Tissue lipid content affects…
  - Flavor, mainly intensity
  - Texture
    - Tissue lipid and moisture content are inversely related
    - As fillet lipid level goes up, moisture goes down
    - Proportion of lipid and moisture affects texture
- Influence of nutrition on fillet lipid content depends on species of fish

Consumers expect farmed fish to be equivalent to wild fish as sources of omega-3 fatty acids

- Omega-3 fatty acids of interest are EPA (C20:5) and DHA (C22:6) produced by marine algae
- Consumers obtain most of their omega-3 fatty acid intake from fish
- Wild fish is typically high in omega-3 fatty acid content, expressed on a percentage basis
  - Wild salmon fillet lipids are typically 25+% omega-3
  - Farmed salmon fillet lipids are typically 20-25% omega-3
- Farmed fish are typically higher in fillet fat level
- Therefore, consumer intake of omega-3 fatty acids is equivalent or higher from farmed fish that wild fish when expressed on a g omega-3 / 100g serving

Fish oil use in aquafeeds a global problem

- Global production averages 1,200,000 mt per year
- Aquafeeds utilize >80% of annual production, most of which is used in salmon, trout and marine fish feeds
- Salmonids grow normally when fed diets containing alternate oils
  - Diets must contain 1-2% of diet as omega-3 fatty acids
- Salmon feeds now contain blends of plant and fish oils; fish oils are fed during final grow-out stages to raise omega-3s in fillets
- Fillet fatty acid profiles reflects dietary fatty acids
  - Tissue deposition follows a simple dilution model

Selected fatty acid levels in fish, poultry fat and plant oils (% total fatty acids)

Oil or fat source	Palmitic C16:1	Stearic C18:1	Oleic C18:1ω9	Linoleic C18:2ω6	Linolenic C18:3ω3	EPA C20:5ω3	DHA C22:6ω3
Menhaden	19	4	13	1	0.3	11	9
Poultry	22	6	37	19	1	-	-
De-hulled flax 70 kernel	4.3	3.3	20.4	13.1	58.1	-	-
Canola	3	2	60	20	12	-	-
Soybean	5	4	22	54	7	-	-

Plant oils in aquafeeds

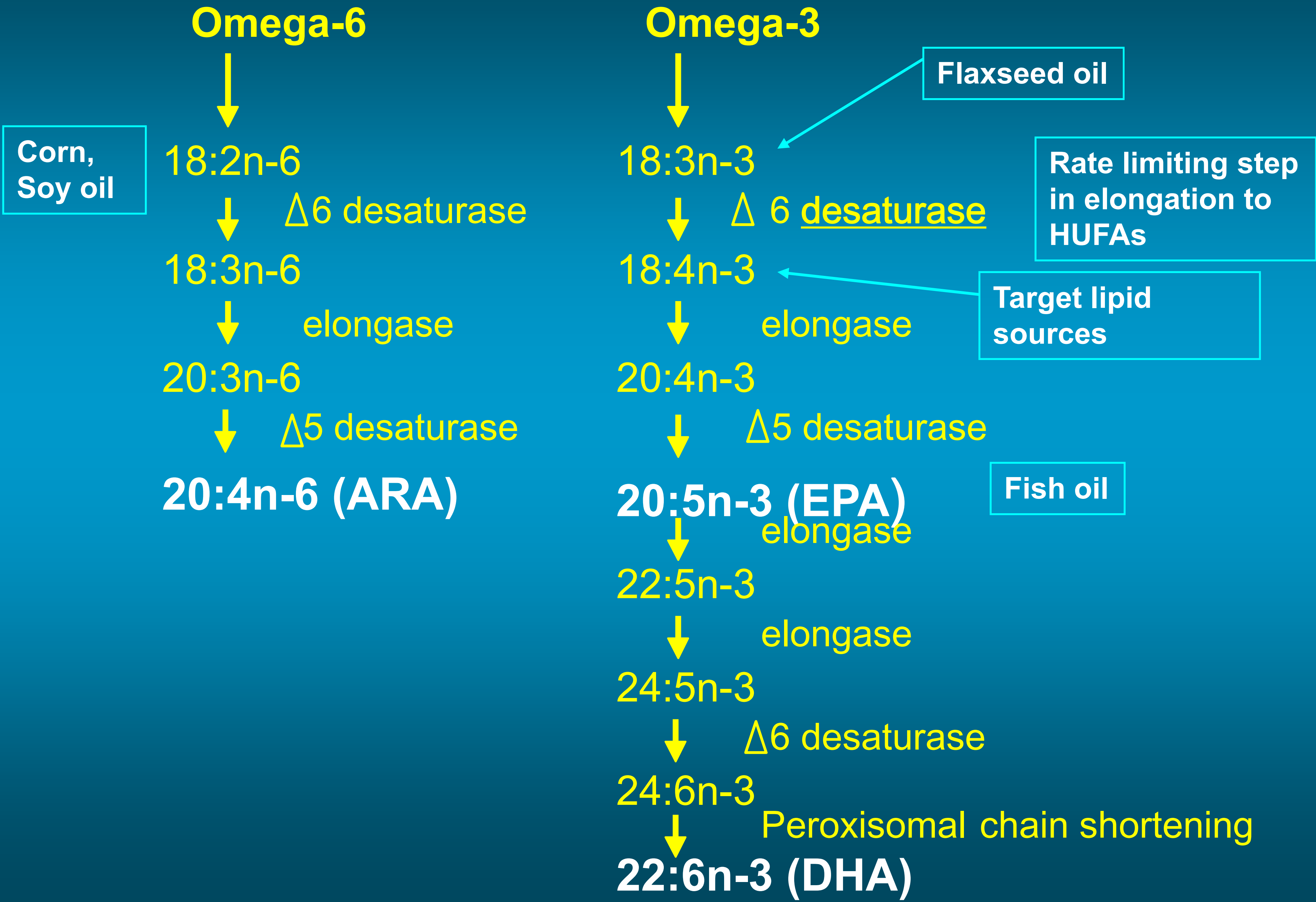
- Plant oils with desirable fatty acid profiles (high in oleic acid (C18:1) and low in linoleic acid (C18:2, omega-6)
  - Canola/rapeseed oil
  - Linseed (flax) oil
  - Sunflower oil
  - Olive oil
- Plant oils with undesirable fatty acid profiles
  - Soy oil
  - Corn oil
  - Palm oil
- Total production of plant oils in 2003 was 105.5 million mt, or 100x annual fish oil production, so supply is not an issue

Soy oil	31.1 mmt
Rapeseed oil	11.9 mmt
Sunflower oil	8.5 mmt

Strategies to maintain omega-3s levels in fillets when plant oils are used in feeds

- Phase feeding with fish oil in feeds fed at the end of the grow-out period and blends of fish and plant oil sources fed earlier
- Breed trout family lines with high expression levels of fatty acid desaturase enzymes
- Identify novel lipid sources that provide substrates for elongation to HUFAs
  - Marine algae, linseed (flax) oil
  - GMO oilseeds or grains

Fatty Acid Desaturation and Elongation



Omega-6

Corn, Soy oil

18:2n-6

↓ Δ6 desaturase

18:3n-6

↓ elongase

20:3n-6

↓ Δ5 desaturase

20:4n-6 (ARA)

Omega-3

Flaxseed oil

18:3n-3

↓ Δ 6 desaturase

Rate limiting step in elongation to HUFAs

18:4n-3

Target lipid sources

↓ elongase

20:4n-3

↓ Δ5 desaturase

20:5n-3 (EPA)

Fish oil

↓ elongase

22:5n-3

↓ elongase

24:5n-3

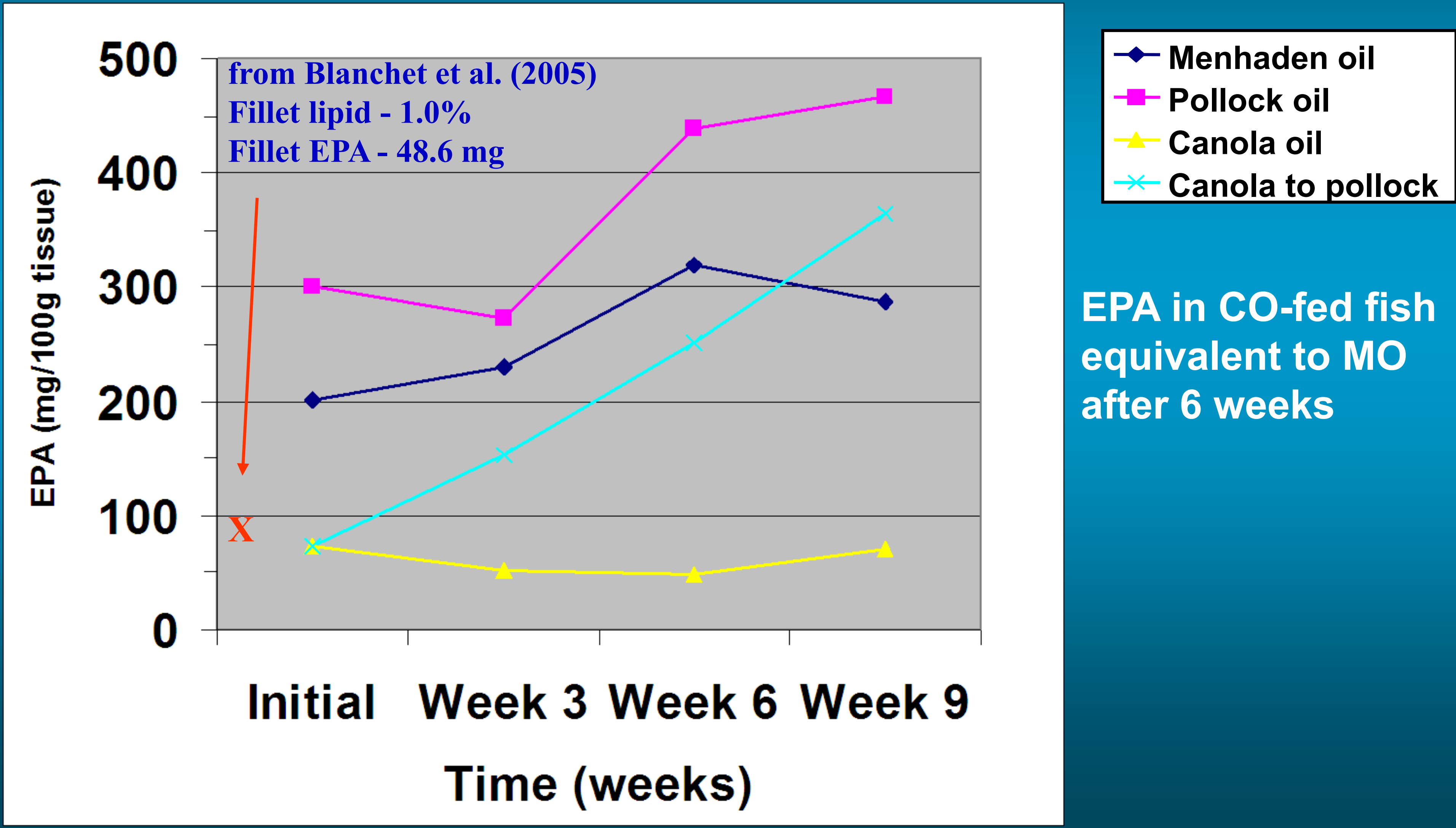
↓ Δ6 desaturase

24:6n-3

↓ Peroxisomal chain shortening

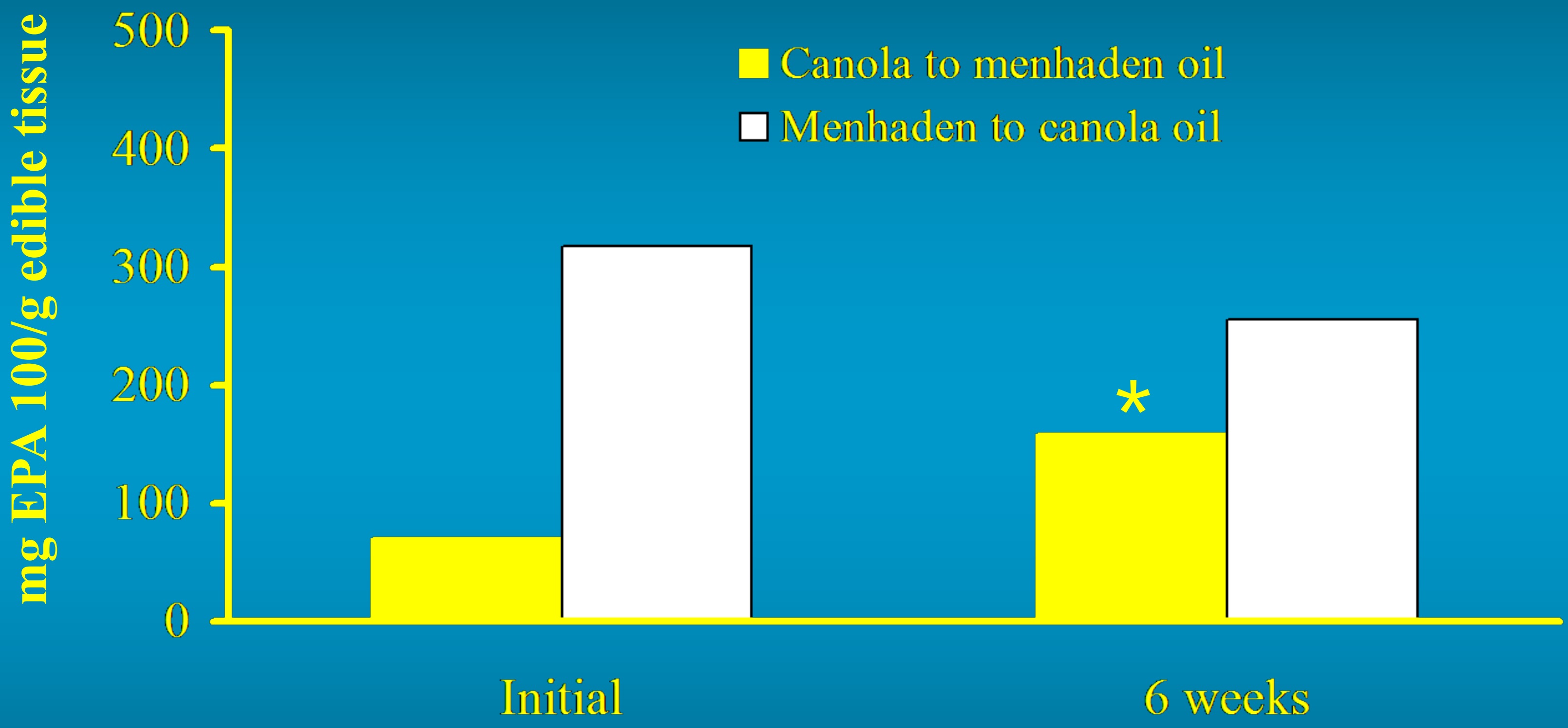
22:6n-3 (DHA)

Phase feeding different oil sources EPA levels in trout fillets



EPA in CO-fed fish equivalent to MO after 6 weeks

Trout fillet eicosapentenoic acid (EPA) level after switching oil source in feed for six weeks

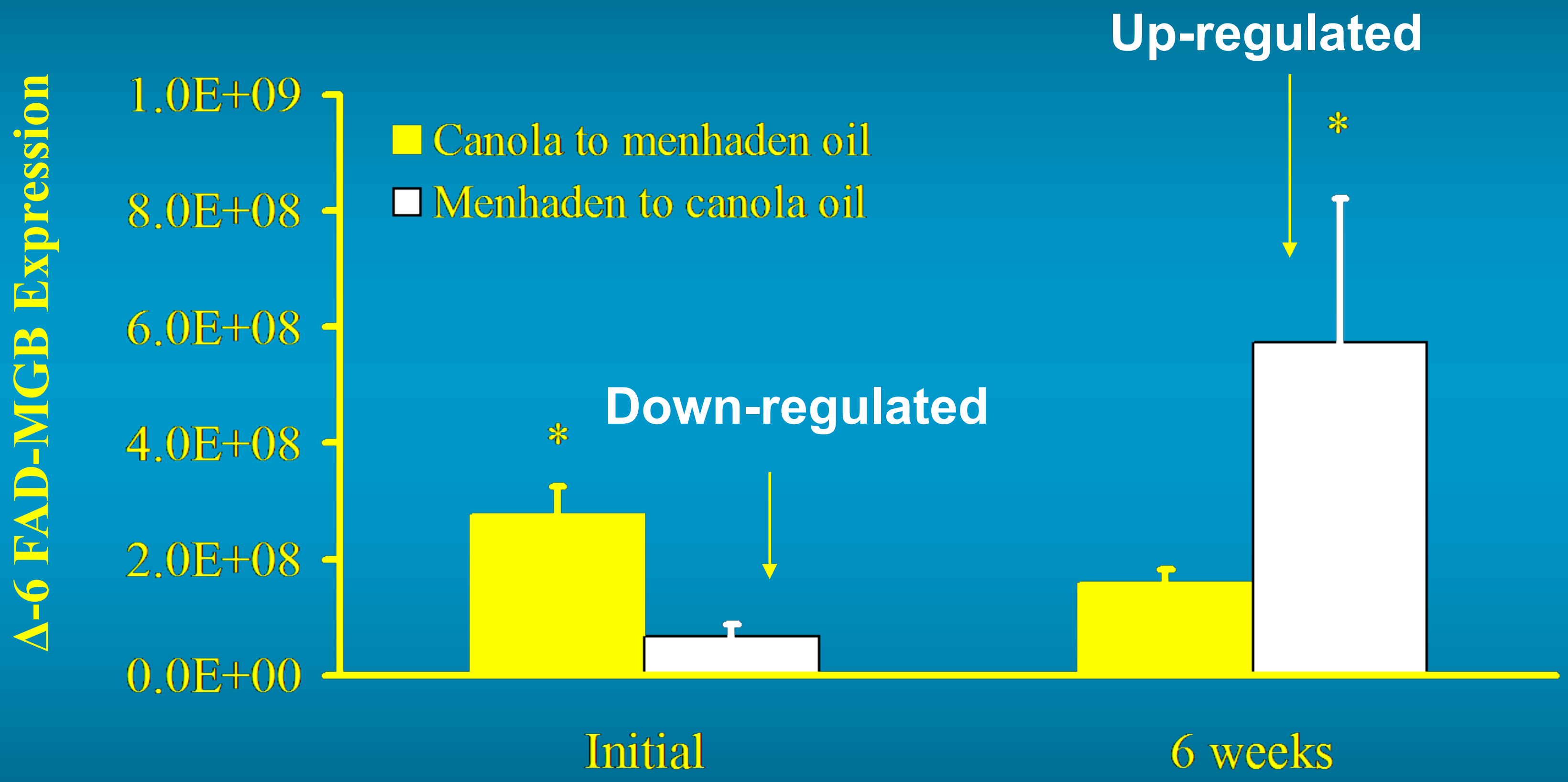


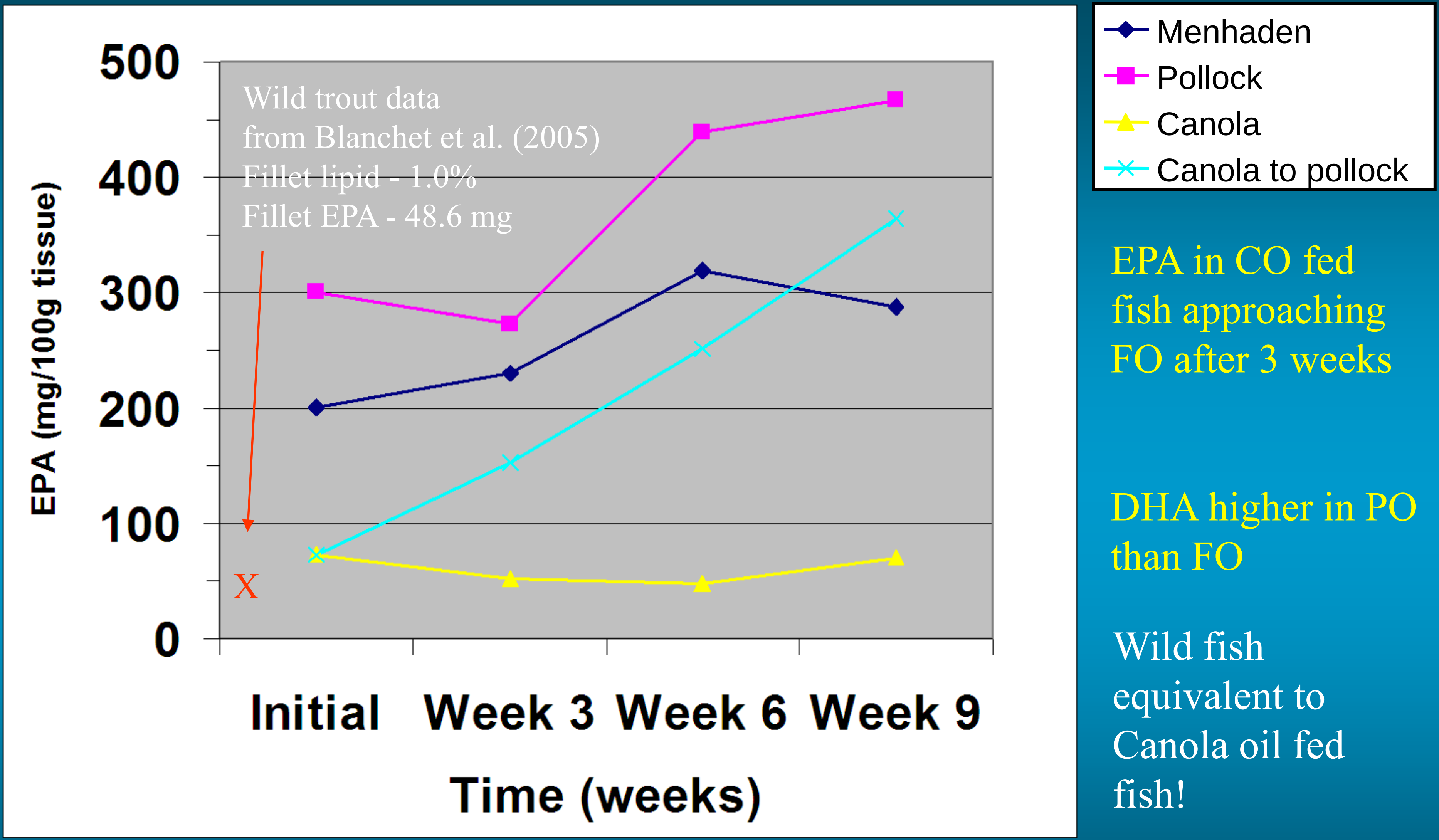
had attained an average weight of 1118 g fish-1 with an SGR of 0.905 and an FCR of 1.17 * Denotes significant differences within dietary groups over time (One factor ANOVA, *P*<0.05).

Diet history: Previously fed oil source for 17 weeks then switched

Initial weight 807g. At the completion of the experiment fish

Δ-6 fatty acid desaturase expression in trout after switching dietary oil source for six weeks





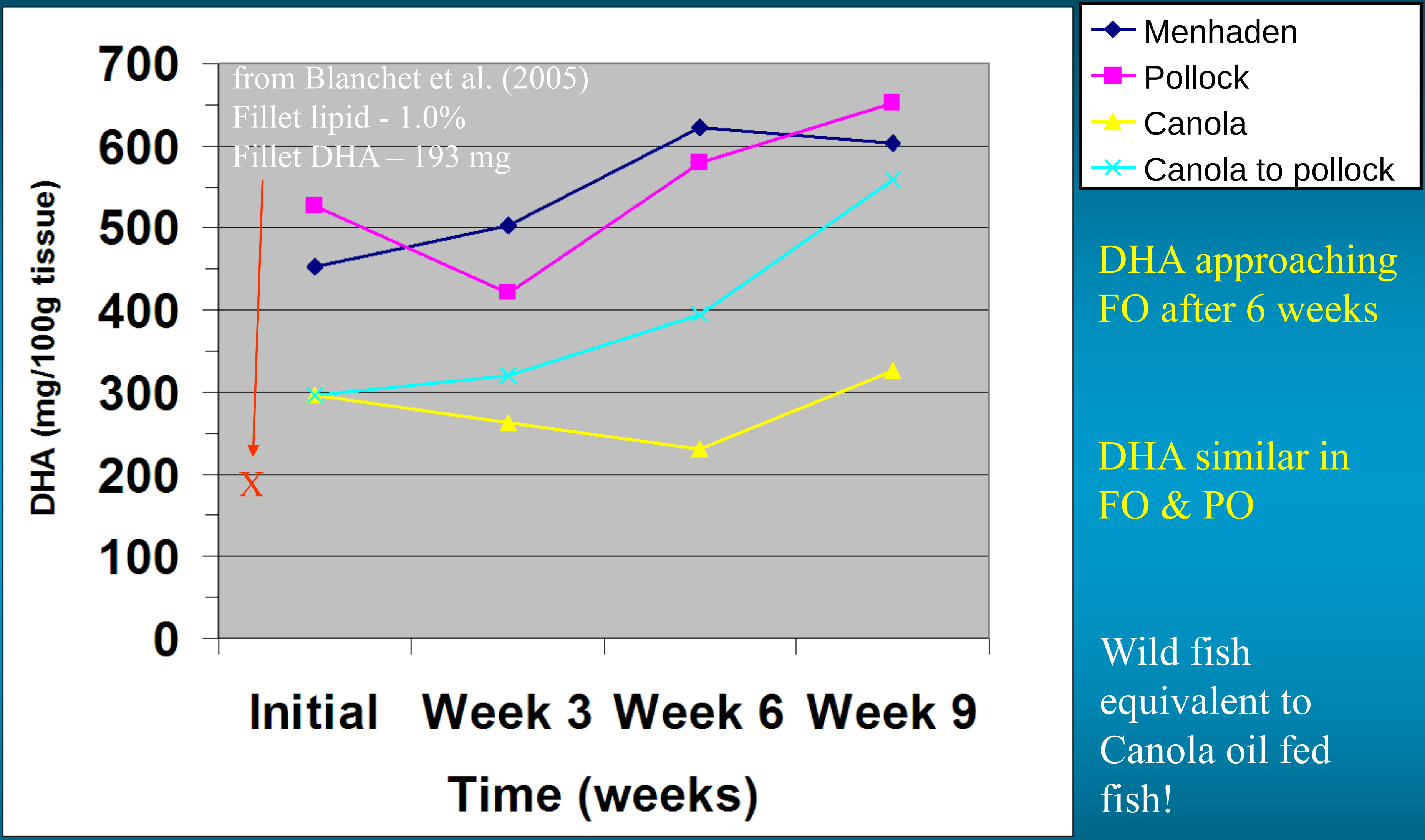
EPA in CO fed fish approaching FO after 3 weeks

DHA higher in PO than FO

Wild fish equivalent to Canola oil fed fish!

Phase II: EPA levels in trout fillets

Phase II: DHA levels in fillets of trout



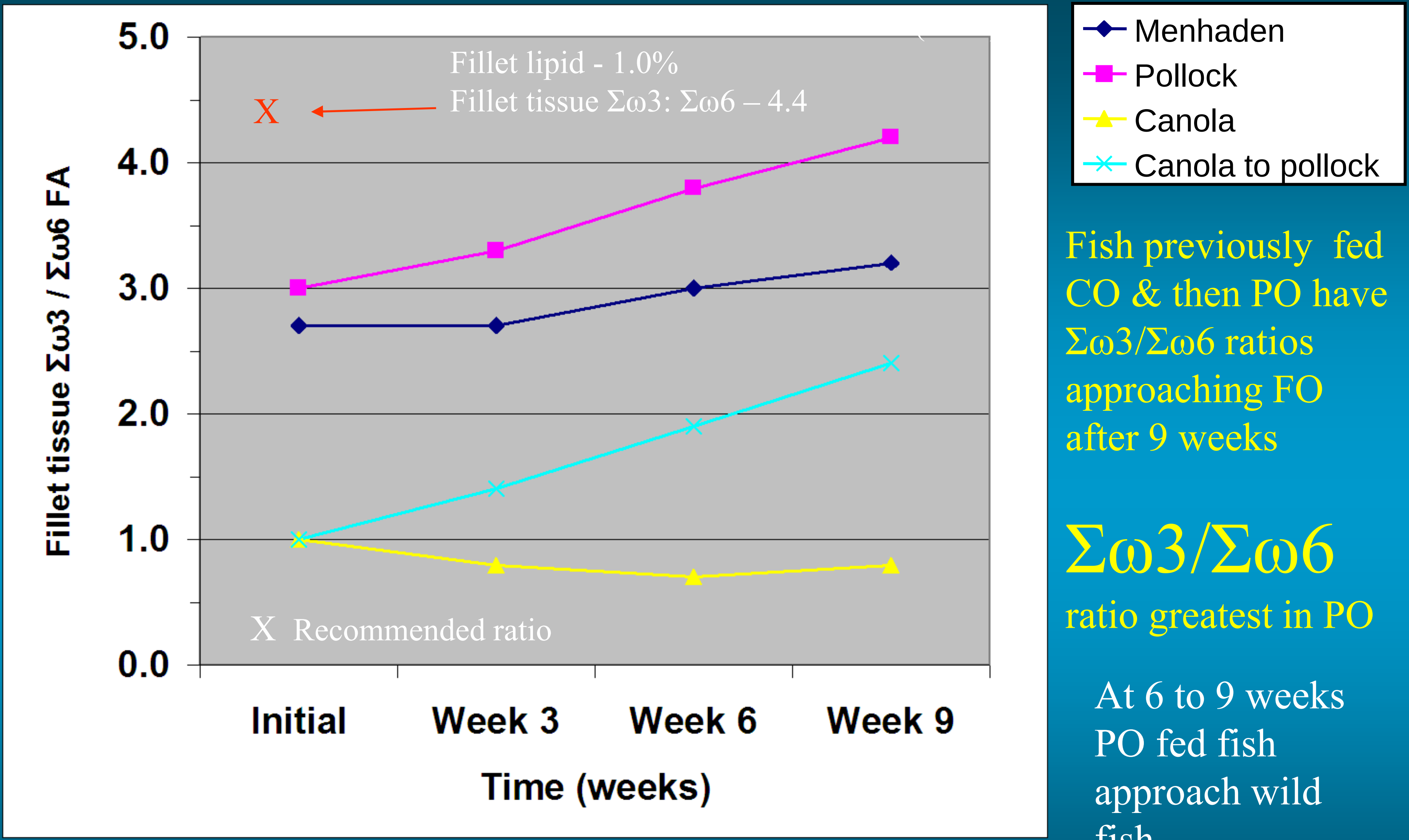
from Blanchet et al. (2005)
Fillet lipid - 1.0%
Fillet DHA – 193 mg

DHA approaching FO after 6 weeks

DHA similar in FO & PO

Wild fish equivalent to Canola oil fed fish!

Phase II: Ratio of fillet tissue Σω-3/Σω-6 FA.



Fish previously fed CO & then PO have Σω3/Σω6 ratios approaching FO after 9 weeks

Σω3/Σω6 ratio greatest in PO

At 6 to 9 weeks PO fed fish approach wild fish

Recommended EPA & DHA intake

For primary prevention of coronary heart disease the International Society for the Study of Fatty Acids & Lipids recommends consumption of **500 mg of EPA + DHA daily**

(Blanchet et al. 2005)

The importance or the ratio of ω-3 to ω-6 fatty acids in human nutrition & health

- ω-3 & ω -6 FA compete for the same metabolic enzymes
- Thus the ω-3:ω-6 ratio will influence the ratio of the ensuing eicosanoids (hormones)
- This will alter the body's metabolic function
- Typical Western diets are dramatically skewed to high ω-6 FA intake, leading to ratios ranging from 1:10 to 1:30 (Simopoulos 2000)
- For human health, the ideal ratio of ω-3 to ω-6 FA is reported to be approximately 1:4

Omega-3 content of various fish fillets

required amount of fillet to be consumed to meet the recommended daily intake level of 500 mg EPA+DHA/day

Source of fillet	Amount of fillet (g)	Σω-3/Σω-6
Wild fish	*202*	4.4
Pollock oil *400g fish*	61	3.0
Pollock oil *800g fish*	45	4.2
Canola to pollock oil *800g fish*	54	2.4
Recommended		1:4

Sensory attributes in fish

- Color
  - Salmon and trout fillets (muscle)
  - External (skin) coloration in selected marine fish species
  - Color is caused by carotenoid pigments, mainly astaxanthin and canthaxanthin
  - Fish cannot synthesize carotenoid pigments; they must be supplied in the diet
- Taste and odor
  - Humans can detect five tastes (sweet, sour, salty, bitter and unami)
  - Humans can detect 5000+ odors using sense of smell
  - Most people associate flavor with taste, but it is actually odor
- Texture
  - Amount of connective tissue in beef muscle affects texture, but this is not much of a factor affecting texture in fish
  - Muscle fiber number correlated with texture in fish
  - Muscle lipid content associated with texture in fish

Sensory attributes in fish

- **Fillet color**
  - Salmon and trout deposit astaxanthin in muscle during post-juvenile growth stage
  - Astaxanthin withdrawn during maturation process
    - Deposited in eggs in females
    - Deposited in skin in males
  - Astaxanthin levels of at least 4 ppm are required to properly pigment salmon fillets to meet consumer expectations
  - High intake of astaxanthin results in crystal formation in the fluid in the eye – problem in France for sunbathers taking ultra-high amounts of astaxanthin as a skin tanning agent
  - A human could not eat enough salmon to equal the dose of astaxanthin known to cause crystals in the eye

- Skin pigmentation occurs at lower intake levels
- Shrimp (*P. monodon*) supplied with dietary astaxanthin to ensure proper color after cooking

Sensory attributes in fish

- Flavor is a combination of taste and odor
  - Humans taste buds are located on the tongue and they can detect five tastes (sweet, sour, salty, bitter and unami)
  - Humans can detect >5000 odors using sense of smell
  - Flavor is caused by release of odor by cooking and when chewing fish
- Flavor of fish can be altered by diet and by environmental factors in pond fish (off-flavors)
- Dietary oil source is responsible for most of the flavor of fish
- Trained taste panelists learn “descriptors” to use when tasting fish, such as fishy, nutty, grassy, and so on.
  - Fishy flavor when menhaden oil or tuna oil used in feed
  - Fishy flavor diminished or disappears when plant oils replace fish oil in the feed

Storage stability of farmed fish

- Fresh fish spoilage
  - Associated with inadequate temperature control during storage
  - Caused by surface bacterial spoilage and enzymatic degradation of tissues
  - Not something that can be altered by diet of farmed fish
- Frozen fish spoilage
  - Associated with lipid oxidation
  - Caused by slow freezing, freezing at borderline temperatures (should by -20C or below)
  - Associated with slow thawing and long holding before cooking
  - Can be detected by smell, but more often by a metallic aftertaste in the mouth after swallowing

Modifying frozen storage stability of farmed fish fillets

- Frozen fish spoilage is from lipid oxidation
- Oxidation occurs once natural antioxidants in tissues are used up
- Oxidation can proceed while product is frozen if temperature of freezer cycles to near thawing
- Oxidation can occur rapidly after thawing
- Oxidation can be delayed by feeding high doses of α-tocopherol (vitamin E)
  - Dietary requirement is 20-50 mg/kg feed
  - Dietary level to increase tissue levels to delay oxidation is time-dependent, but ranges from 500-1500 mg/kg feed

Safety of farmed fish

Consumers expect farmed fish products to be free of contaminants

- Contaminants of concern
  - Heavy metals, mainly methylmercury
  - Persistent organic pollutants (POPs)
  - Chemotherapeutics, hormones
  - Naturally-occurring toxins
- Parasites, worms
- Microbial spoilage

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Methylmercury facts

- Heavy metals include lead, cadmium and mercury, but mercury is the main element of concern
- Most mercury in the environment is of natural origin
- Elevated mercury associated with human activity is primarily downwind of coal-powered electrical plants and gold mines
- Mercury in seafood is present as methylmercury and is associated with protein fraction of tissues
- Methylmercury levels are high in long-lived, large predacious species such as marlin, swordfish, blue-fin tuna, sharks, but this depends on size of fish, location, and other factors
- Methylmercury levels are low in short-lived, small pelagic species such as anchovy, herring, sardines, capelin
- All fish bio-accumulate methylmercury in their tissues

Methylmercury in farmed fish

- Most farmed fish are relatively short-lived and not reared to large sizes, so dietary intake is insufficient to result in significant accumulation of methylmercury
- Fish meal is the only significant potential source of methylmercury in the diet of farmed fish
- Other dietary ingredients are not sources of methylmercury
- Fish meal levels in aquafeeds are decreasing due to increasing costs and development of viable alternate proteins
- As a result, methylmercury levels in farmed fish products are virtually nil, making this a non-issue

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POP facts

- Persistent organic pollutants (POPs) are primarily chlorinated or brominated compounds
- Some are pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex and toxaphene)
- Some are industrial chemicals (hexachlorobenzene, PCBs)
- Some are produced by combustion (PCBs, dioxin, furans)
- POPs of concern in fish are dioxin, dioxin-like compounds and PCBs, and also PBDEs (flame-retardant)
- All are lipophilic, meaning that they are lipid-soluble and found in lipid fraction of fish tissues
- Fish oil and fish meal are the only ingredients in aquafeeds that are likely to contribute POPs to farmed fish

POPs in farmed fish

- POPs are present in some fish oils and fish meals, mainly those from polluted areas (Baltic and North Seas)
- POPs are virtually absent from anchovy meal or Alaskan fish meals
- EU has implemented strict regulations on POP levels in fish feeds
- Several European fish meal producers now treat all fish meal and oil with activated carbon to removed POPs so that fish feed producers can meet EU standards
- POP levels in farmed fish are very low, according to recent studies in Canada comparing wild and farmed salmon
- POP levels in aquafeeds can be minimized by ingredient choice and feed formulation

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Safety of farmed fish

- Chemotherapeutics include antibiotics and drugs added to rearing water
  - Illegal use of chemotherapeutics can lead to residues in farmed fish
  - This is not a problem in well-regulated aquaculture industries
- Naturally-occurring toxins occur in wild fish products, but are absent in farmed fish
- Parasites and worms are also generally absent in farmed fish compared to wild fish
- Not much to add in terms of nutritional approaches

Advances in research pertaining to fish quality and nutrition

- Nutritional genomics (nutrigenomics) involves measuring expression of genes that respond to different dietary factors
  - Digestion, nutrient transport, metabolism, nutrient partitioning, protein synthesis, protein turnover, and so on respond to nutritional inputs
  - Studying expression of regulatory genes in various pathways will provide insight into physiological processes
- Nutrigenomics is relevant to growth, immune function, reproduction, and quality of farmed fish

Two examples of current research that may lead to nutritional approaches to fish quality

- Studies of genes associated with muscle fiber number and how to influence via nutrition
- Studies of nutrient transport mechanisms and how to influence via nutrition

University of Idaho team has discovered the first evidence that the capacity for muscle hyperplasia is nutrient (dietary CHO) sensitive in fish: potentially high impact to affect muscle fiber numbers and thereby affect texture of fillets

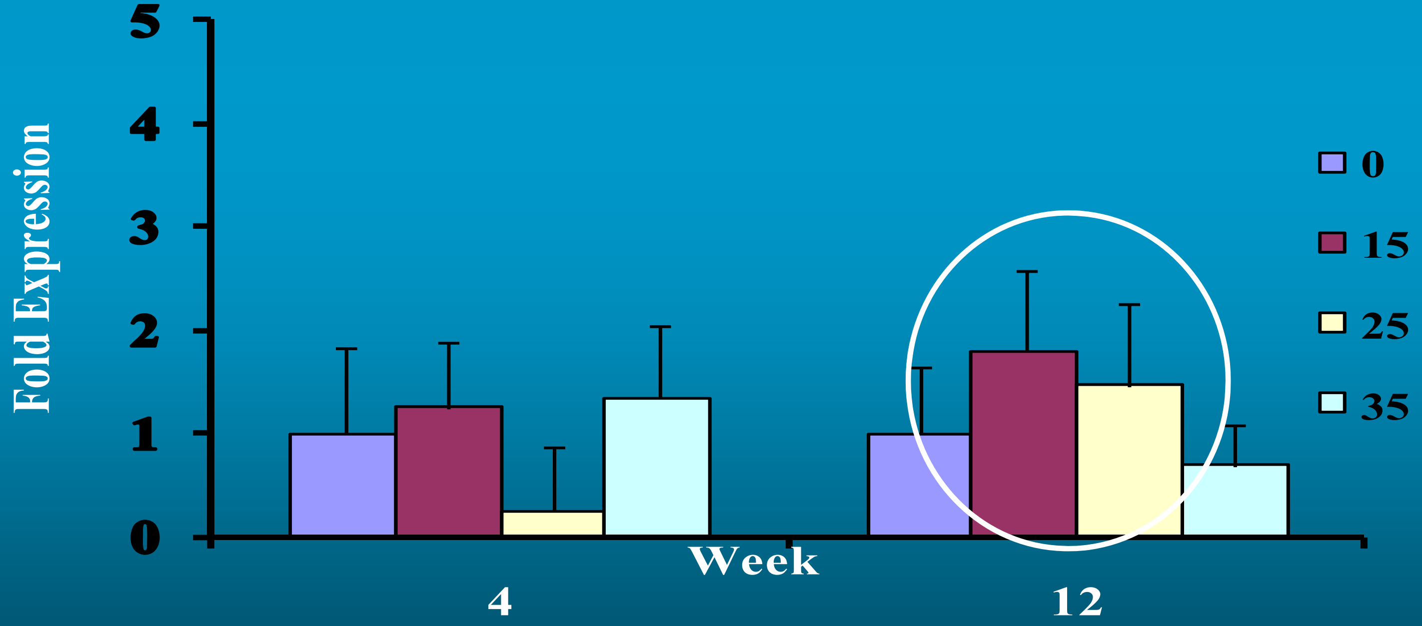
Context: Fish are classified as "indeterminate or semi-determinate" in their muscle growth

- **Determinate: increase muscle by hypertrophy**
- **Indeterminate: increase muscle by hyperplasia**
  - **Hyperplasia = increased muscle cell number**
  - **Hypertrophy = increased muscle cell size**

Mammals are determinate and can only increase the muscle cell size (hypertrophic growth) after birth

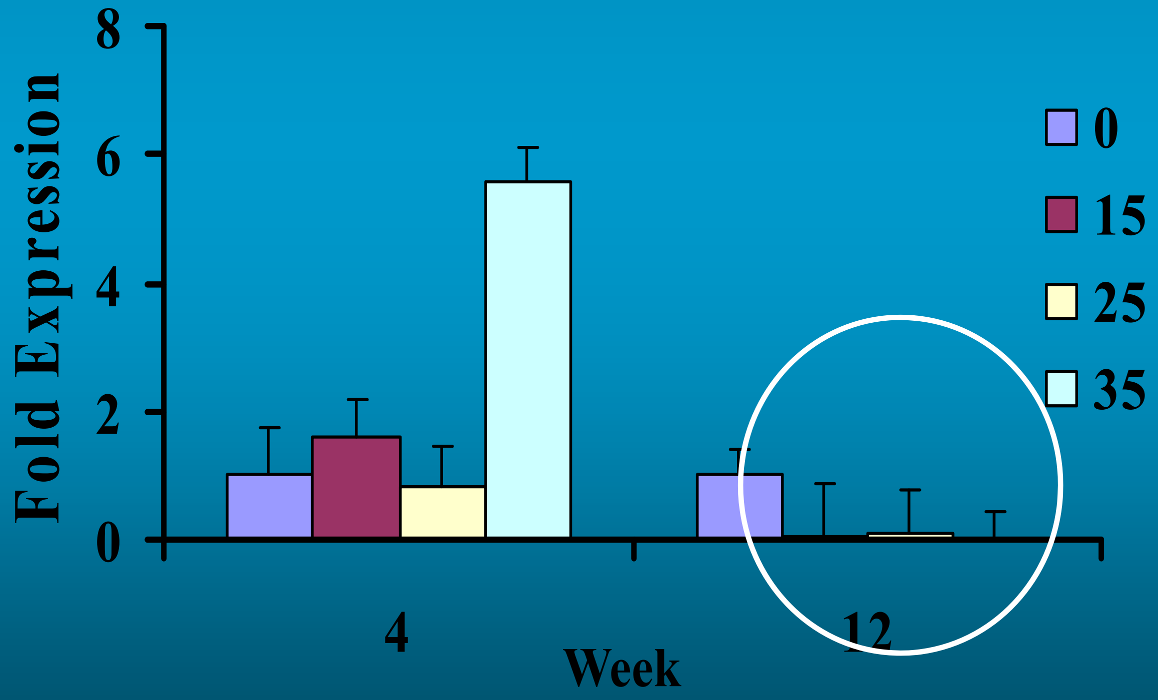
Pax7 Expression

- Muscle satellite cell marker
- Higher expression = more lifetime growth potential



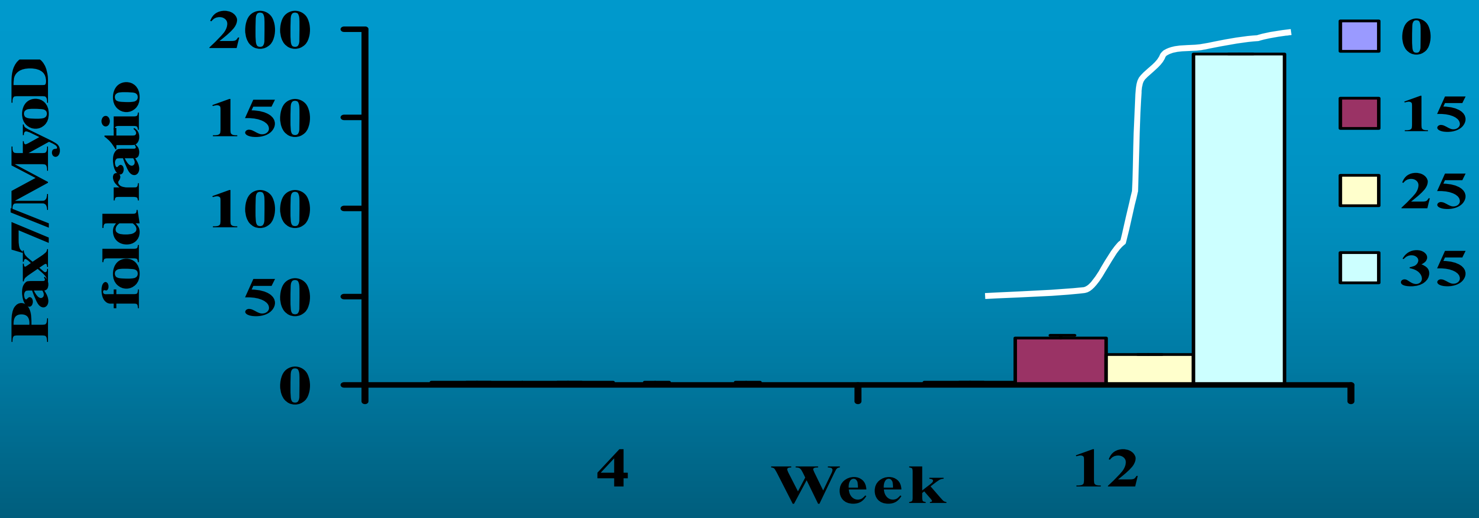
MyoD Expression

- Myogenic lineage determinant (more satellite stem cells become muscle cells)
    - Drives satellite cell differentiation



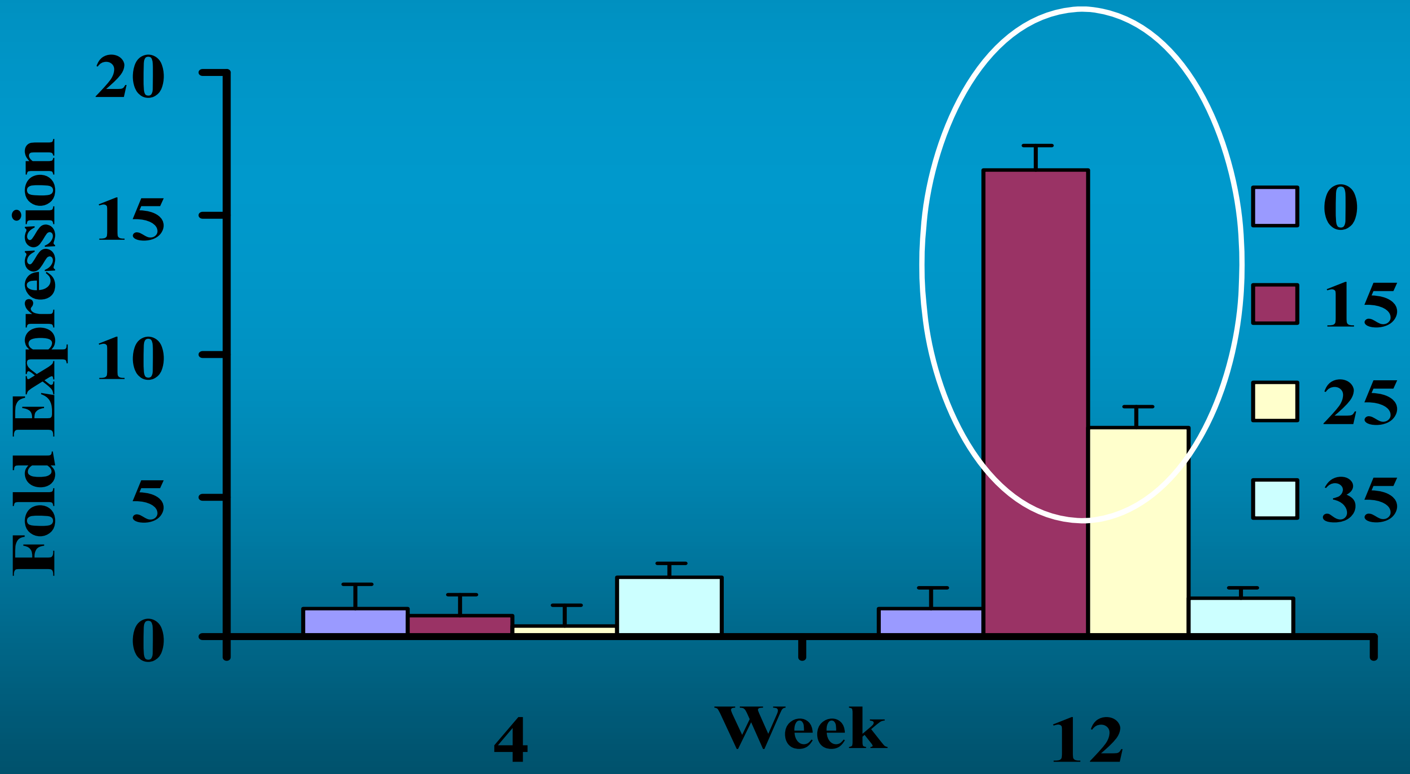
Pax7/MyoD

- Higher ratio = More satellite cell self-renewal
- Lower ratio = More satellite cell differentiation



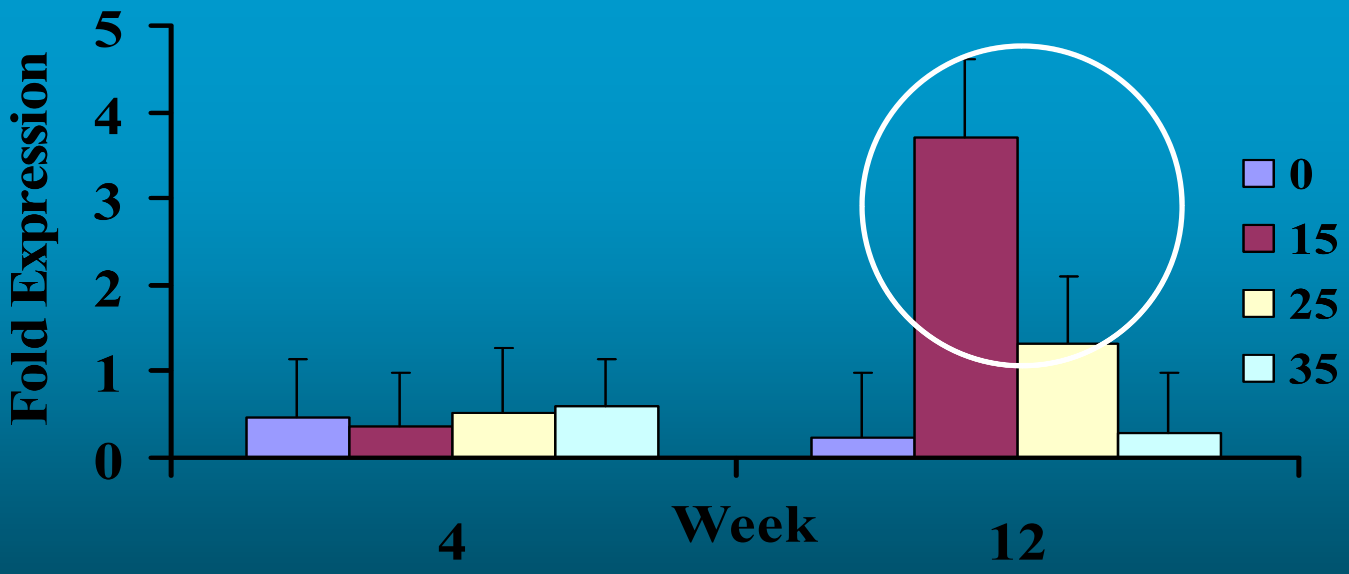
Mrf4 Expression

- Muscle fiber differentiation



Myogenin Expression

- Differentiation marker
- Higher expression = more muscle



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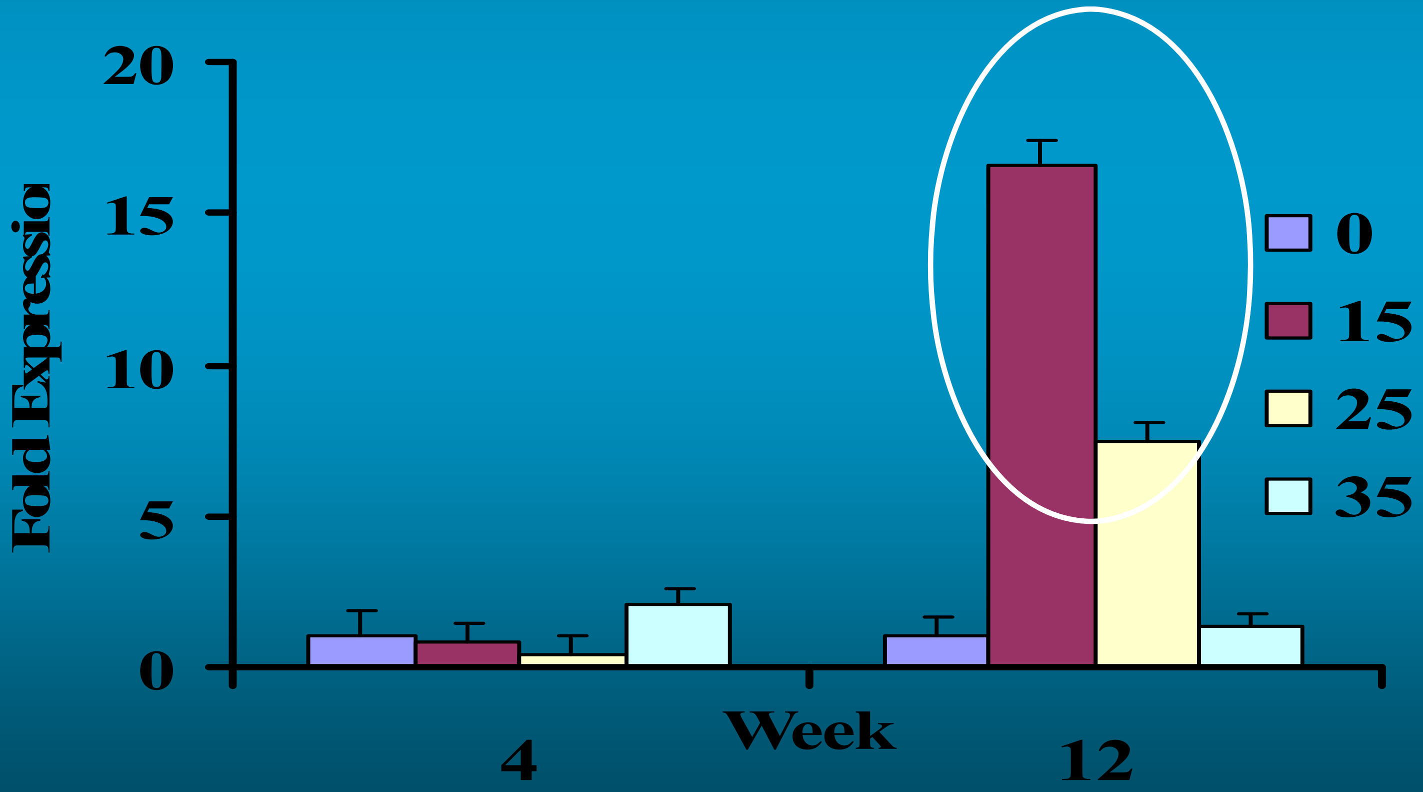

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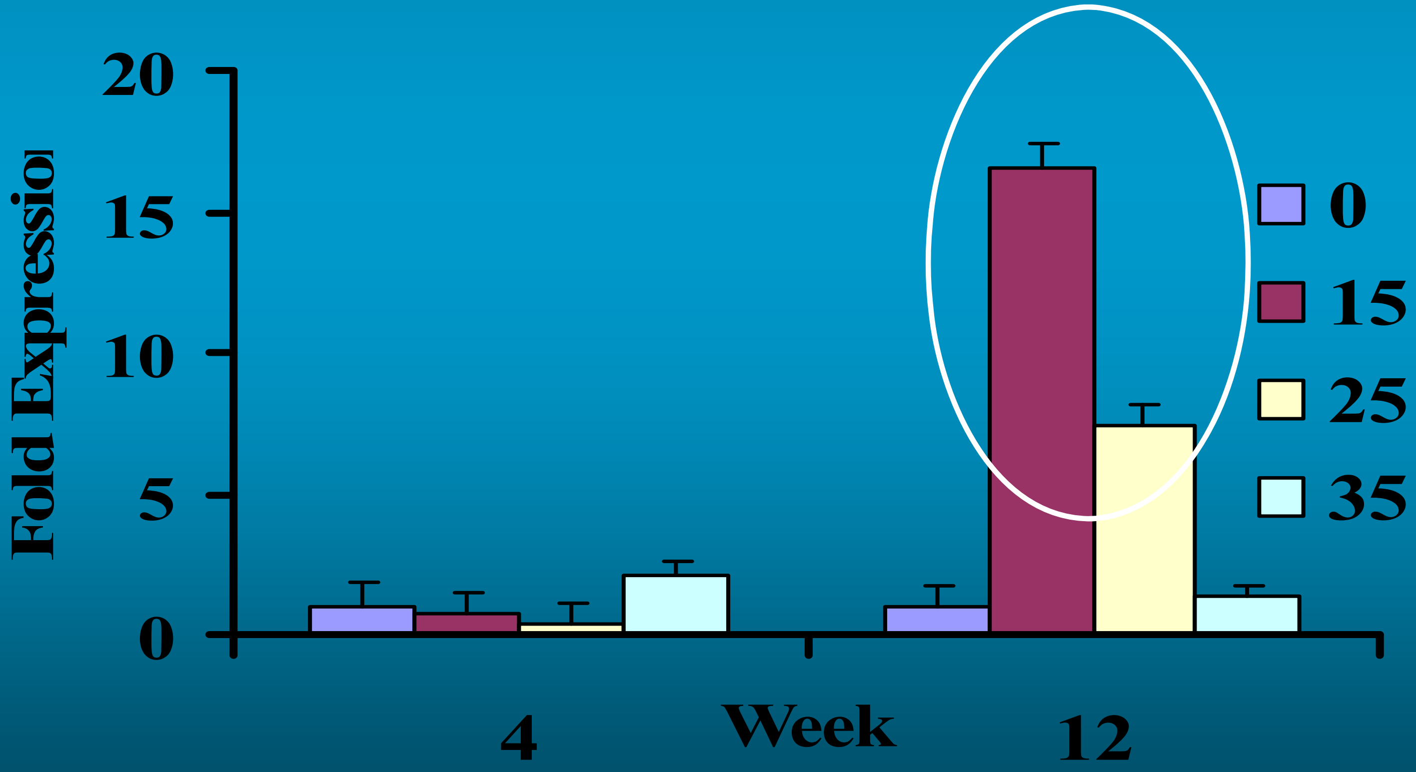

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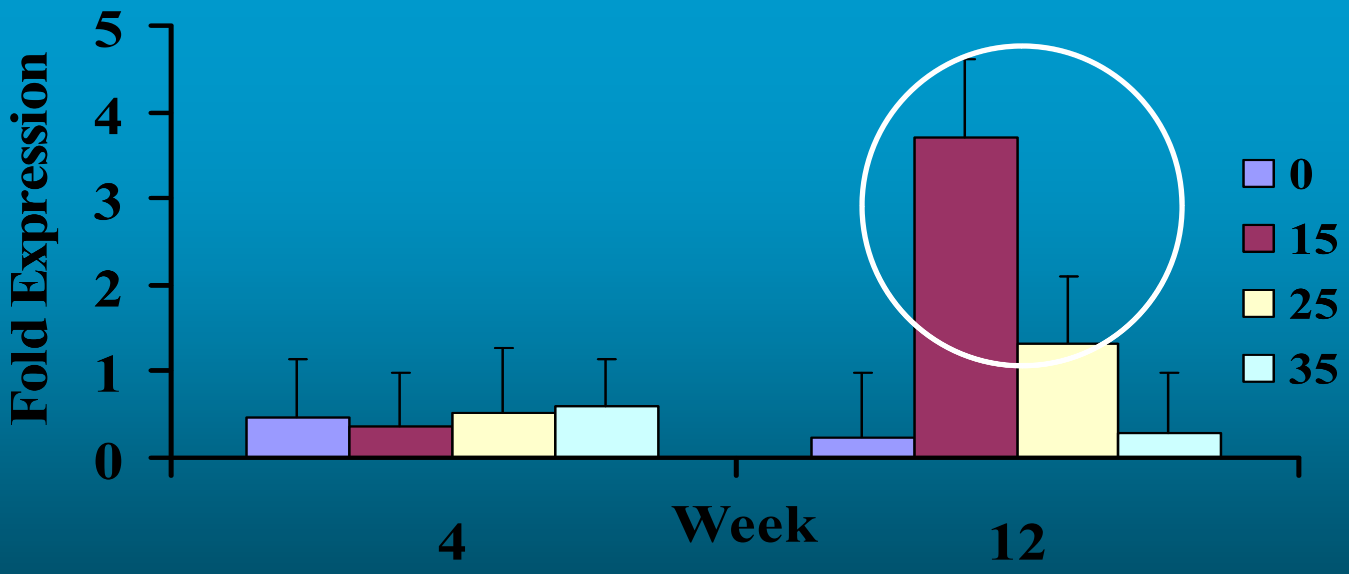
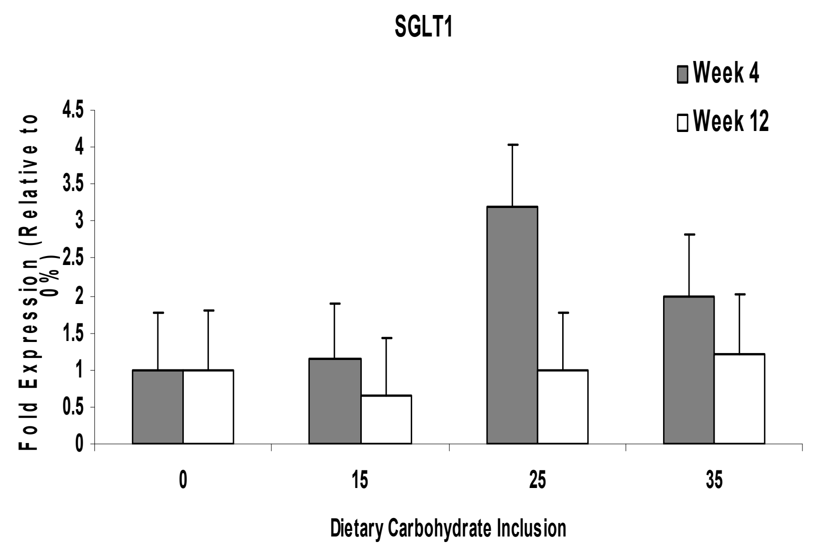
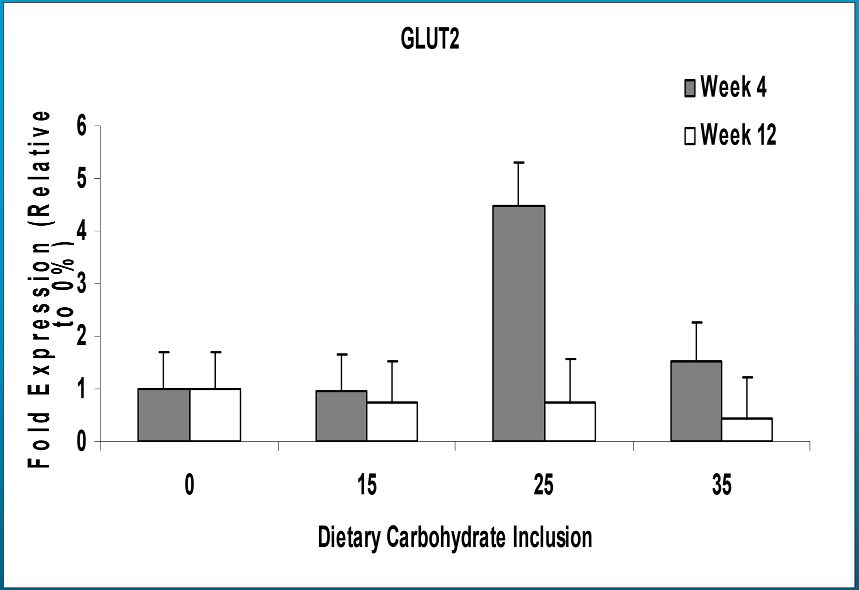


Gene expression of intestinal nutrient transporters

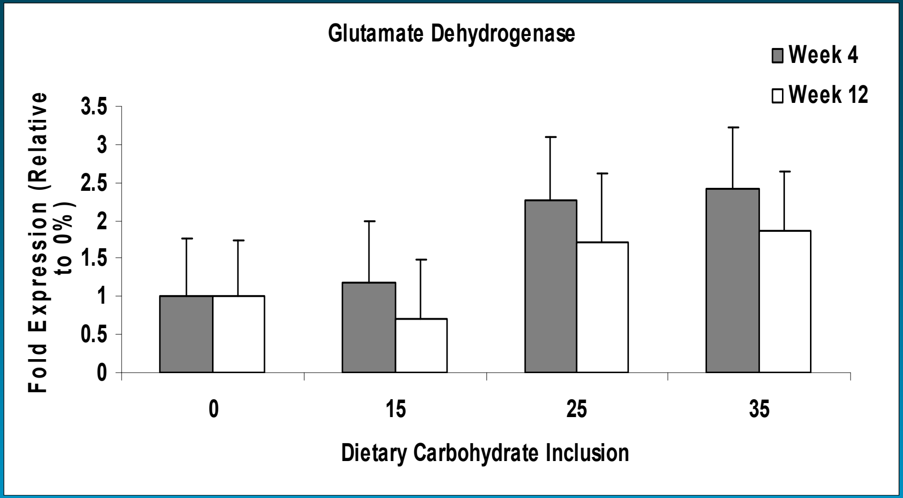
- Trout were fed diets varying in available starch levels (0%, 15%, 25% and 35%)
- Gene expression of intestinal transport systems was measured
- Gene expression of metabolic enzymes was also measured

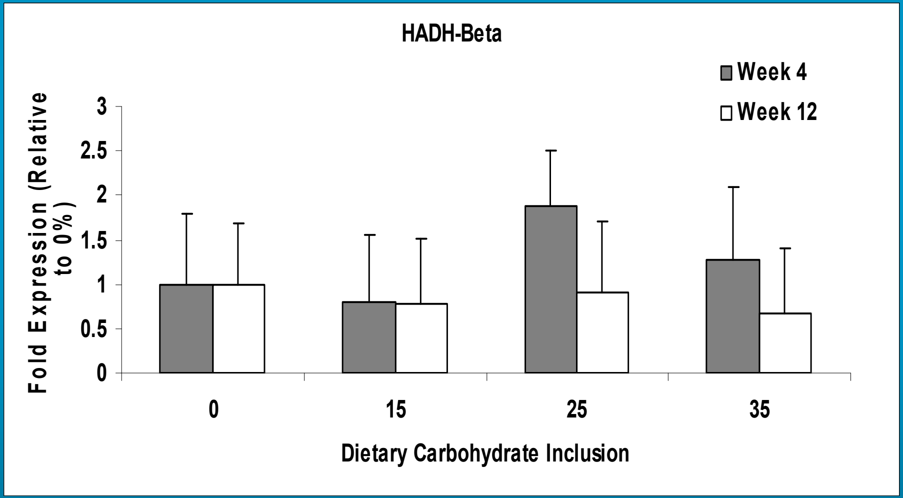
- SGLT1 & GLUT 2 account for 100% of luminal dietary glucose uptake
- Week-4 → expression significantly increases in fish fed 25% CHO
- Solute carriers, PepT1 & GLUT5 follow a similar pattern.
- Week-12 → No difference between treatments





- Amino acid catabolism increased in PC of fish fed > 15% CHO at week-4
- Beta oxidation remained constant
- No differences identified in either by week-12
- Acclimation to the diets





Concluding remarks

- Aquaculture has an advantage over wild fisheries products in that dietary inputs can be controlled
- Quality of farmed fish can be enhanced for the consumer
  - Farmed fish products as functional foods (mainly omega-3s)
  - Reduction of risk associated with contaminants to levels below detection
- Current estimates of benefit - risk ratio for fish consumption is 100:1; nutritional control in farmed fish can maintain or enhance this ratio for the consumer