

# The influence of dietary marine proteins, peptides and water soluble nitrogenous compounds on fish health and performance



MIC 2013, Holmenkollen, Norway

Katerina Kousoulaki

# *MARINE water-soluble components*

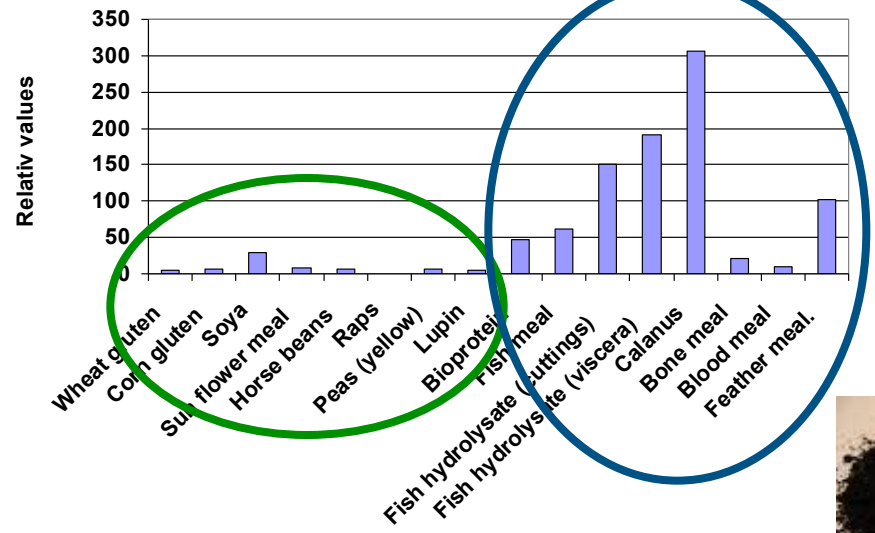
Main focus on water soluble **nitrogenous** compounds in feed ingredients with potential performance promoting effect

- Free amino acids
- Hydroxyproline
- Taurine
- Anserine / Carnosine
- Nucleotides
- Polyamines
- Peptides
- Glutamine

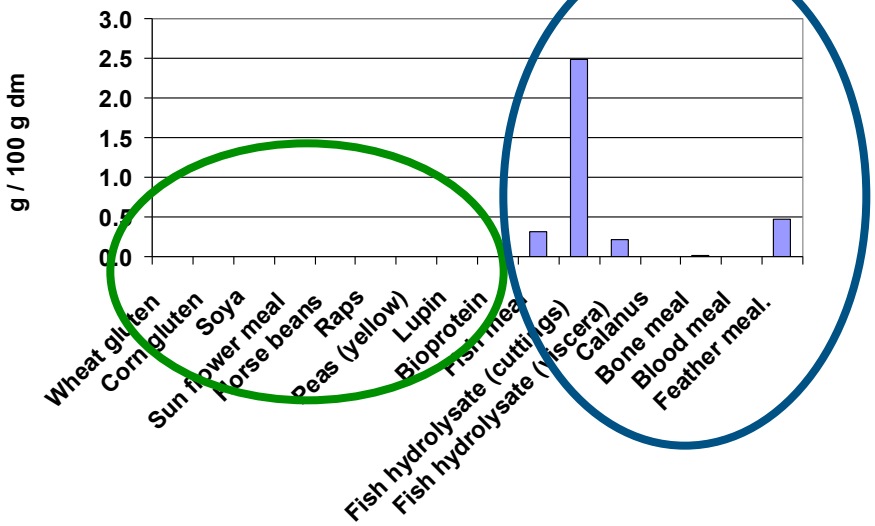


Photo by Gregersen, Nofima

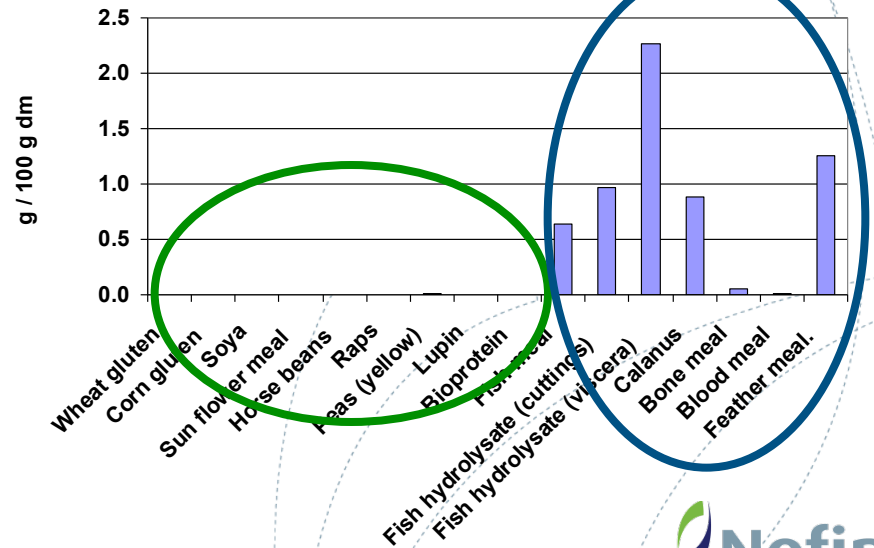
Sum Free Nucleotides in feed ingredients



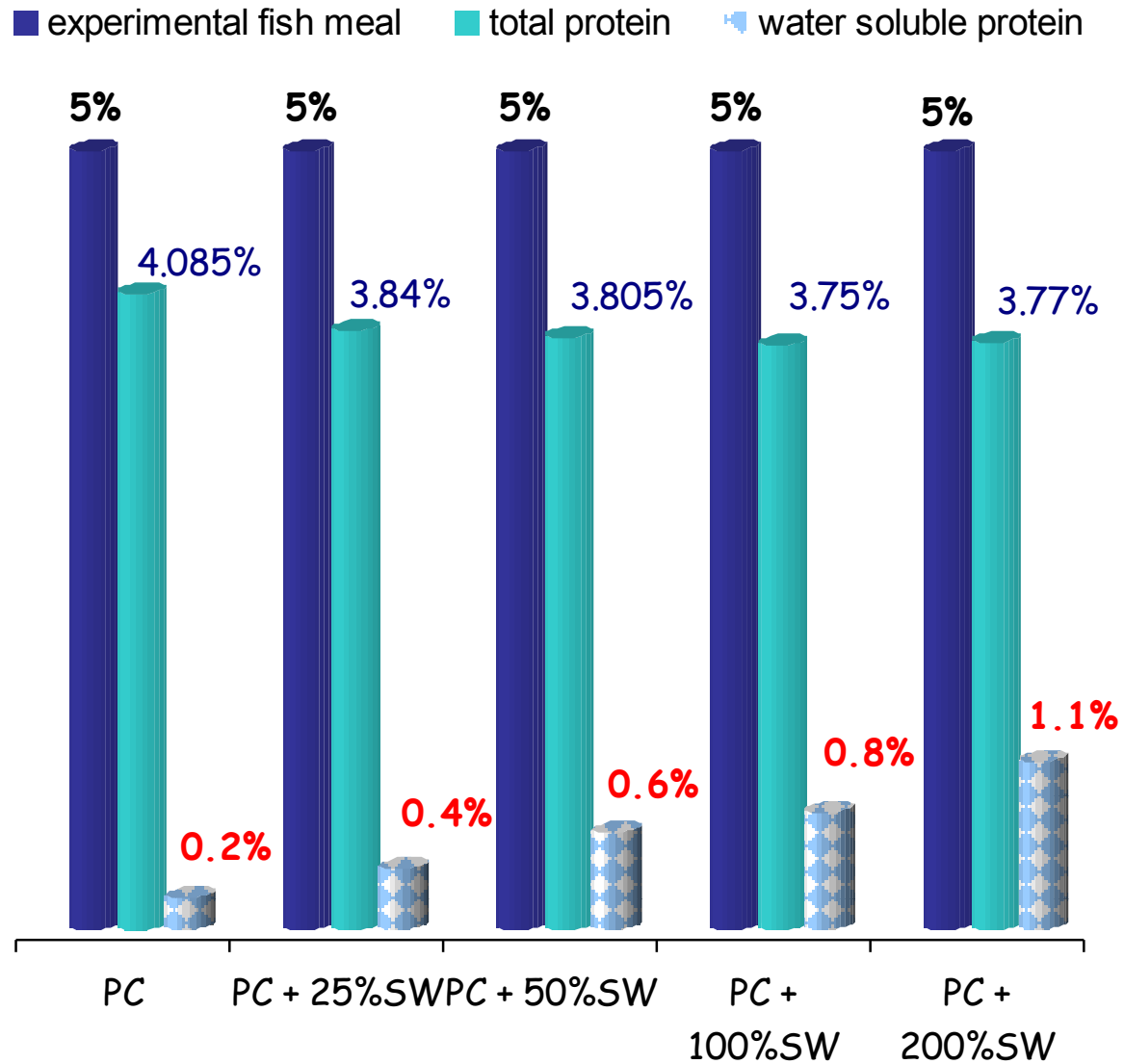
ANSERINE in feed ingredients



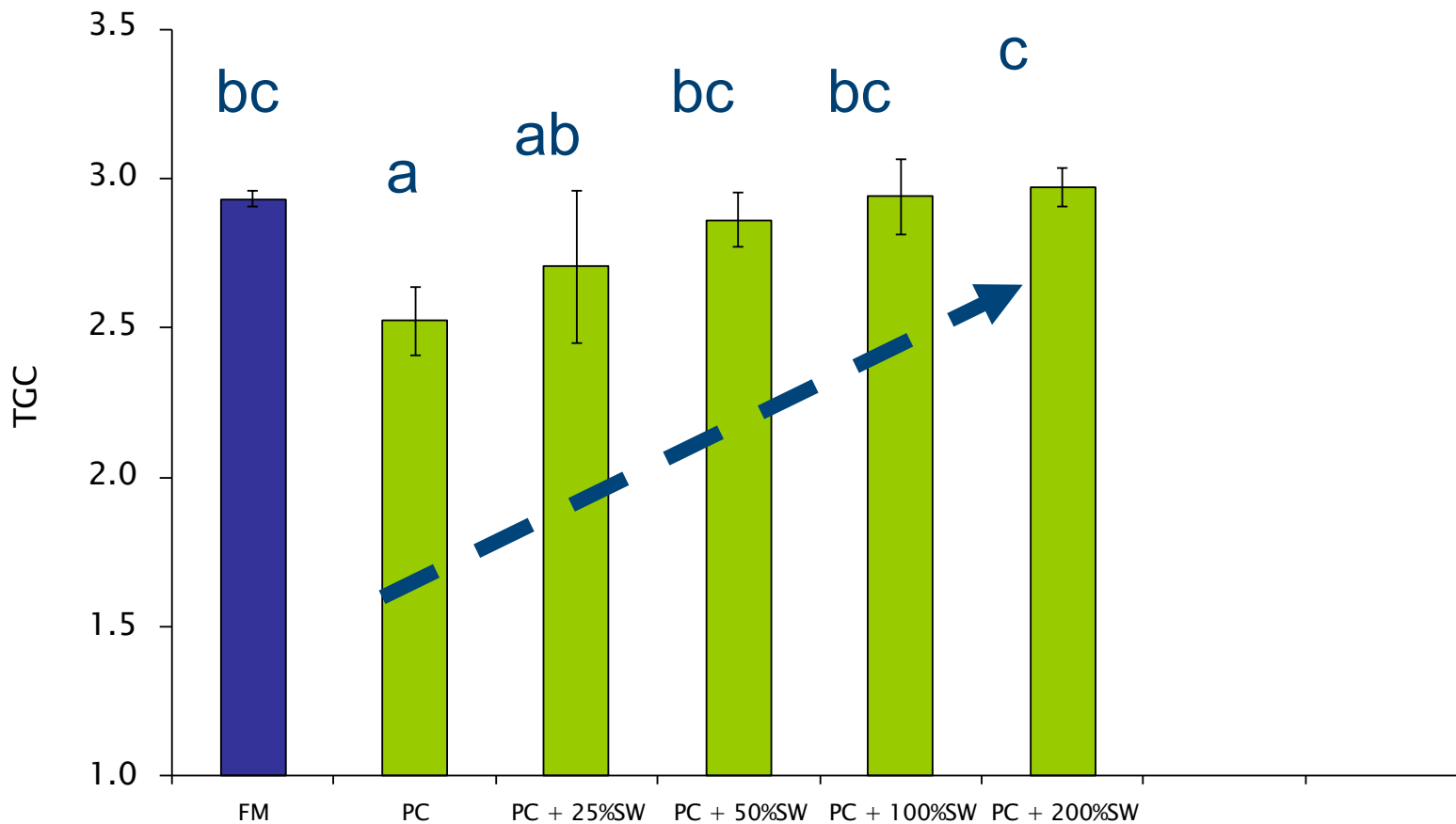
TAURINE in feed ingredients



# Low fish meal diets (5+5%)

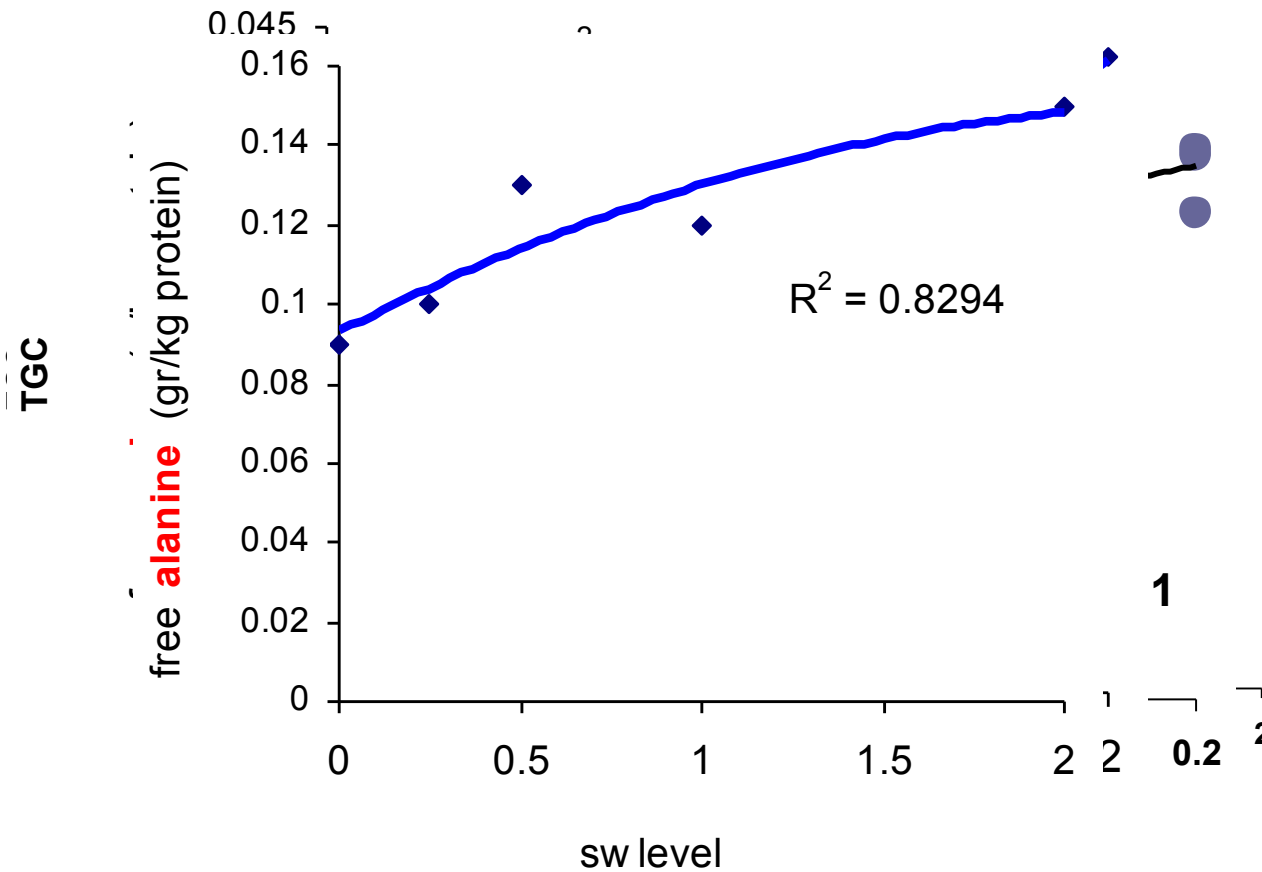


# Results - GROWTH

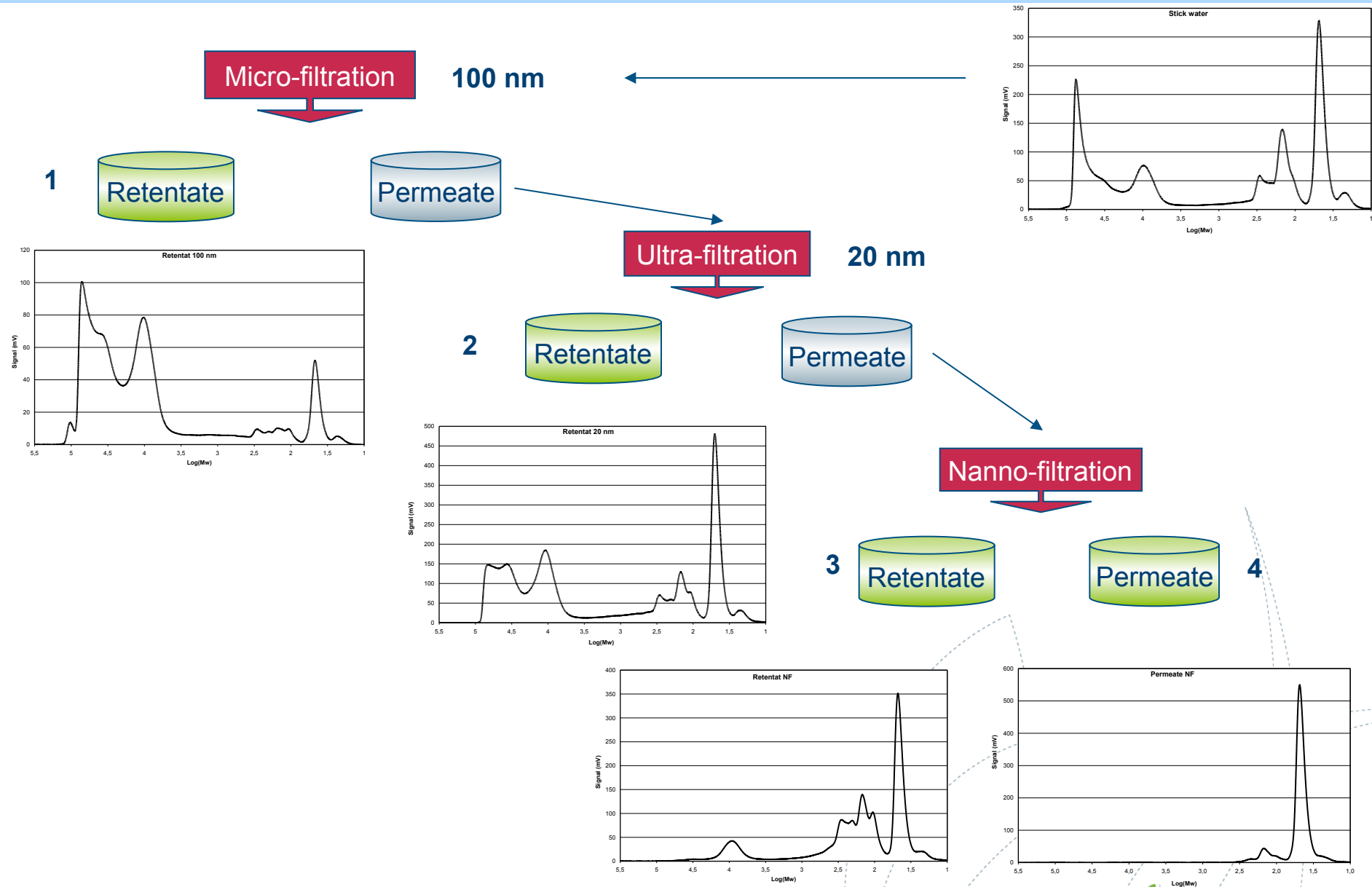


$P < 0.05$

# Marine soluble compounds in feed correlating with growth

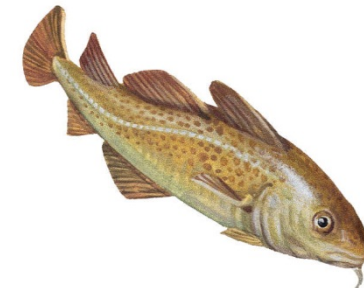


- MWS
- Tau
- Hyp
- Free Lys
- Free Gly
- Free Ser
- Free Ala





Nofima



## Dietary protein hydrolysates and free amino acids affect the spatial expression of peptide transporter PepT1 in the digestive tract of Atlantic cod (*Gadus morhua*)

Snorre Bakke<sup>a,\*</sup>, Ann-Elise Olderbakk Jordal<sup>a</sup>, Pedro Gómez-Requeni<sup>a</sup>, Tiziano Verri<sup>b</sup>, Katerina Kousoulaki<sup>c</sup>, Anders Aksnes<sup>c</sup>, Ivar Rønnestad<sup>a</sup>

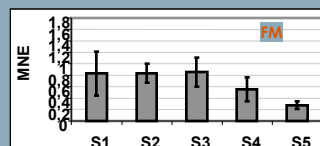
<sup>a</sup> Department of Biology, University of Bergen, NO-5020 Bergen, Norway

<sup>b</sup> Department of Biological and Environmental Sciences and Technologies, University of Salento (formerly University of Lecce), I-73100 Lecce, Italy

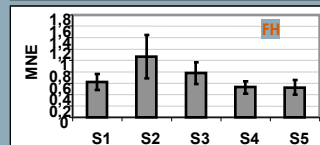
<sup>c</sup> NOHMA, NO-5141 Fyllingsdalen, Bergen, Norway



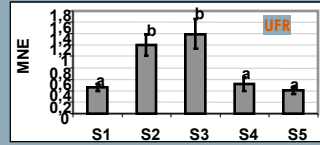
### Dietary comparison



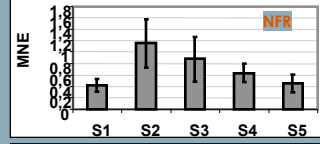
P = n.s



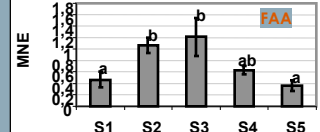
P = n.s



P < 0.01

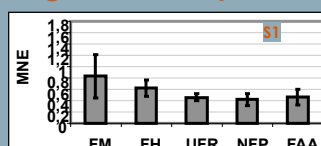


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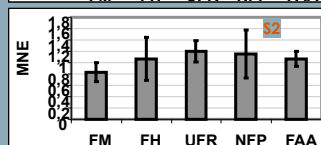


P < 0.01

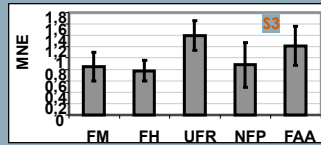
### Segmental comparison



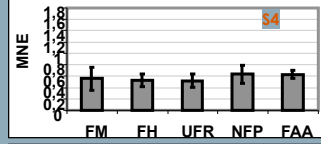
P = n.s



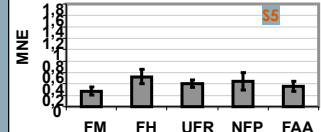
P = n.s



P = n.s



P = n.s



P = n.s

### Conclusion

**PepT1 mRNA expression is variably affected by dietary peptides as well as FAA.**

**Dietary hydrolysates, peptides in various chain length as well as free amino acids affect segments relative expression of PepT1 along the whole intestinal tract.**

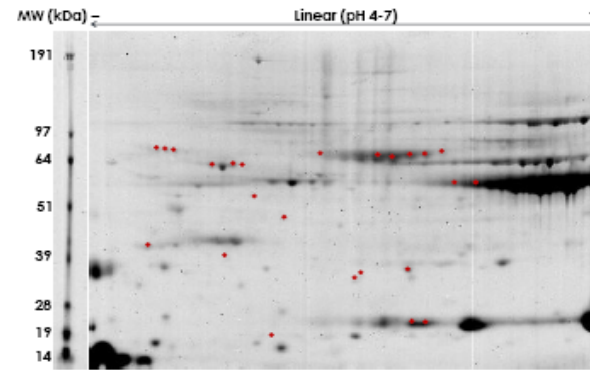
However, the regulation of PepT1 mRNA seems to be highest in the pyloric caeca and proximal segments, where maximal peptide load and maximal peptide variety is experienced by the fish after meal ingestion. The signaling pathways remains to be described.



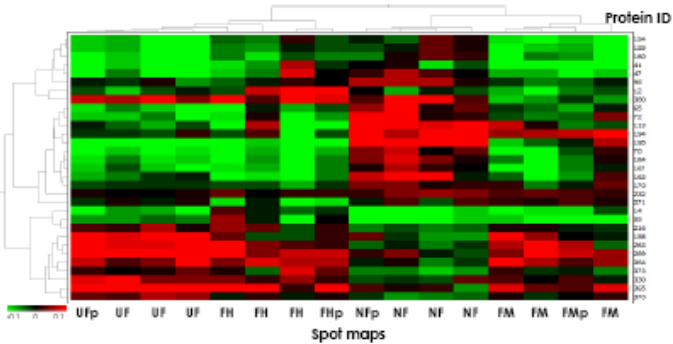
# Growth and muscle proteome response to fish protein hydrolysates in the diet of zebrafish

Mahaut de Vareilles 2,..., Katerina Kousoulaki 3,..., IvarRønnestad 1

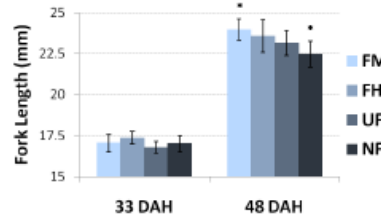
- 1 Department of Biology University of Bergen
- 2 CCMAR Universidade do Algarve
- 3 NOFIMA Ingrediens



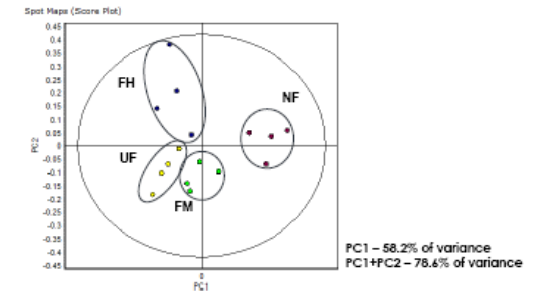
Low-salt soluble proteins (sarcoplasmic fraction) of *D. rerio* white muscle, stained with CBB G-250. Spots being sequenced are shown in red.



Hierarchical cluster analysis of differentially expressed proteins (One-way ANOVA;  $p$ -value<0.01); performed with Pearson's correlation as distance metric and complete linkage, using DeCyler 2-D software V7.0.



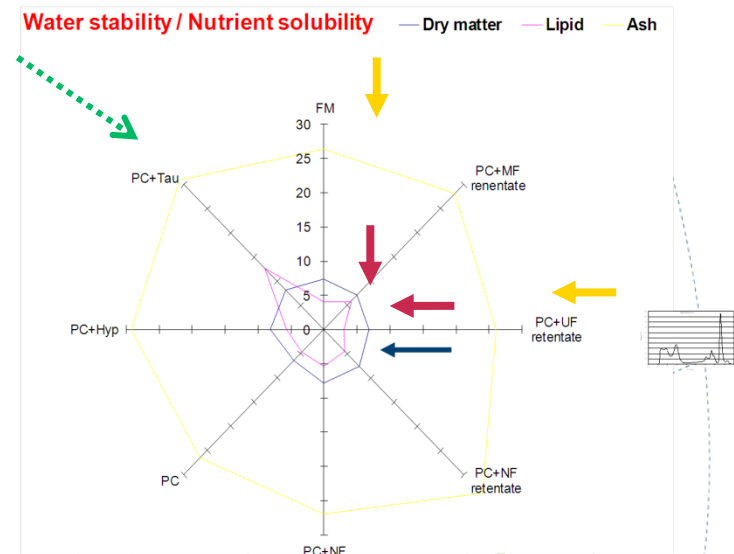
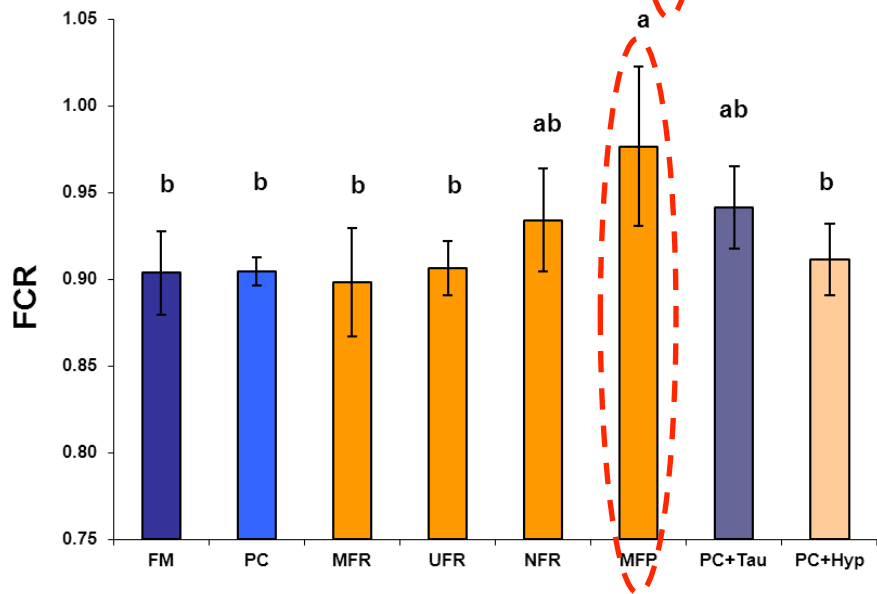
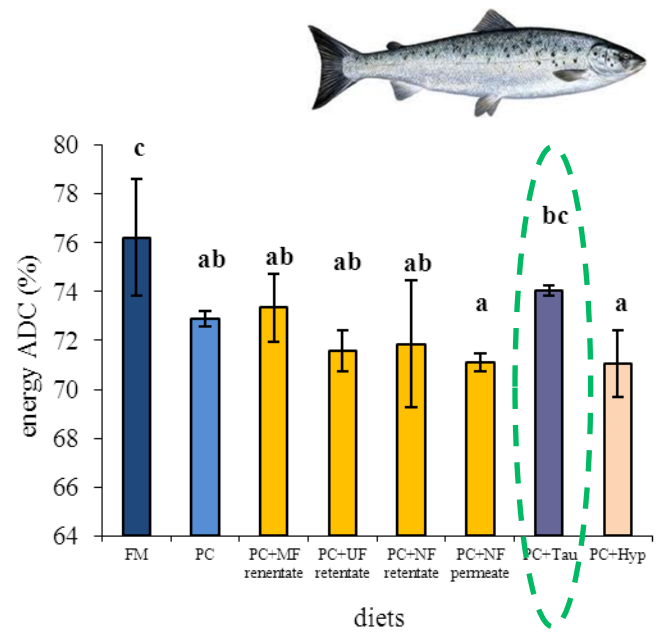
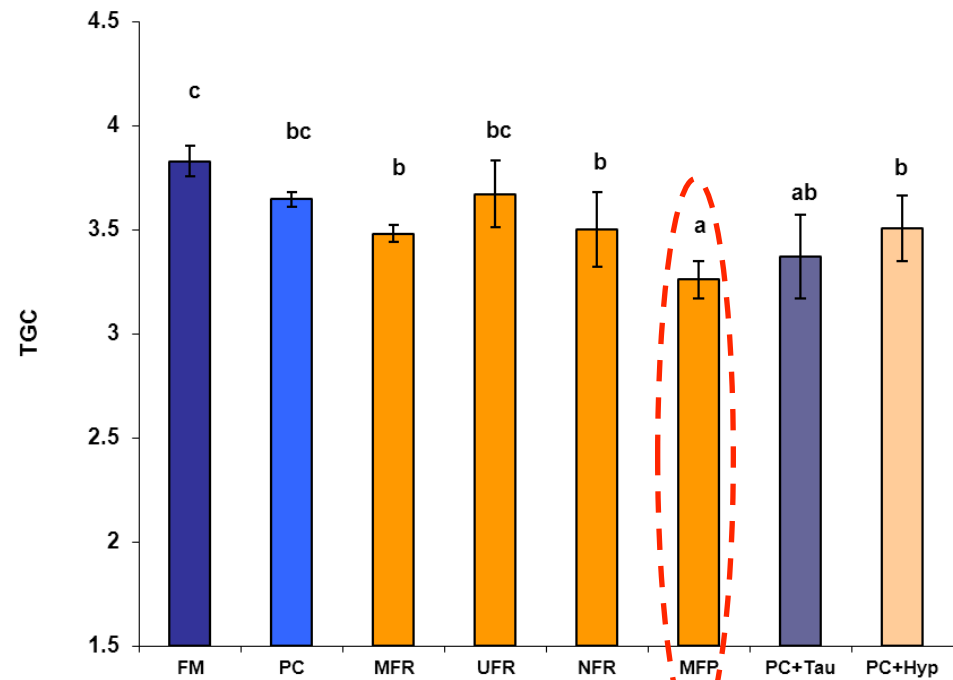
Growth of juvenile zebrafish during 15 day feeding trial, expressed as initial and final fork length (mm  $\pm$  S.D.) \* indicates significantly different values (One-Way ANOVA;  $p$ -value<0.05)



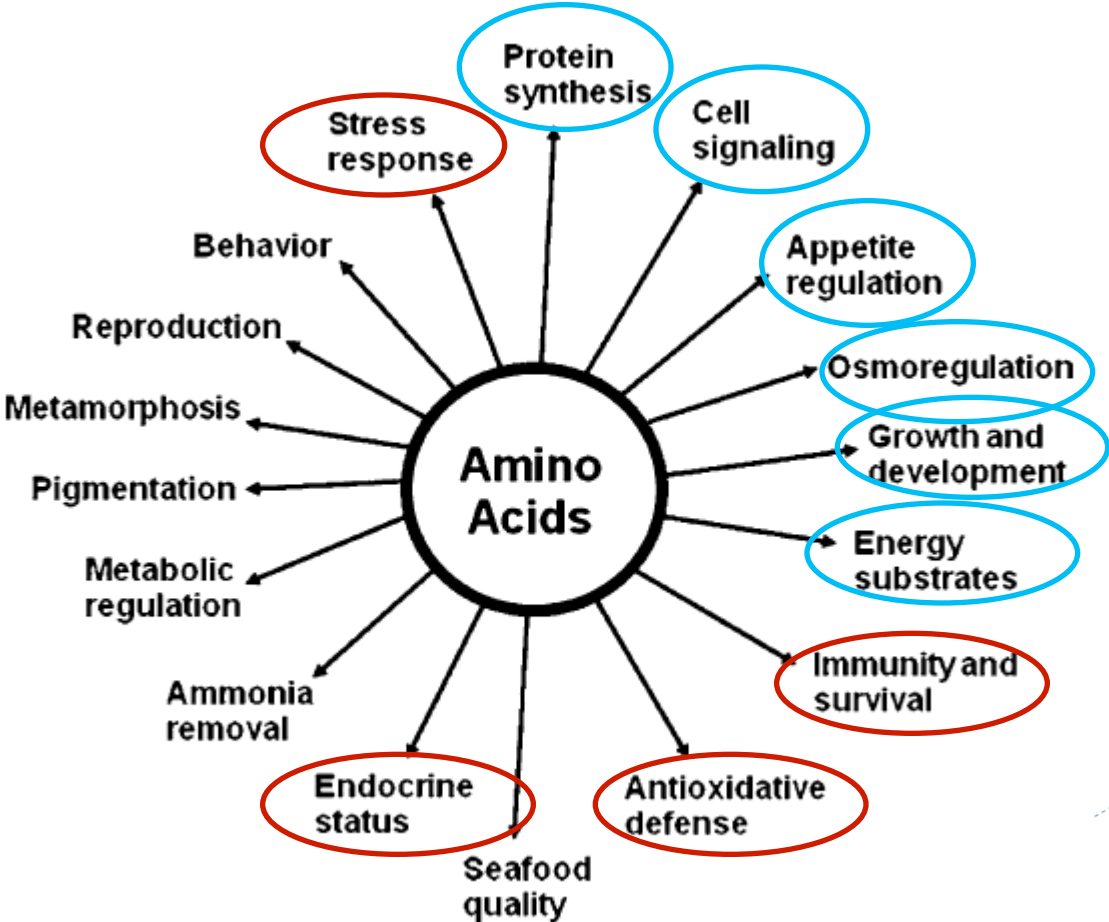
PCA on differentially expressed proteins (One-way ANOVA;  $p$ -value<0.05); DeCyler 2-D software V.7.0 in-built function used, with mean centering and standard deviation scaling, on protein.

## CONCLUSIONS

Growth and sarcoplasmic protein expression in the white muscle of juvenile zebrafish respond to dietary composition in fish protein hydrolysates. Growth is lowest when diet is richest in low MW protein hydrolysate fractions, and this is reflected in muscle proteome as protein expression in fish fed diet NF was clearly different from other treatments. Identification of these proteins is underway and will further elucidate the mechanisms affected by composition and molecular size distribution of proteins and peptides in the diet of juvenile fish.



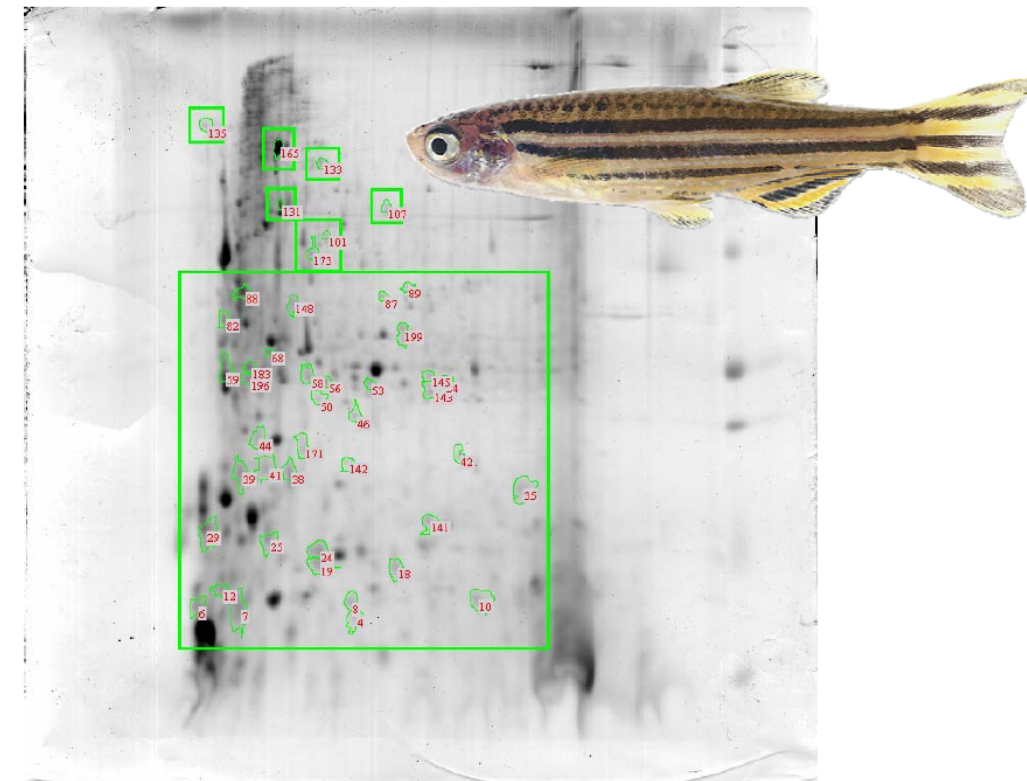
# Amino acid regulation of metabolic pathways



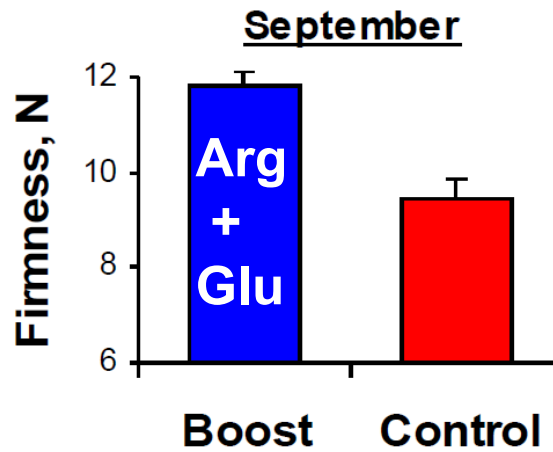
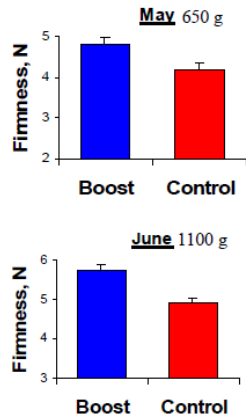
# Lysine metabolism

## Whole body proteome response to a dietary lysine imbalance in zebrafish *Danio rerio*

Pedro Gómez-Requeni <sup>a,\*</sup>, Mahaut de Vareilles <sup>a,b</sup>, Katerina Kousoulaki <sup>c</sup>, Ann-Elise O. Jordal <sup>a</sup>, Luis E.C. Conceição <sup>b</sup>, Ivar Rønnestad <sup>a</sup>



- **Growth rates** (expressed as mm/day) **decreased significantly** in fish fed with diet Lys-.
- 45 proteins were differentially affected by the different dietary treatments.
- **Muscle growth and differentiation** related proteins were indistinctly up- and down-regulated in the 3 groups. Proteins related to **muscle contraction** were down-regulated in Lys- group.
- **Energy production** proteins (aldolase b, triosephosphate isomerase 1b) were up-regulated in FAA and Lys+ groups whereas an ATP-dependent **energy expenditure** protein (NDK-Z2) was up-regulated in Lys- group.
- **Eye lens differentiation** spot proteins were up-regulated in Lys+ and down-regulated in FAA and Lys- groups.
- A **cholesterol efflux promoter** (Apo-A1) was down-regulated in FAA and Lys- groups and up-regulated in Lys+ group, which may reflect an **increased transformation of cholesterol** in bile acids or **possibly production of steroid hormones** for **sexual maturation** purposes.



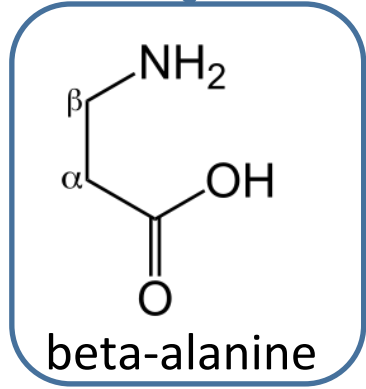
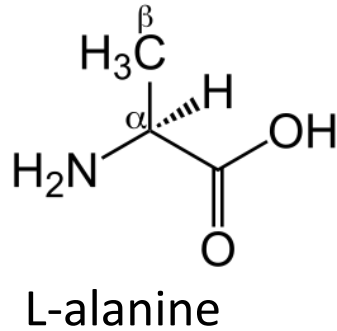
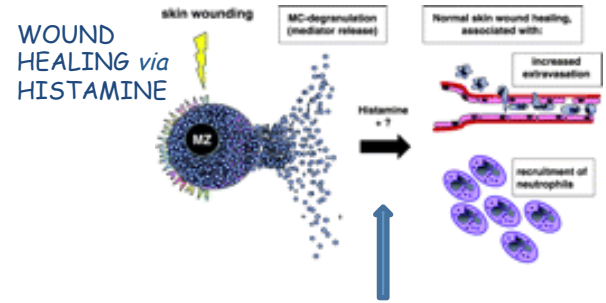
BOOST = för tilsatt en blanding av arginin & glutamat



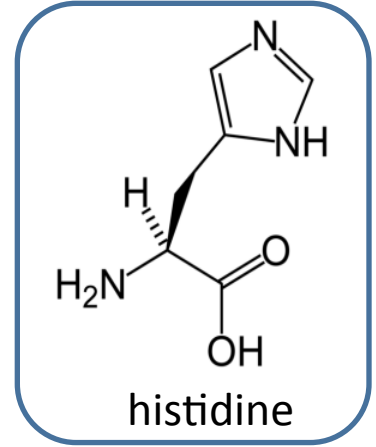
Teksturen kan förbedras genom blant annet fôret. Foto: iStockphoto.

**+ Hydroxyproline**  
(Aksnes, Albrektsen: filed  
Nofima patent 2009)

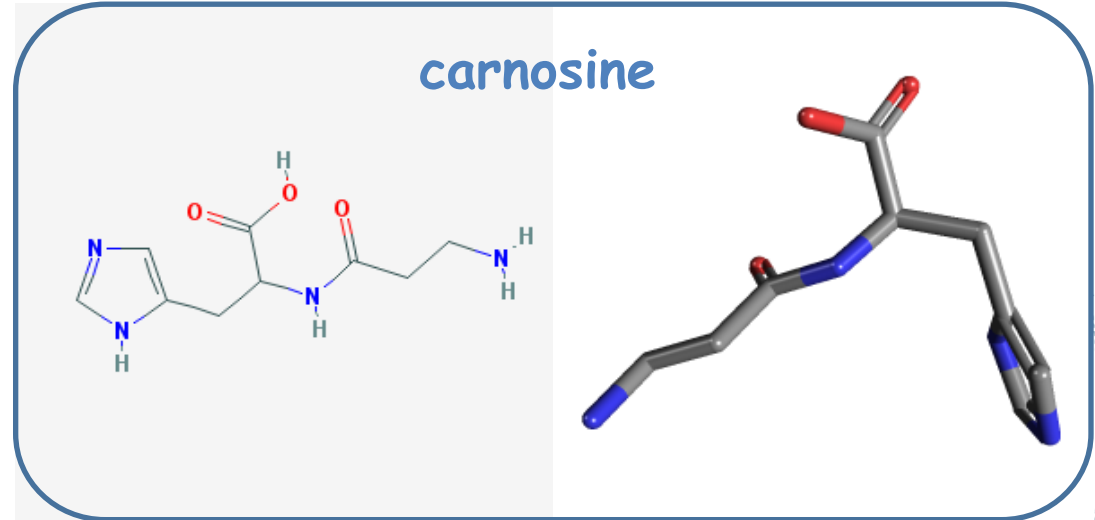
# WOUND HEALING



IUPAC name: 3-Aminopropanoic acid



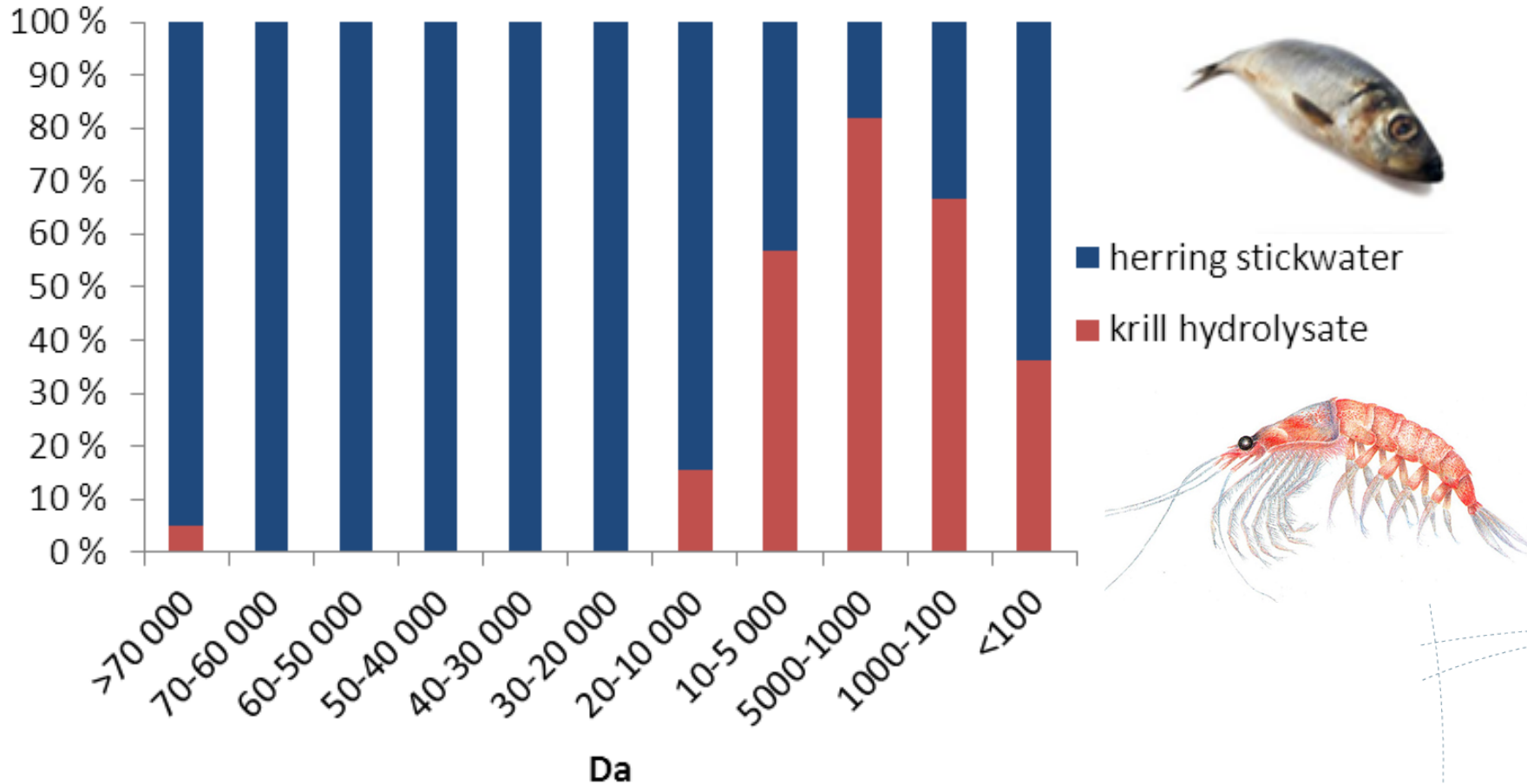
The degradation products of **carnosine** are 1) **histidine** which is metabolised to **histamine** which in turn *enhances the process of wound healing by stimulating effusion at the initial stage of inflammation & 2) beta-alanine* which *stimulates the biosynthesis of nucleic acids and collagen.*



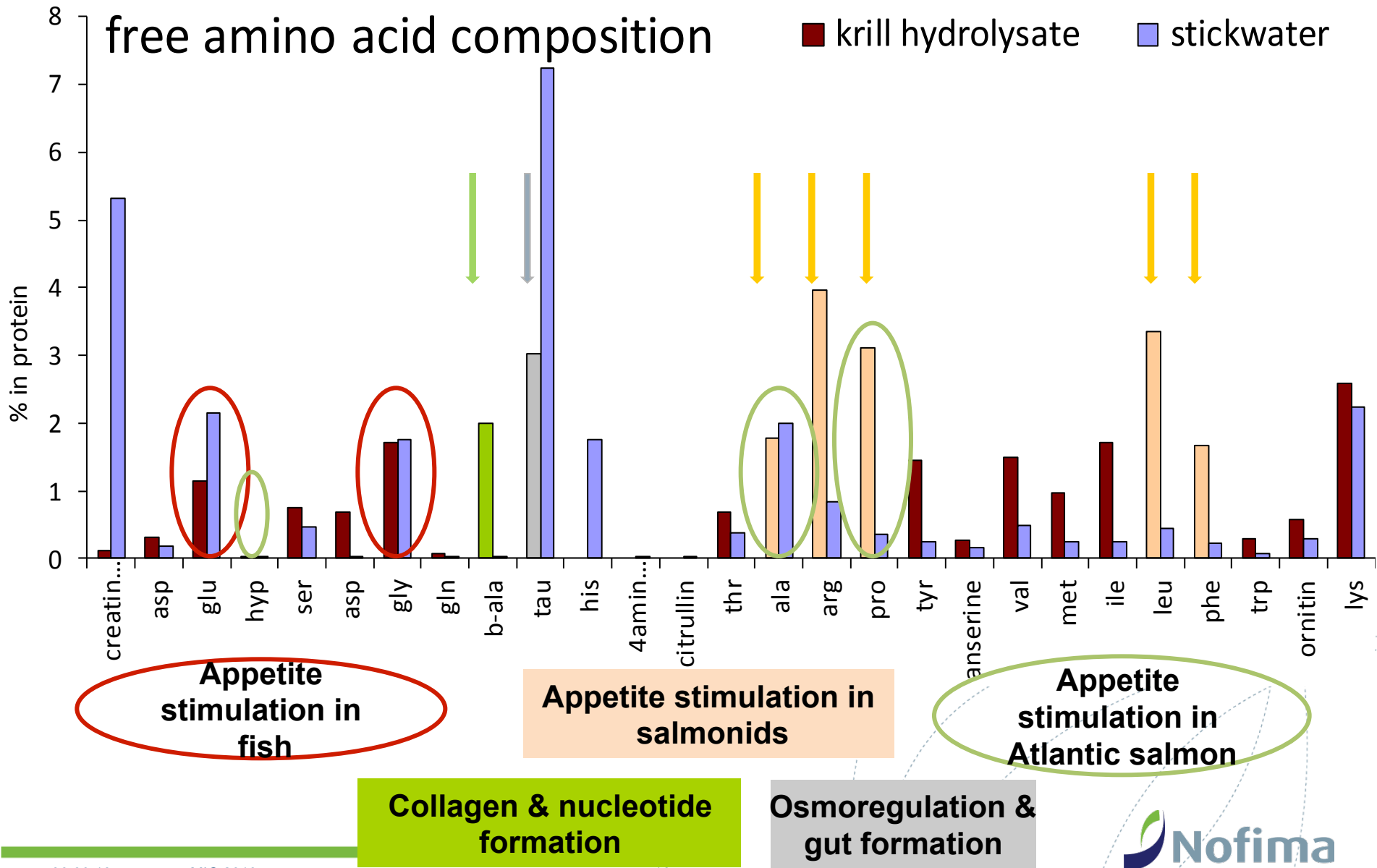
Nagai K, Suda T, Kawasaki K, Mathuura S. 1986. Surgery 100(5):815-21.

# Soluble marine proteins

## Peptide molecular weight distribution

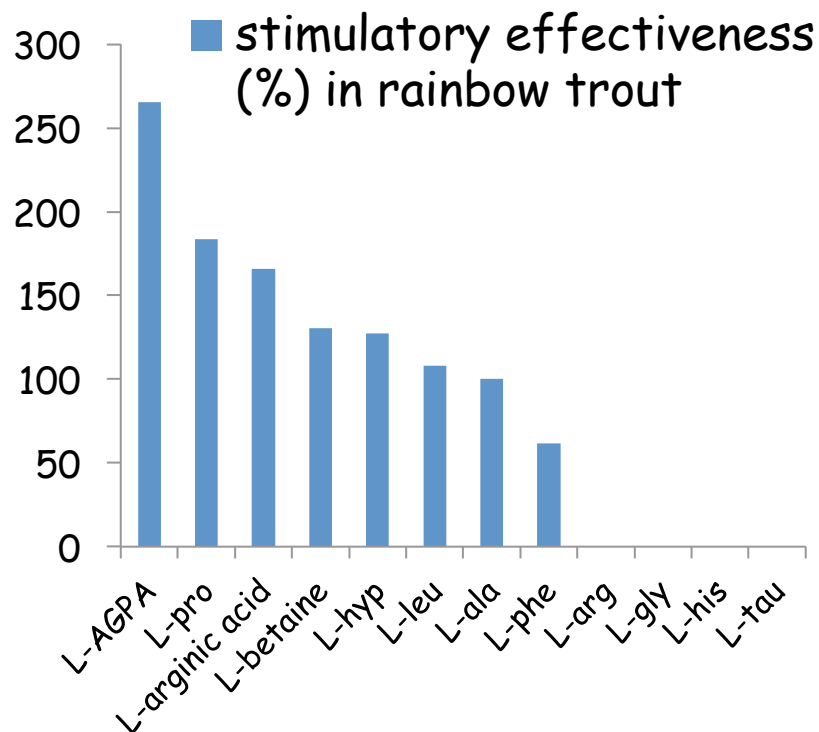


# Feed intake stimulating raw materials



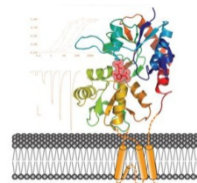


# TASTE STIMULATION IN SALMONIDS



Marui ET AL., J Comp Physiol (1983) 153:423-433

## RECEPTOR SYSTEMS



SALMONIDS

1. L-pro, L-hyp, L-ala
2. L-phe, L-leu
3. L-arg, L-AGPA, betaine

ATLANTIC SALMON

1. L-pro, L-hyp, L-ala
2. L-AGPA

Hara et al. 1994. Journal of Fish Biology 45, 453-465.

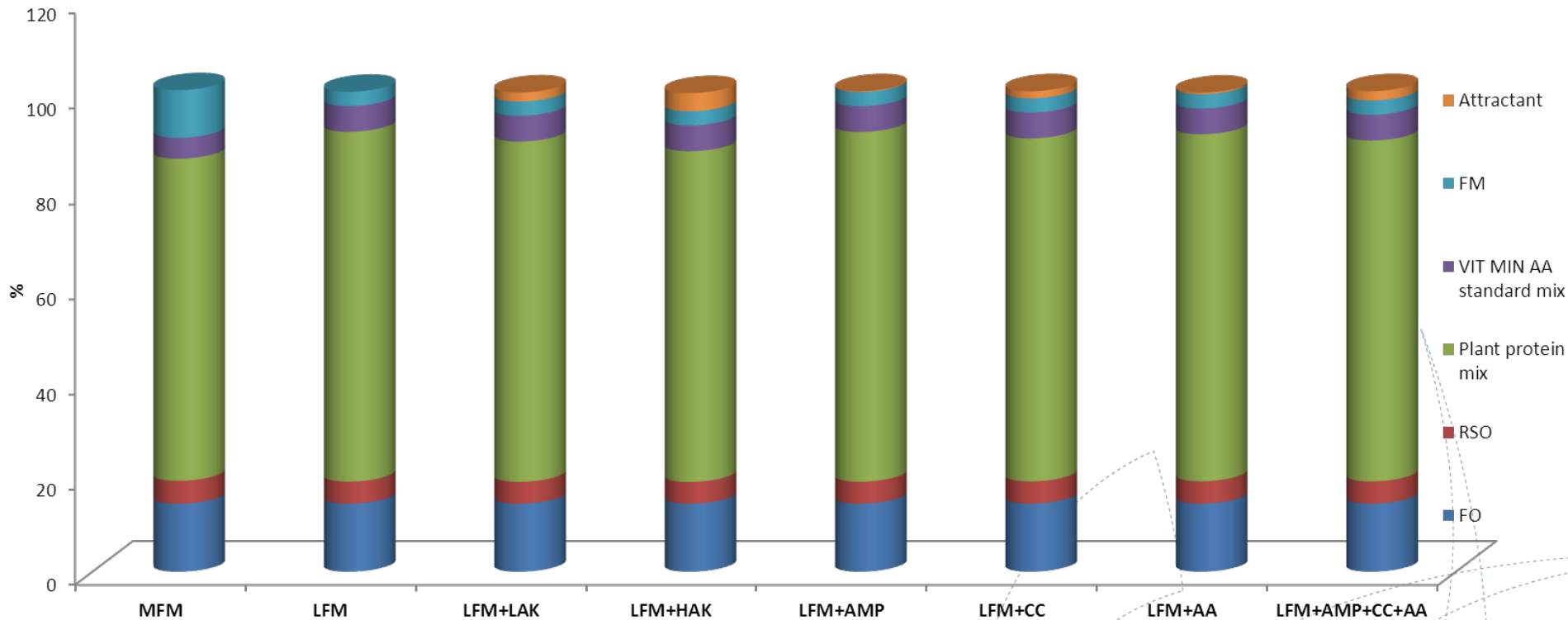
L-AGPA is an arginine (arg) metabolite.

Choline is oxidised to betaine.

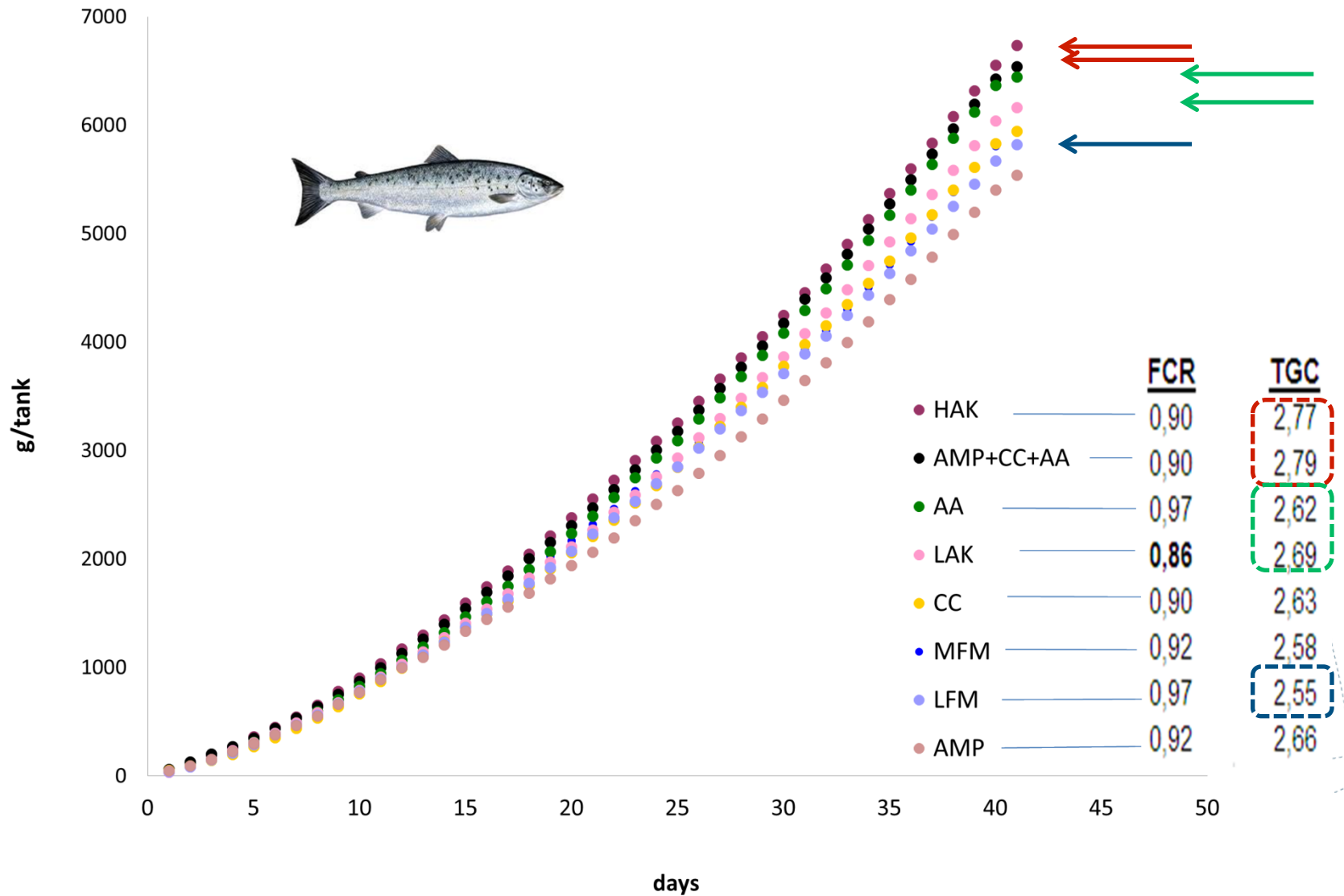




# Feed formulation



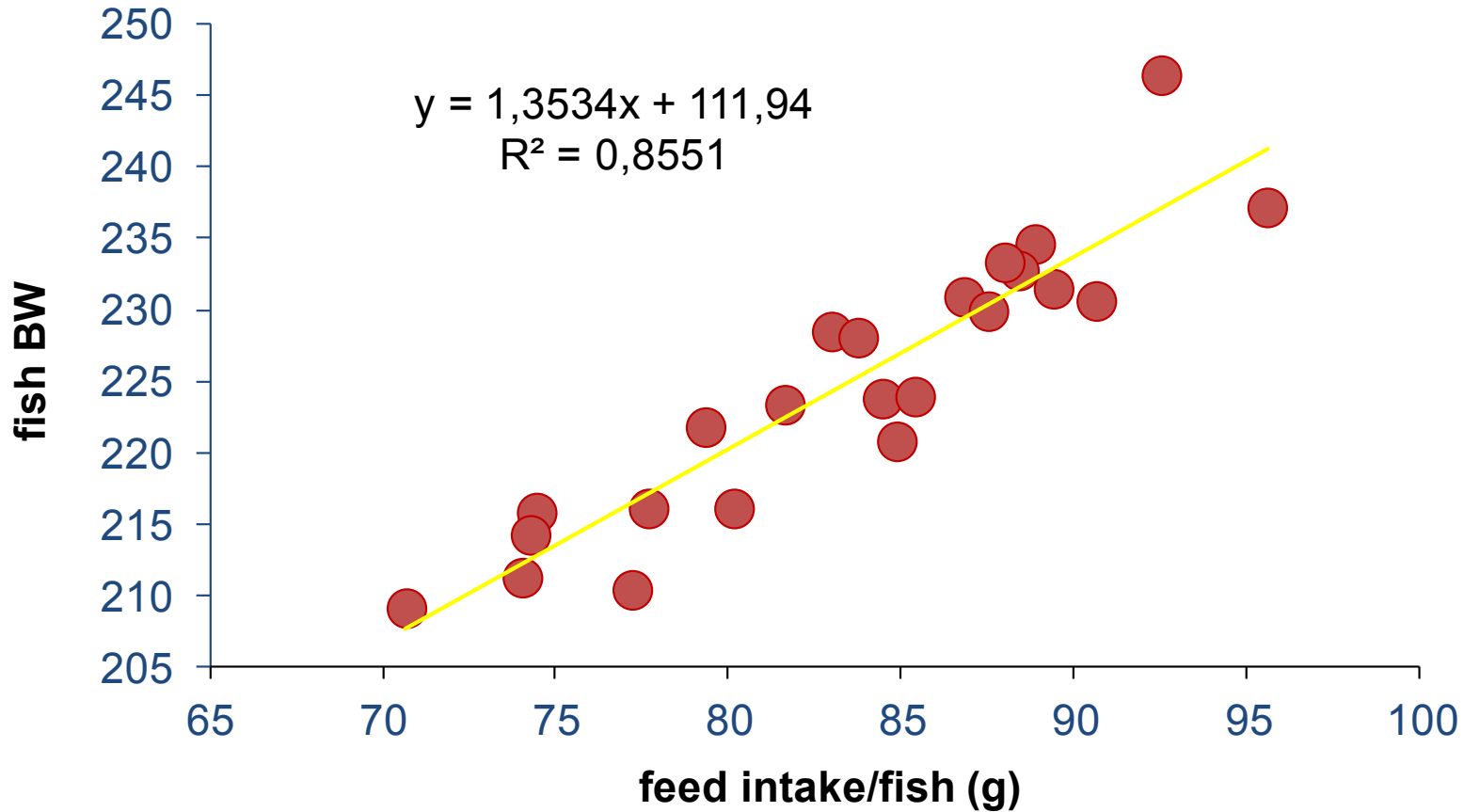
# Results: Cumulative Feed Intake



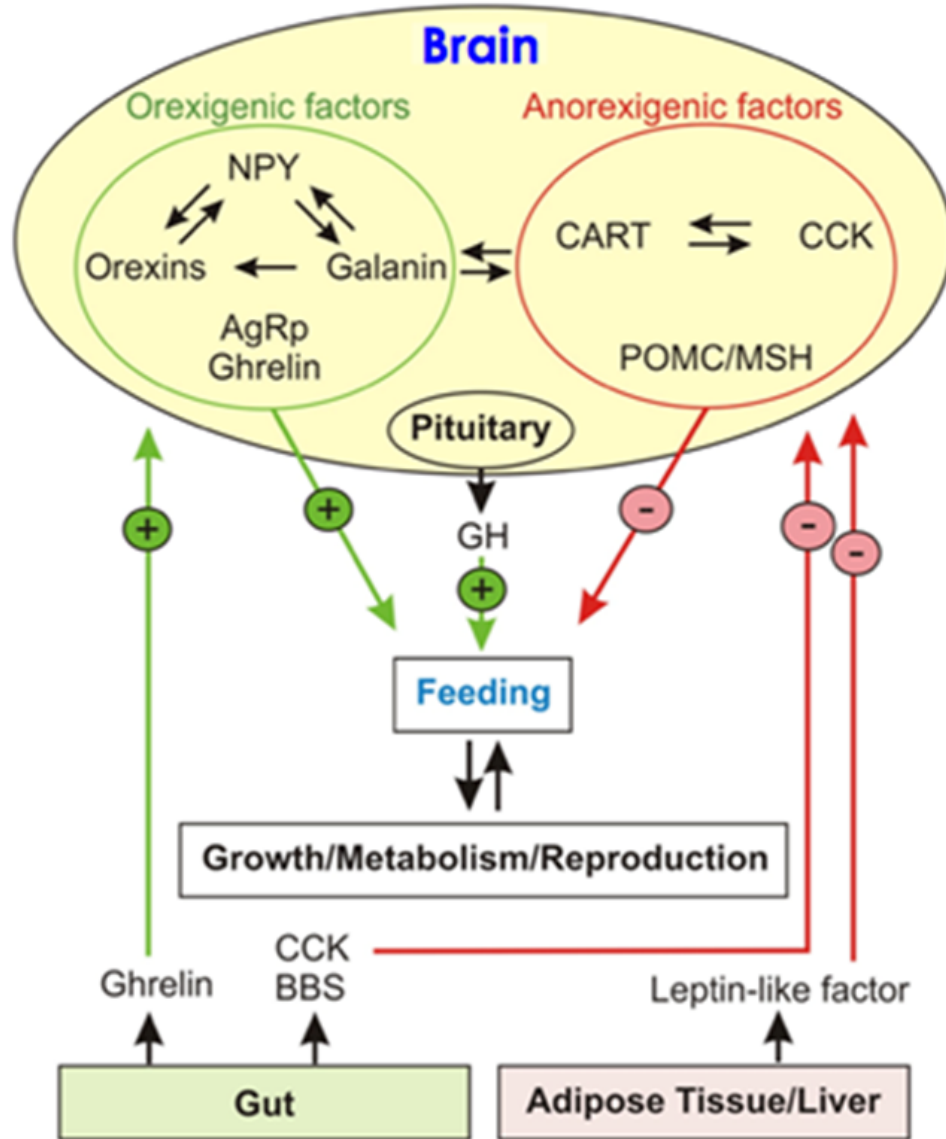
# Results: Feed Intake vs Growth



$P < 0.01$

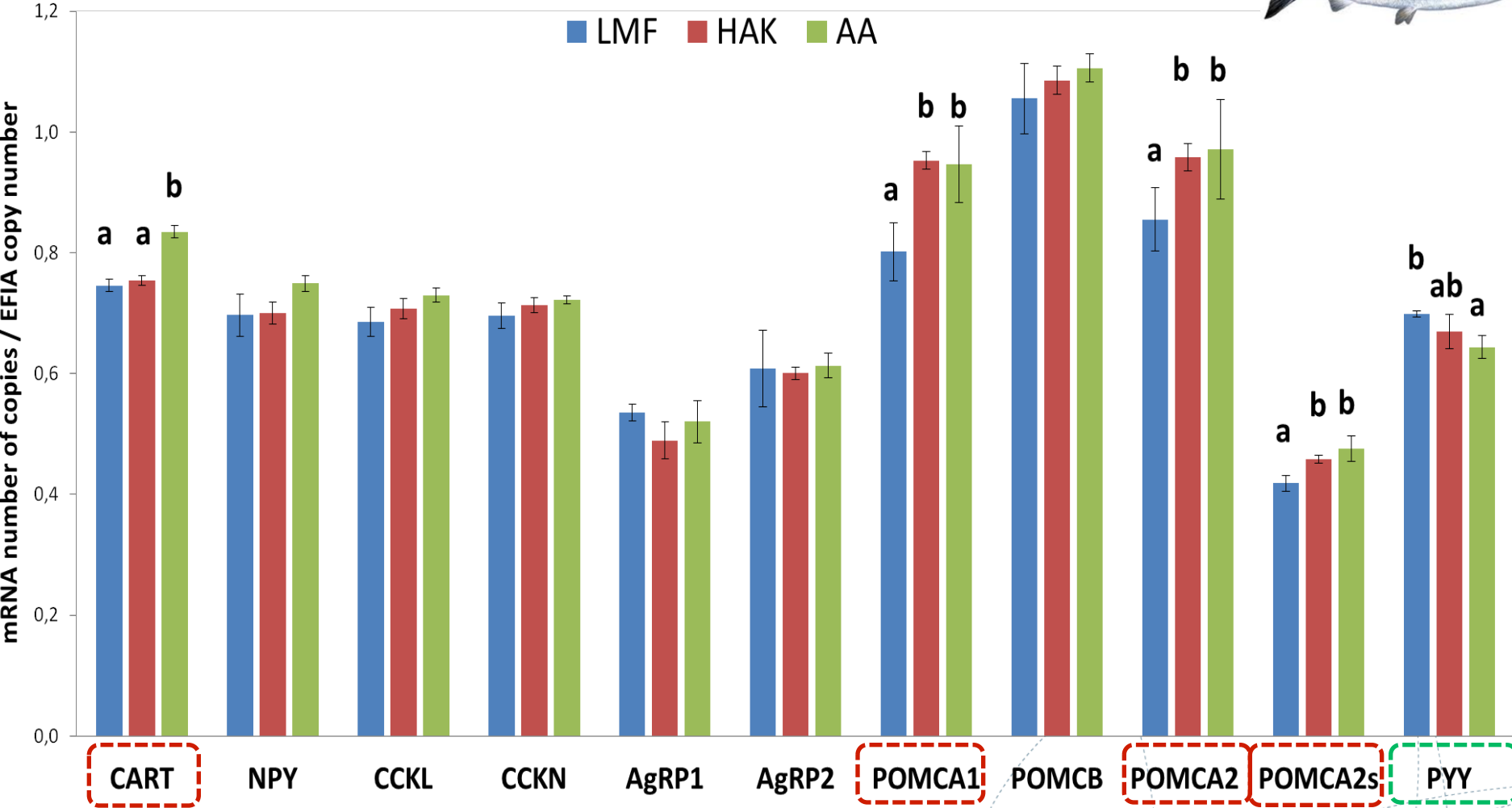


# Appetite regulation in the brain and gut



Appetite (Model in adult teleosts, based on Volkoff et al., 2007- Rennestad 2010)

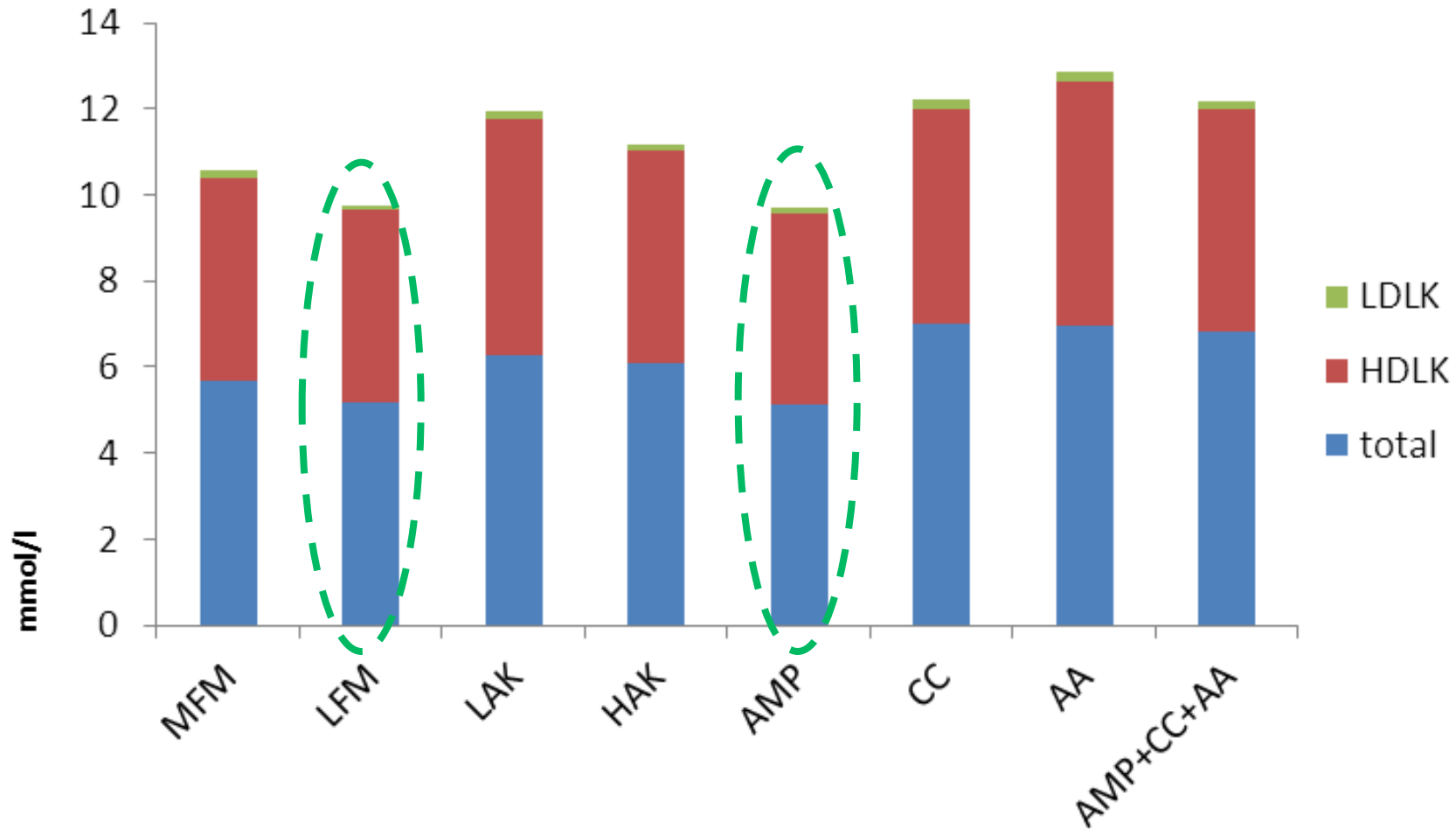
# Apeptide regulating genes' expression



HAK  
AA



# Plasma lipids & cholesterol



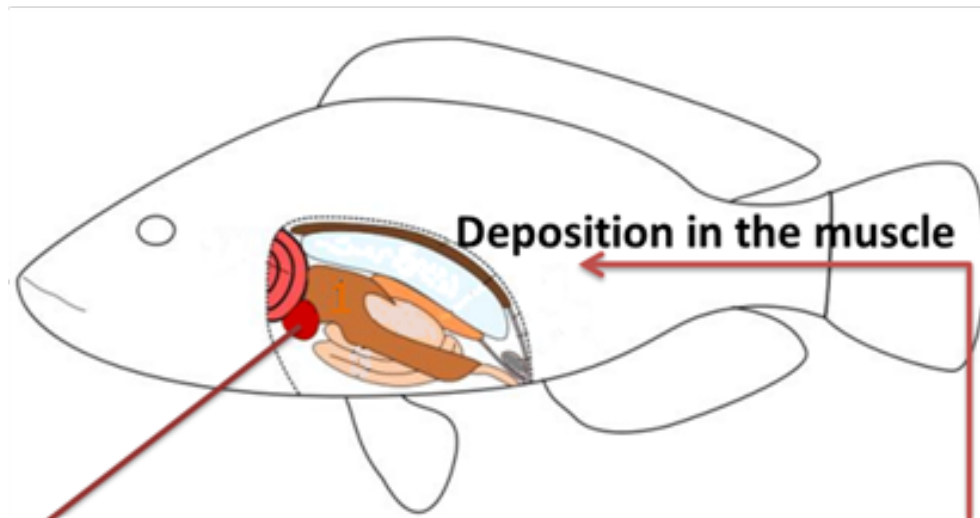
0

AMP+CC+AA



# Choline function

## Lipid stores & transport



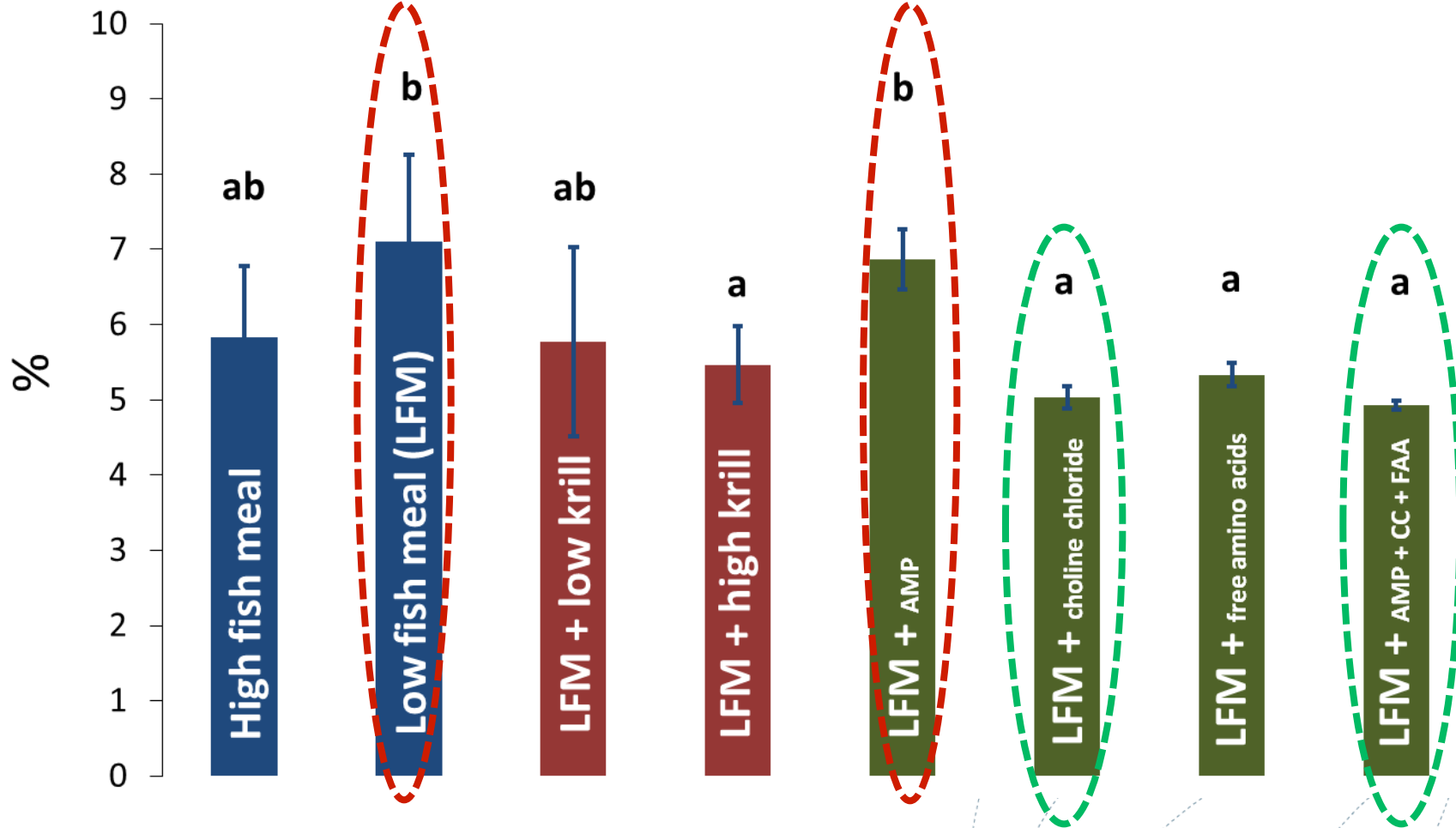
Lipids are transported out of the liver by choline chloride containing VLDL

Energy source

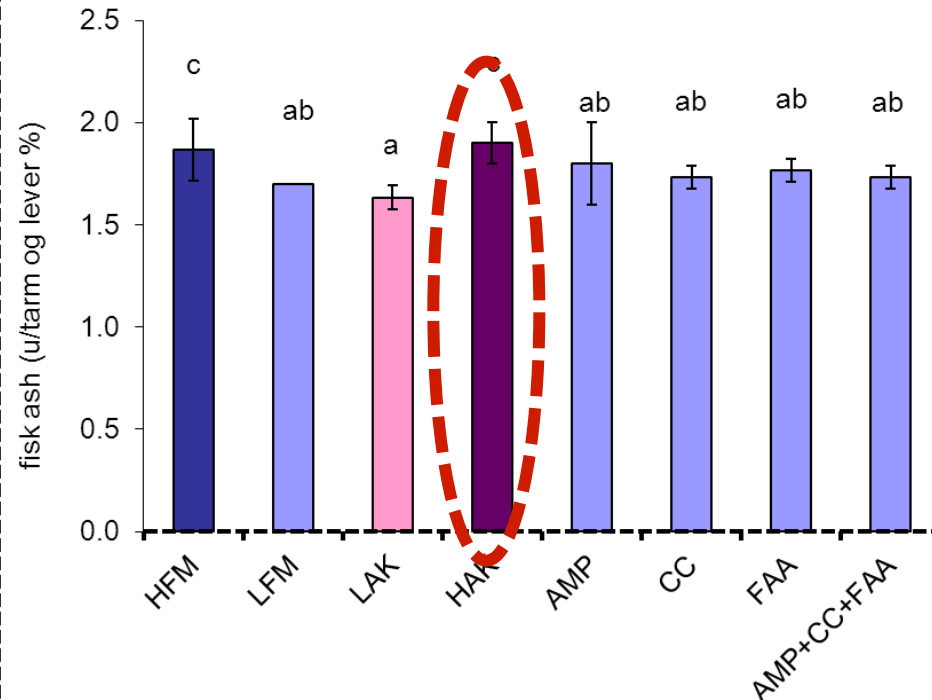
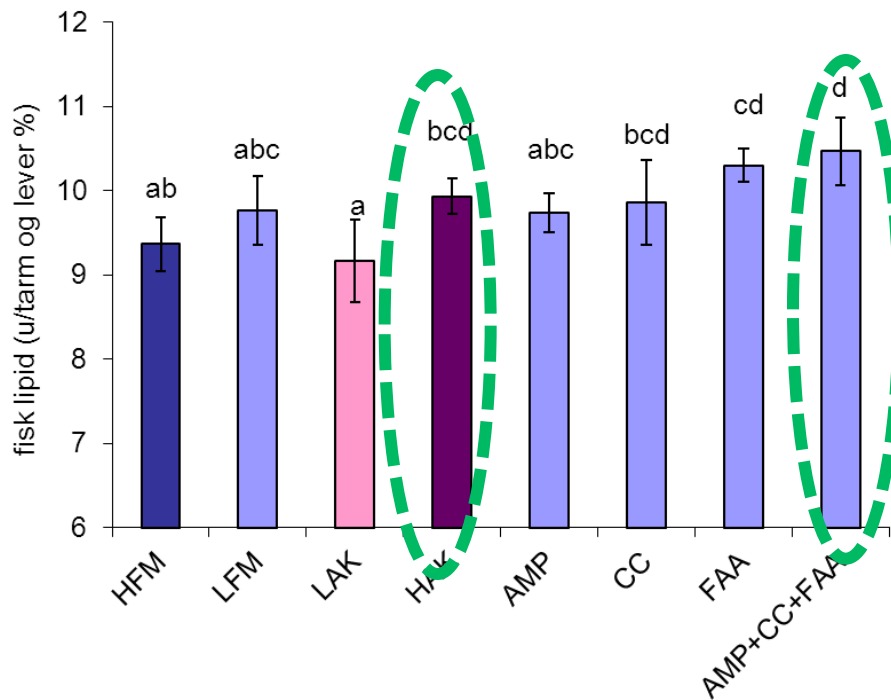
# Liver status



## salmon liver fat

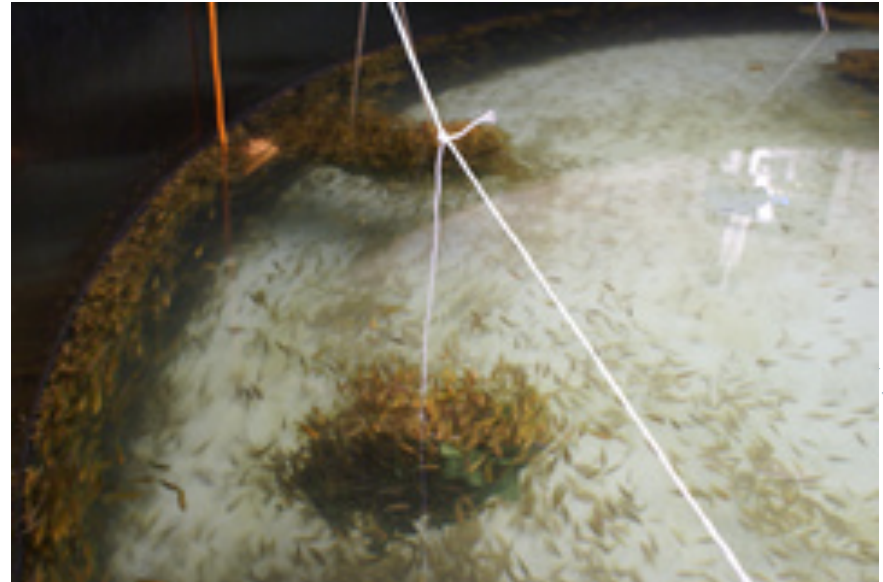


# Gutted body composition





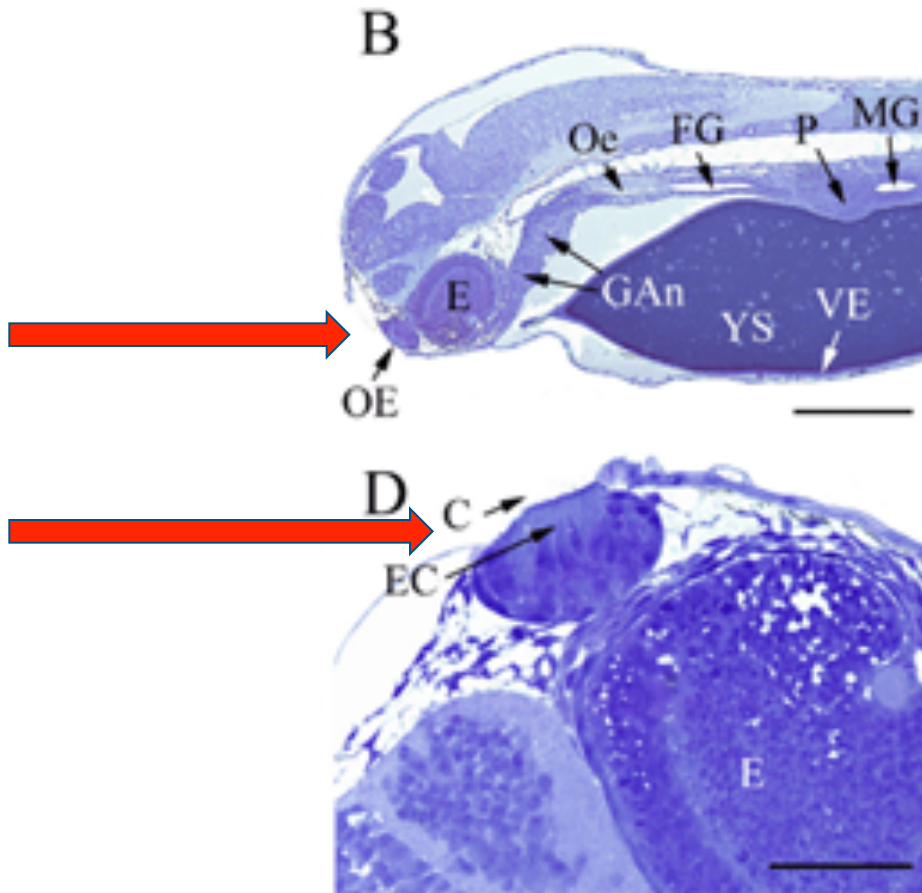
## Ballan wrasse, *Labrus bergylta*



# Ballan wrasse *Labrus bergylta* larvae

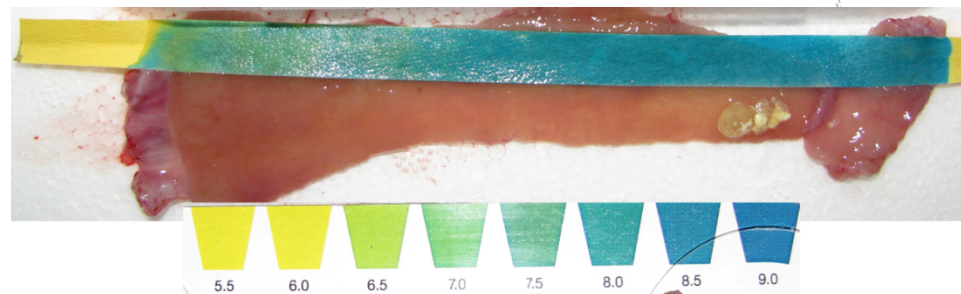


(B) Larva at hatching (D) Olfactory organ at hatching.



Dunaevskaya et al. (2012)

Food items	Ballan wrasse
	%W
Algae	2.26
Porifera	
Cnidaria	0.02
“Worms”	0.58
Maxillopoda	0.01
Amphipoda	0.13
Isopoda	0.10
Decapoda	26.71
Mollusca	2.18
Gastropoda	11.14
Bivalvia	1.47
Echinodermata	45.07
Tunicata	
Pisces	0.09
Fish eggs	
Miscellaneous	0.22



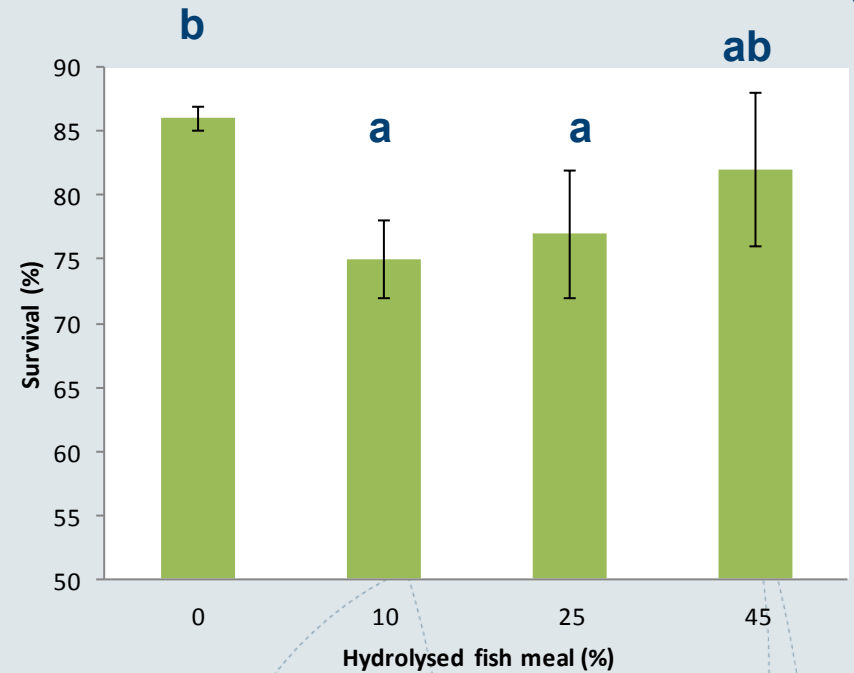
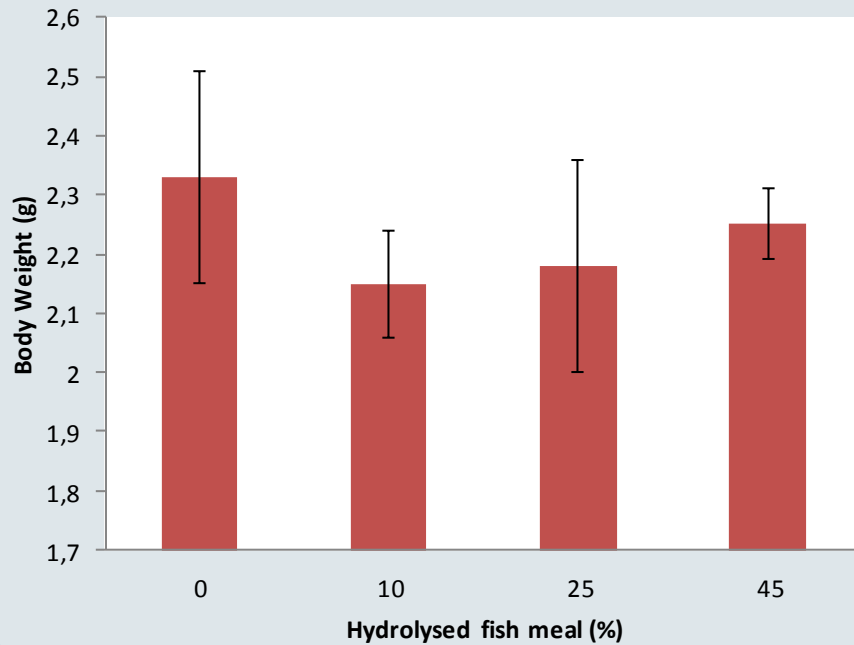
Intestinal bulb

Photo: Saele



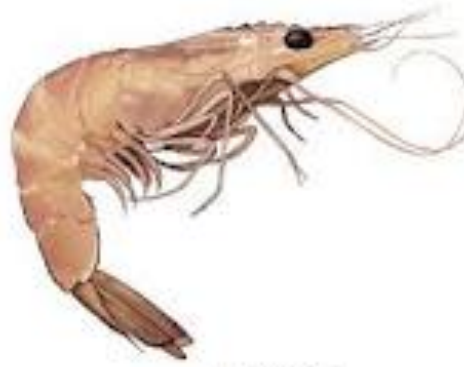


# Increasing amounts of hydrolysed protein





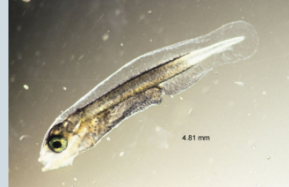
# Raw materials - Attraktant studies



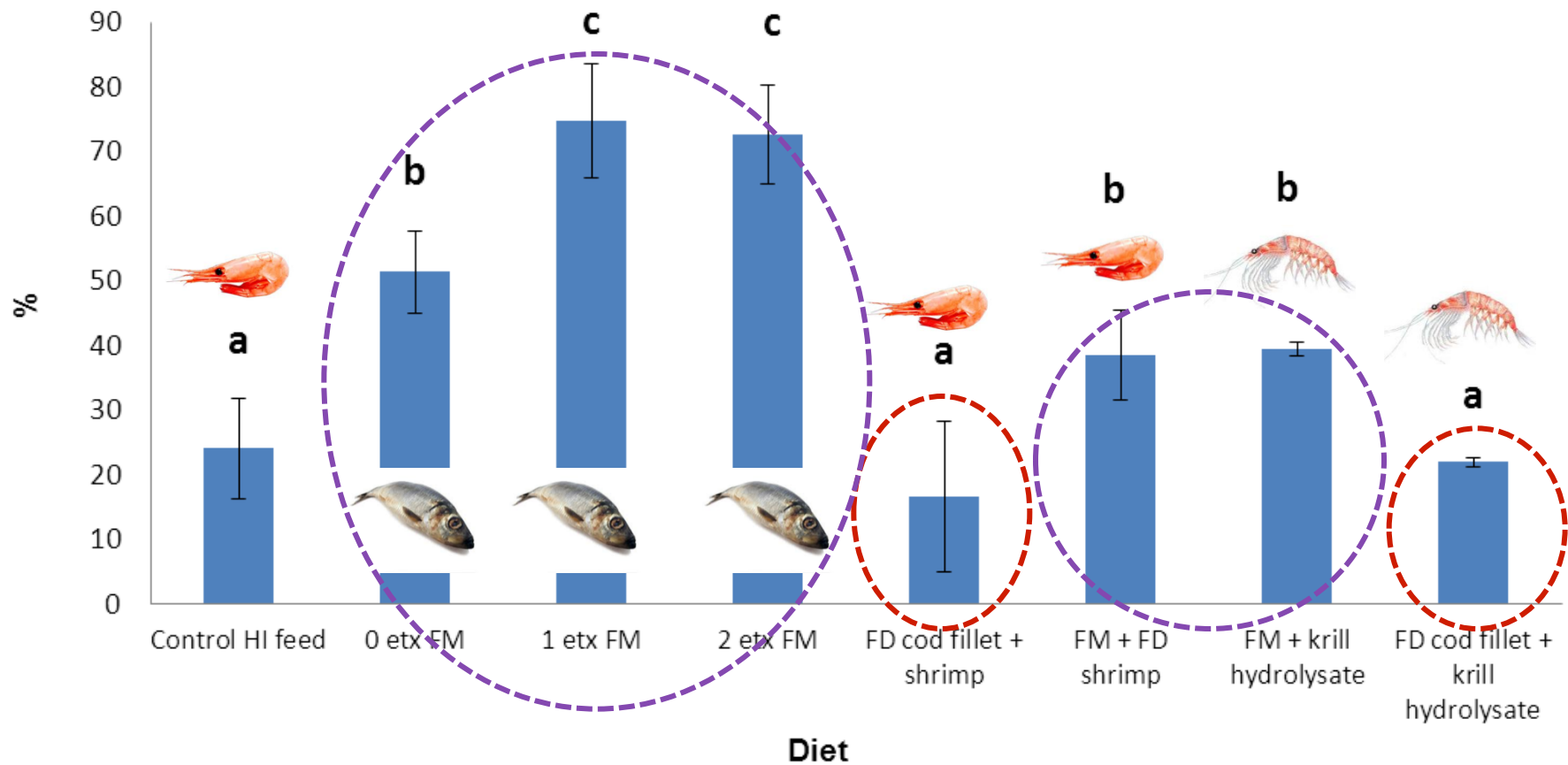
Deepwater shrimp







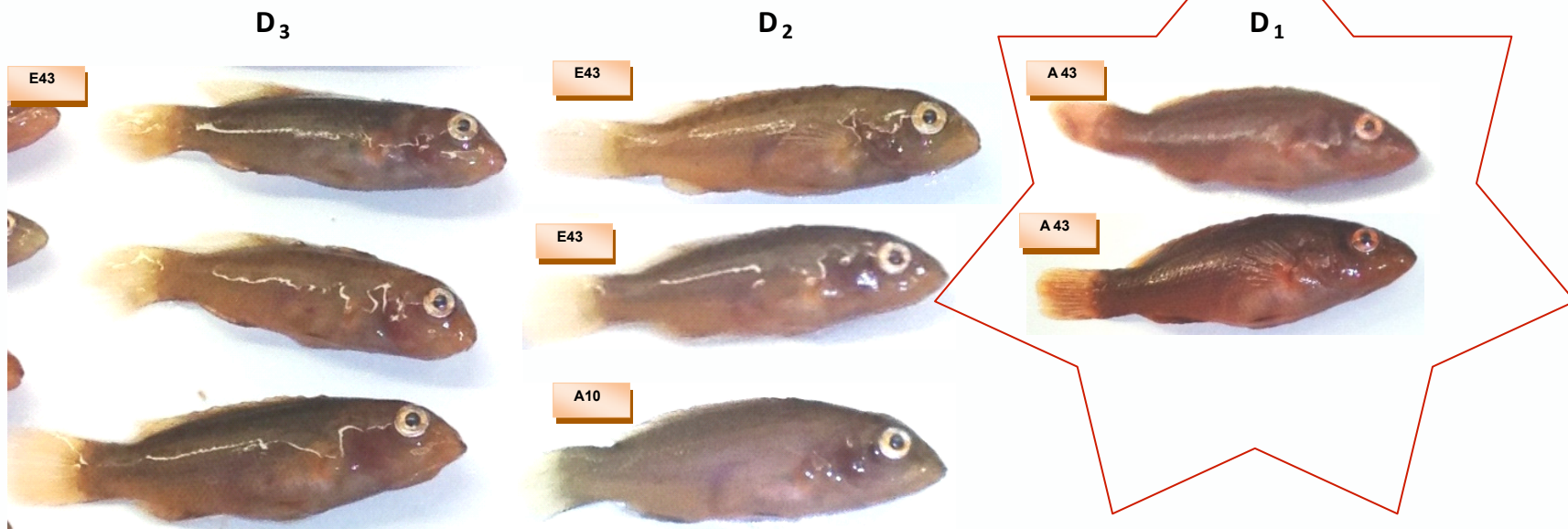
### One month Ballan Wrasse weaning mortality (P<0.000)





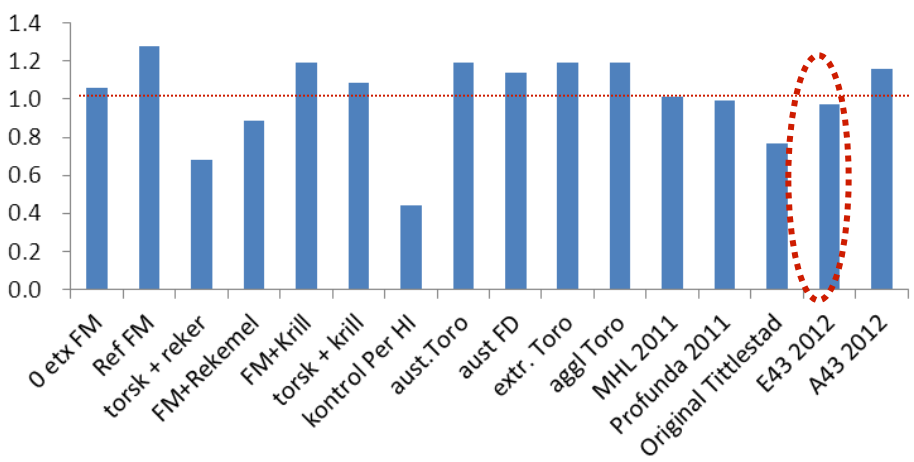
# Head deformities

	<u>Cod muscle + shrimp</u>	<u>Cod Muscle + Stick Water + shrimp</u>
<b>Survival %</b>	<b>62</b>	<b>35</b>
<b>SGR</b>	<b>5.1</b>	<b>4.9</b>
<b>Good fish %</b>	<b>3</b>	<b>32</b>

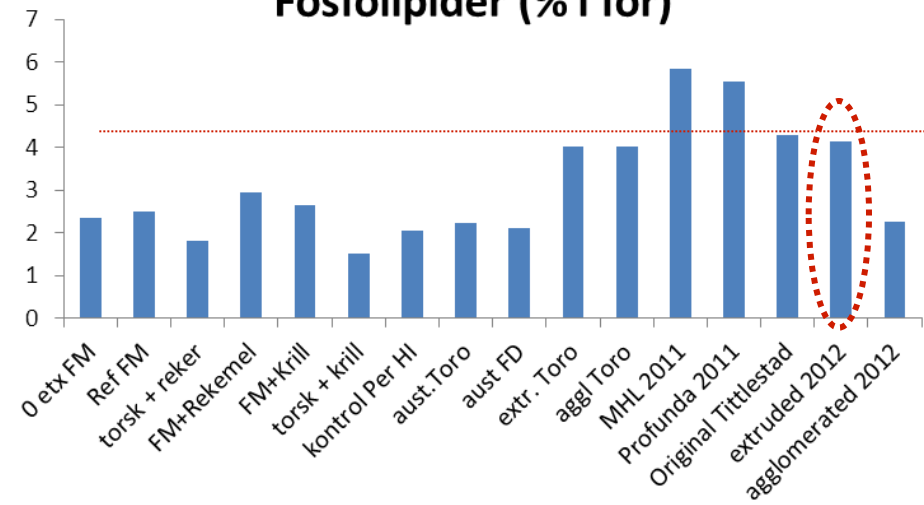


# Weaning diets history

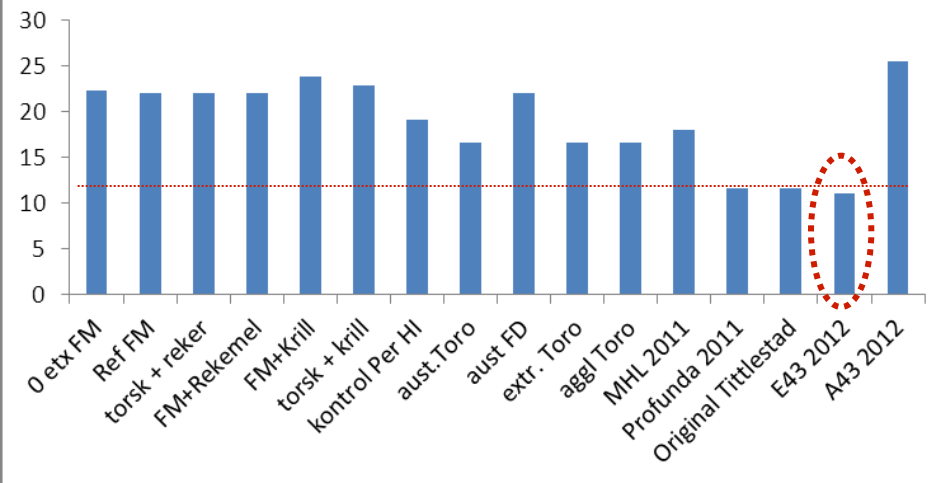
### Løselig P (% tørrstoff)



### Fosfolipider (% i fôr)

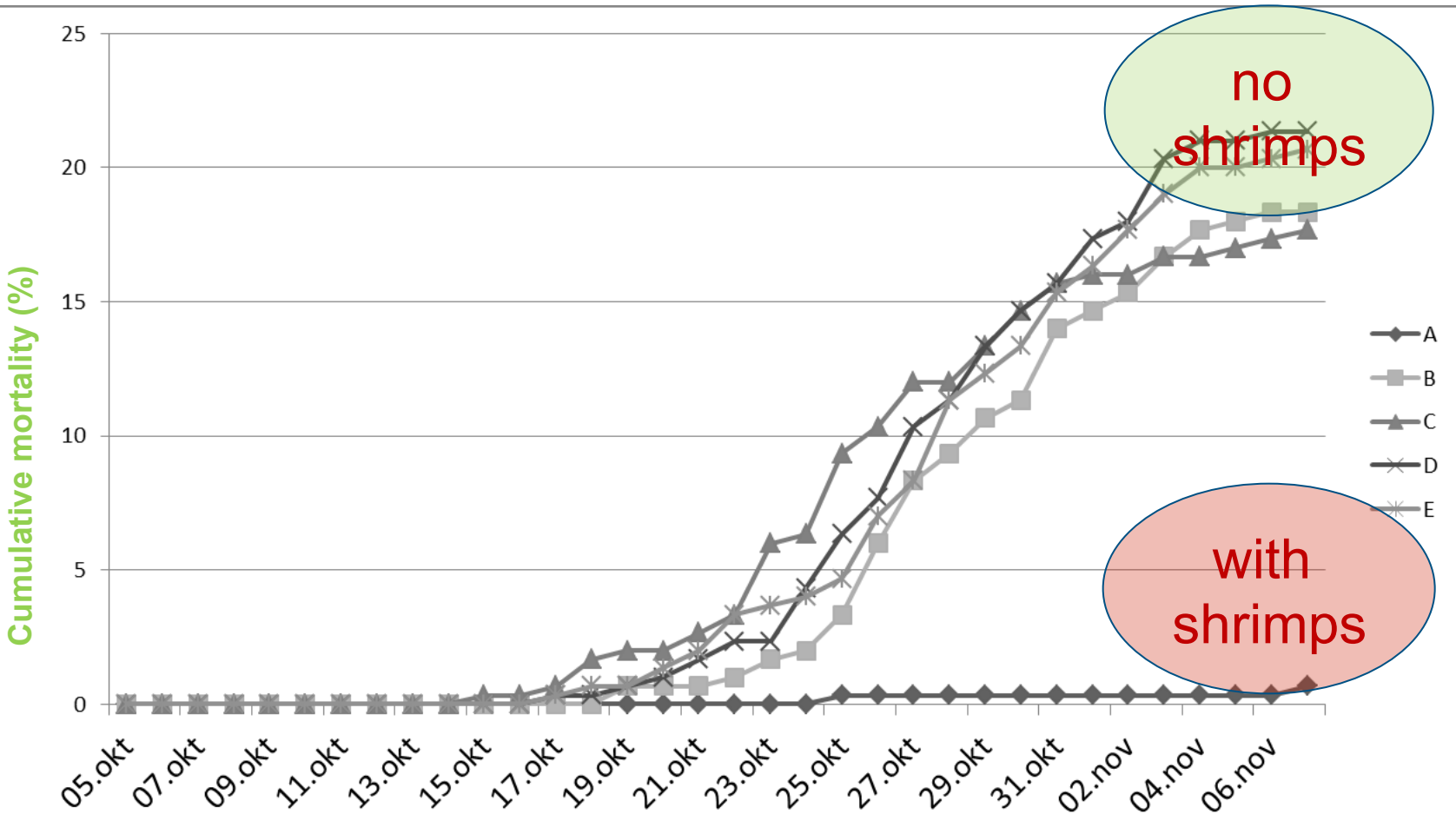


### vannløselig protein (% i fôr)

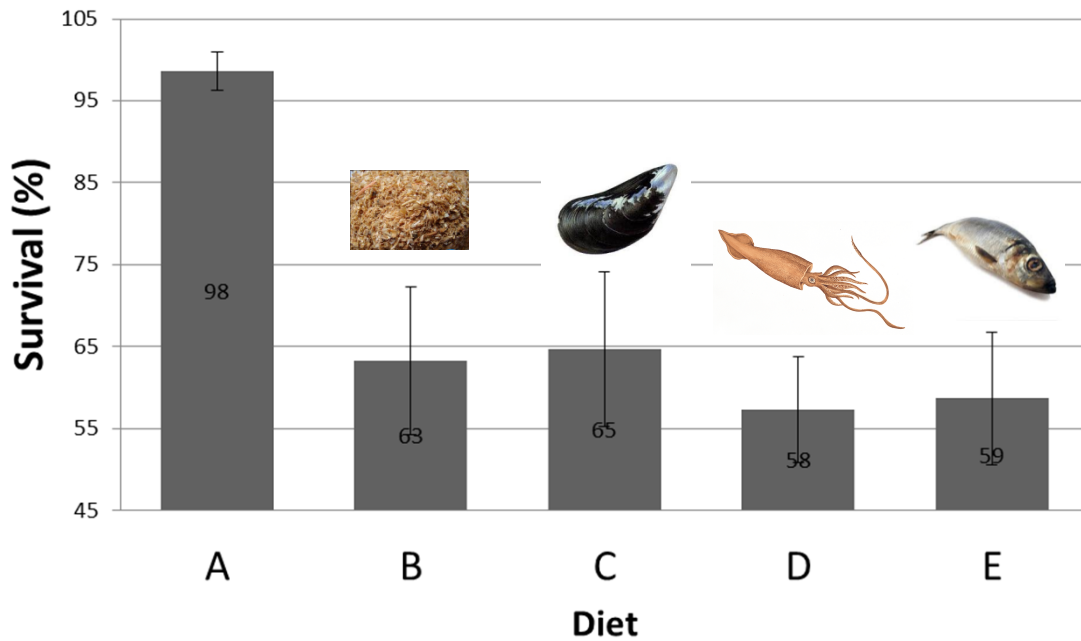
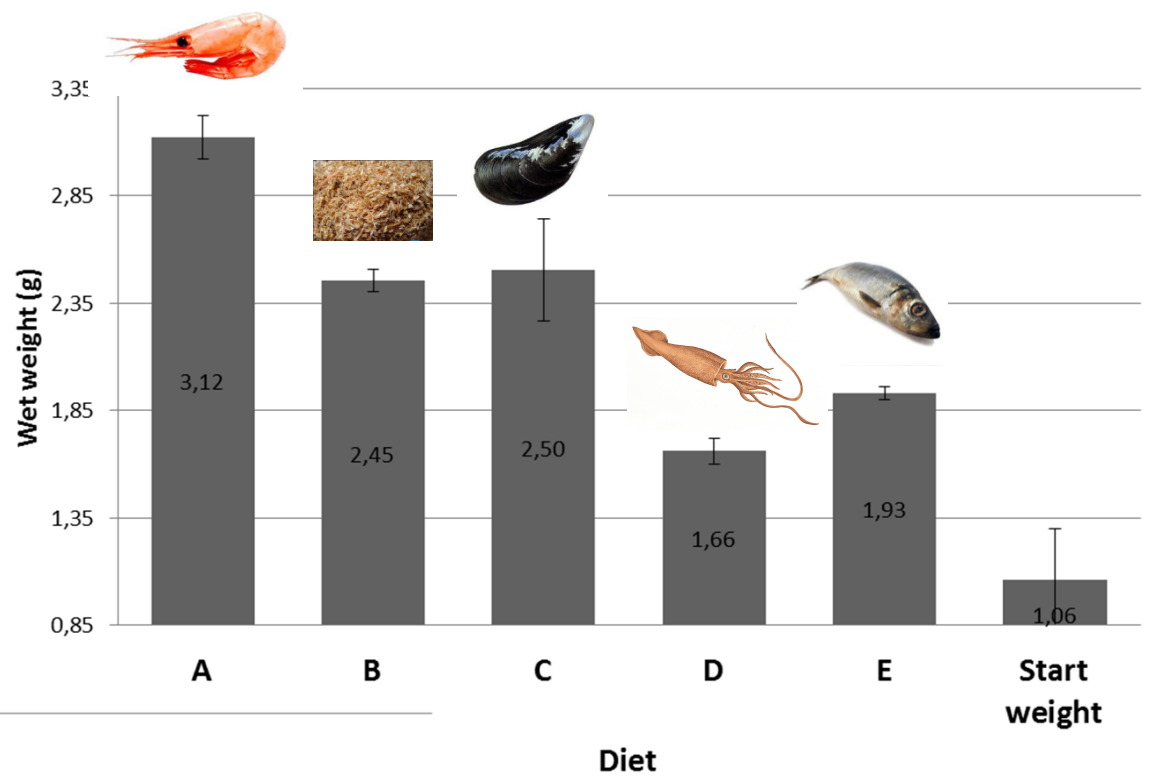




	Shrimp shell				Fishmeal
	Shrimp meal	meal	Blue mussle	Squid meal	only
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>
Ingredient cost NOK/kg	61.0	15.0	19.5	16.0	15.0



# On-growing



# What does Ballan wrasse need?





**Thank you for your attention**