Forskningsrådet marineharvest excellence in seafood villa ORGANIC NIFES Nofima

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

MIC 2013, Holmenkollen, Norway

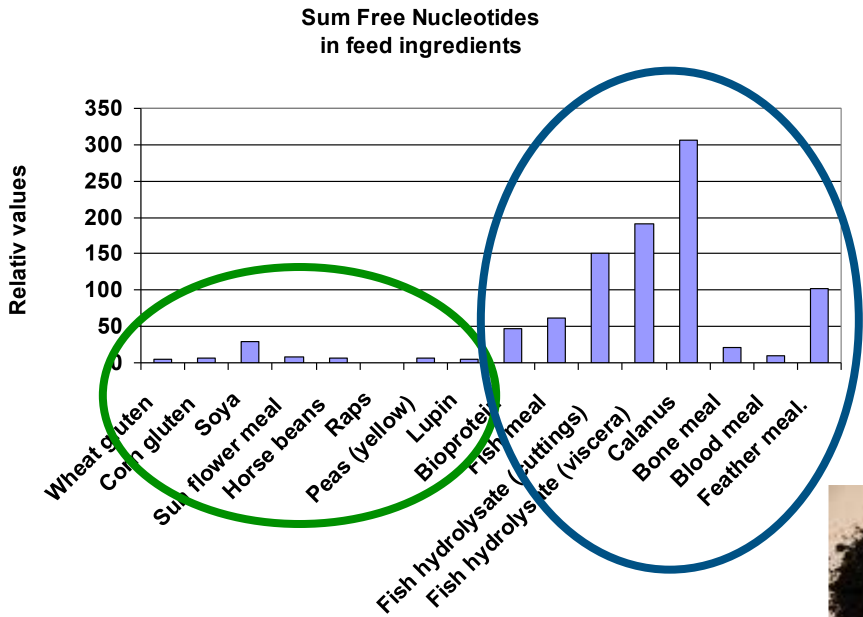
Katerina Kousoulaki

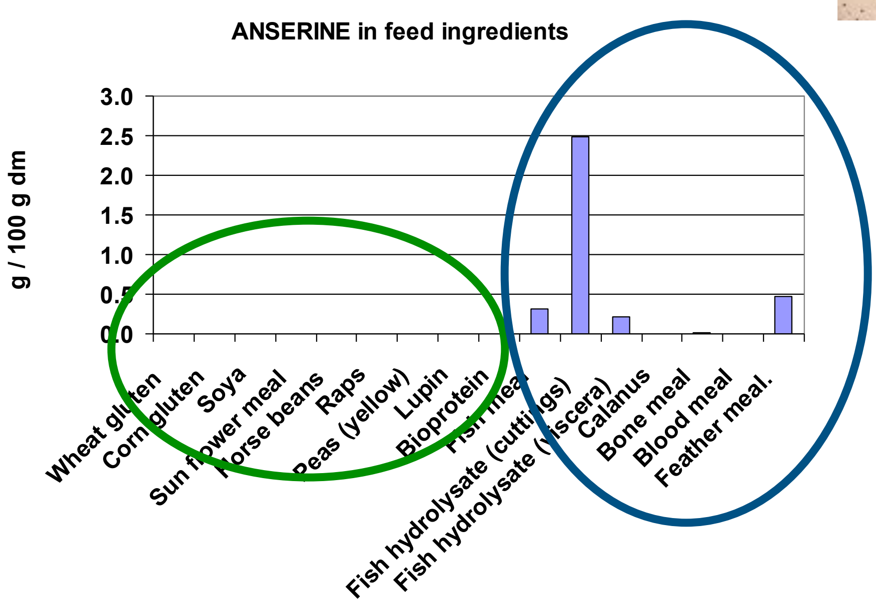
# *MARINE water-soluble components*

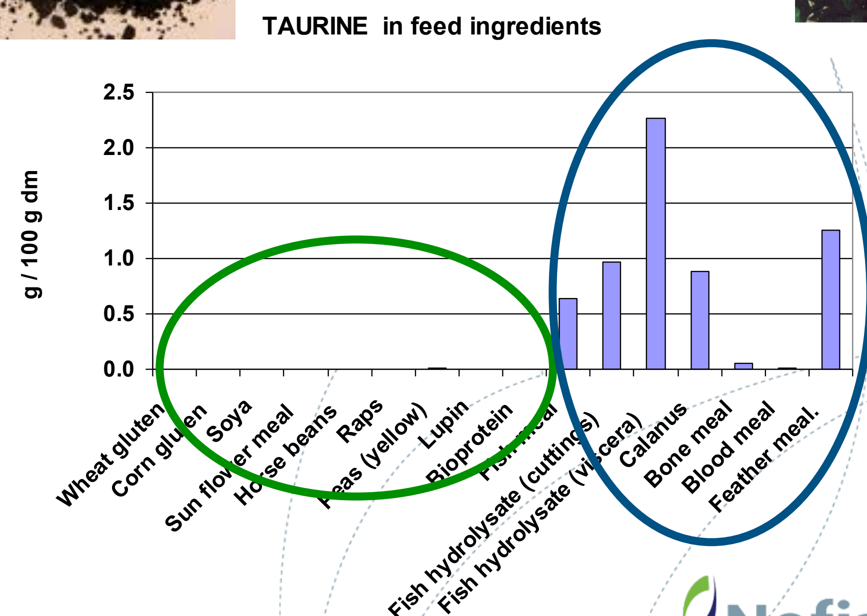
Main focus on water soluble **nitrogenous** compounds in feed ingredients with potential performance promoting effect

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

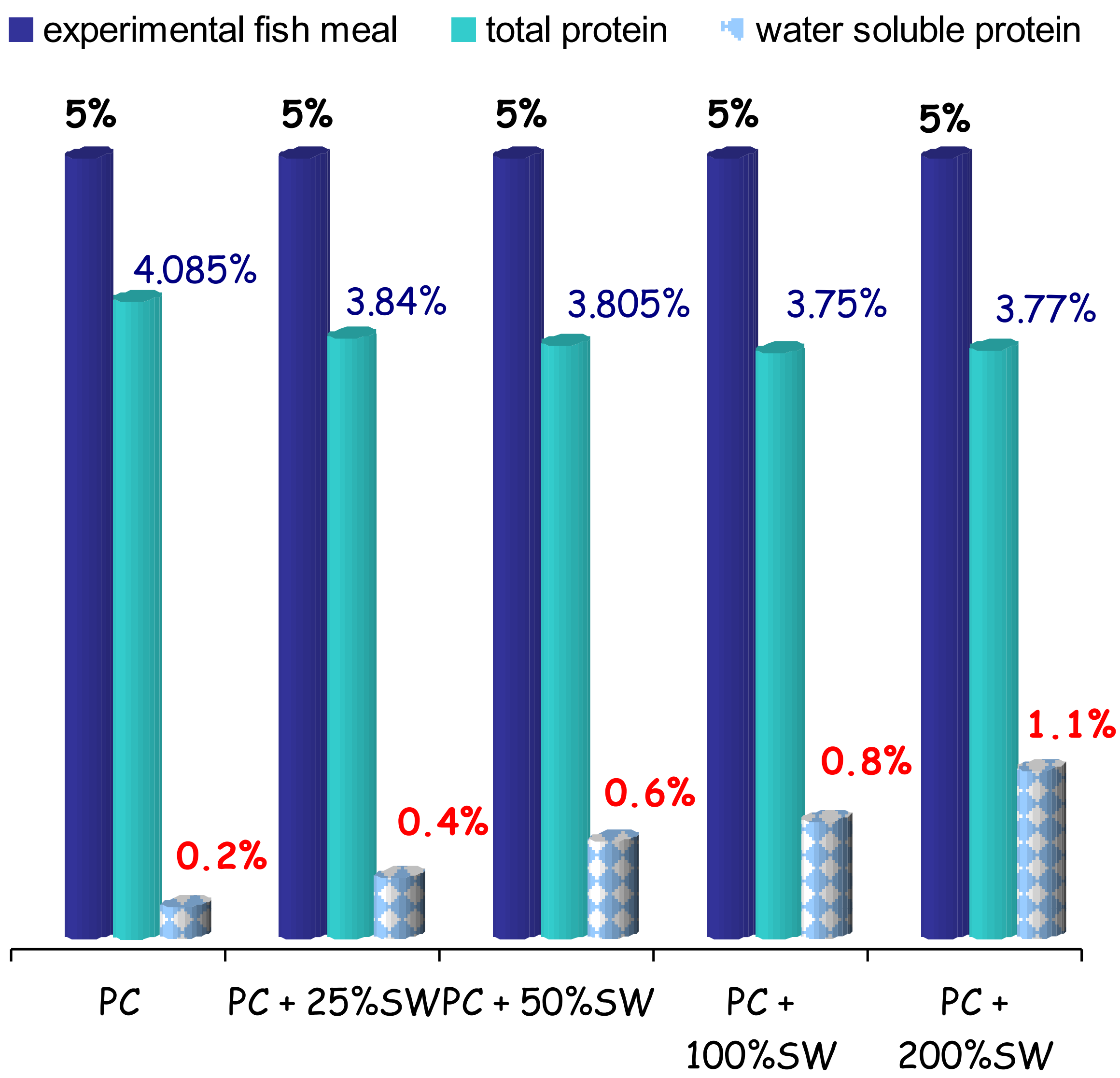
Photo by Gregersen, Nofima


Sum Free Nucleotides
in feed ingredients
Relativ values
350
300
250
200
150
100
50
0
Wheat gluten
Corn gluten
Soya
Sun flower meal
Horse beans
Raps
Peas (yellow)
Lupin
Bioprotein
Fish meal
Fish hydrolysate (cuttings)
Fish hydrolysate (viscera)
Calanus
Bone meal
Blood meal
Feather meal.

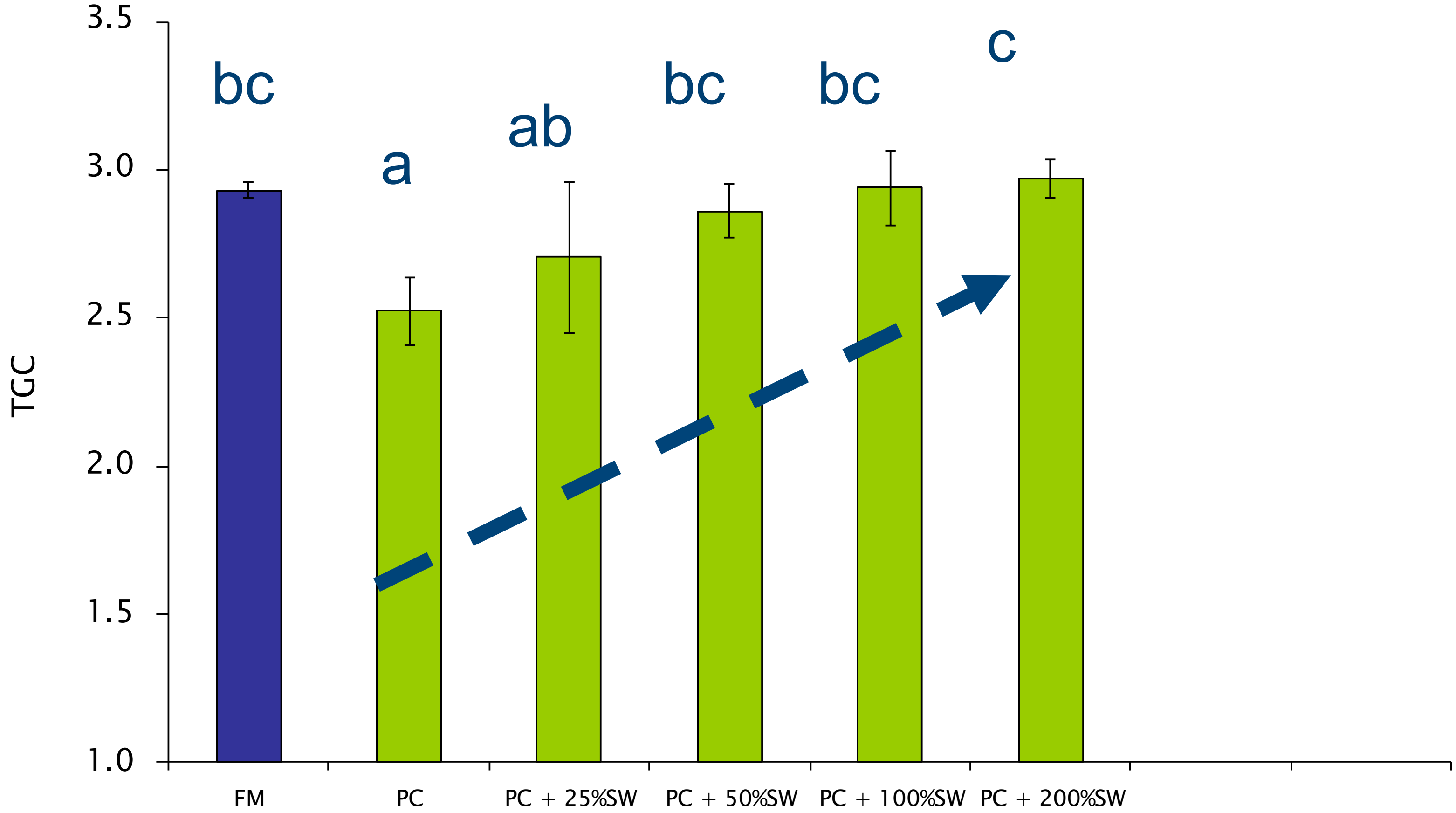

ANSERINE in feed ingredients
g / 100 g dm
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Wheat gluten
Corn gluten
Soya
Sun flower meal
Horse beans
Raps
Peas (yellow)
Lupin
Bioprotein
Fish meal
Fish hydrolysate (cuttings)
Fish hydrolysate (viscera)
Calanus
Bone meal
Blood meal
Feather meal.


TAURINE in feed ingredients
g / 100 g dm
2.5
2.0
1.5
1.0
0.5
0.0
Wheat gluten
Corn gluten
Soya
Sun flower meal
Horse beans
Raps
Peas (yellow)
Lupin
Bioprotein
Fish meal
Fish hydrolysate (cuttings)
Fish hydrolysate (viscera)
Calanus
Bone meal
Blood meal
Feather meal.

# Low fish meal diets (5+5%)

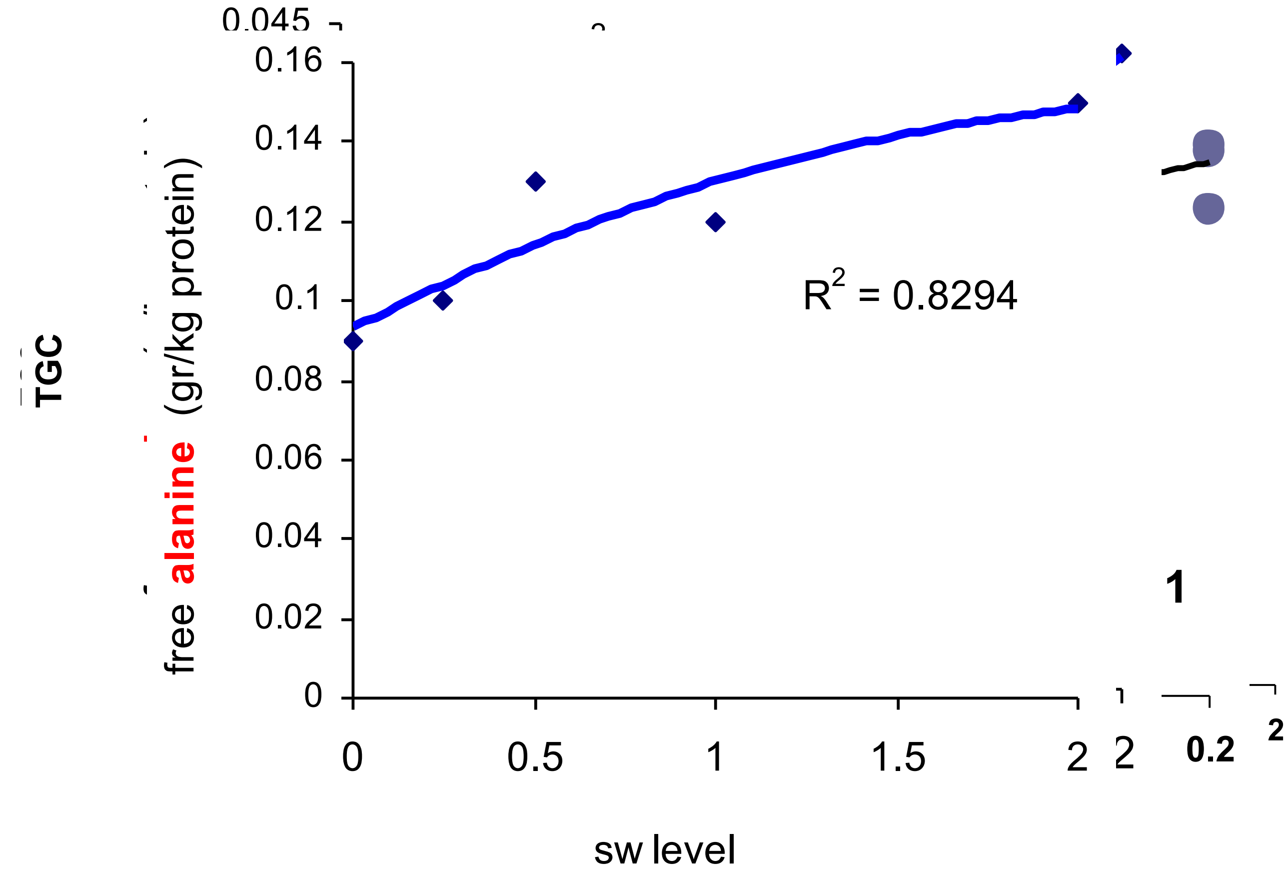


# Results - GROWTH




P<0.05

# Marine soluble compounds in feed correlating with growth




MWS

Tau

Hyp

Free Lys

Free Gly

Free Ser

Free Ala

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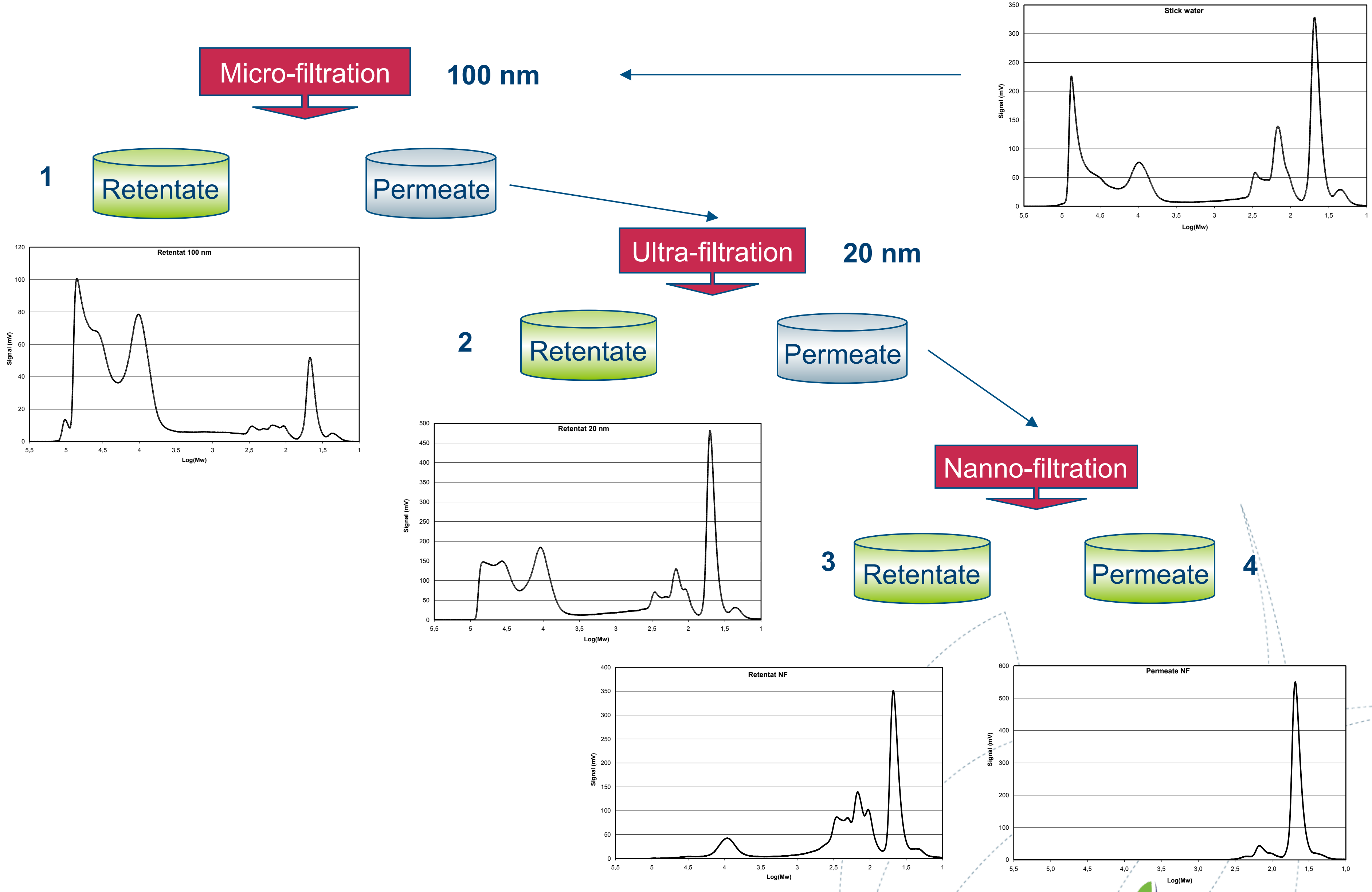
# Water soluble protein **fractionation** SW



Comparative Biochemistry and Physiology, Part B 156 (2010) 48–55

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

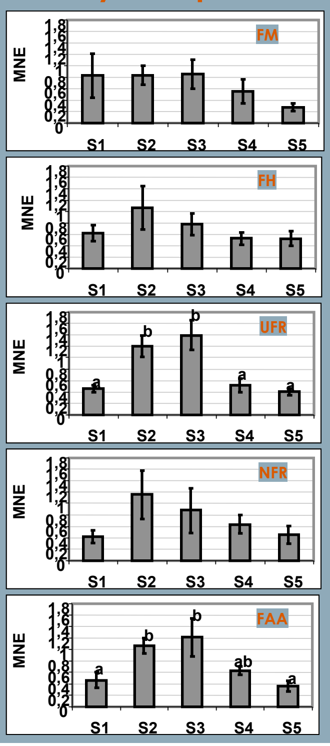
journal homepage: www.elsevier.com/locate/cbpb

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

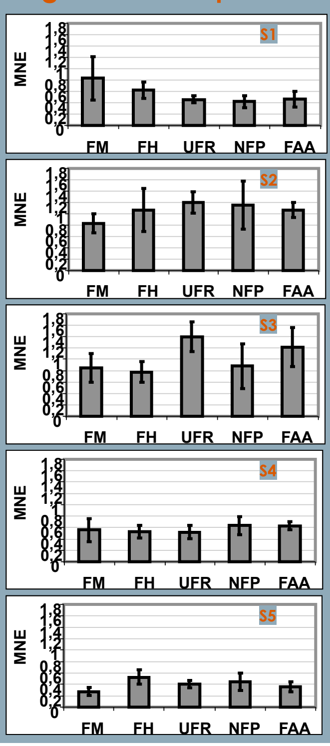
Snorre Bakke [a,*,1], Ann-Elise Olderbakk Jordal [a], Pedro Gómez-Requeni [a], Tiziano Verri [b], Katerina Kousoulaki [c], Anders Aksnes [c], Ivar Rønnestad [a]

[a] Department of Biology, University of Bergen, NO-5020 Bergen, Norway
[b] Department of Biological and Environmental Sciences and Technologies, University of Salento (formerly University of Lecce), I-73100 Lecce, Italy
[c] NOFIMA, NO-5141 Fyllingsdalen, Bergen, Norway


## Dietary comparison




## Segmental comparison




## Conclusion

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

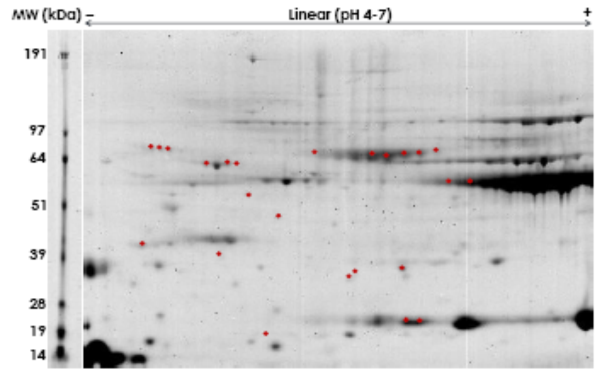
Dietary hydrolysates, peptides in various chain length as well as free amino acids affect segments relative expression of PepT1 along the whole intestinal tract.

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.

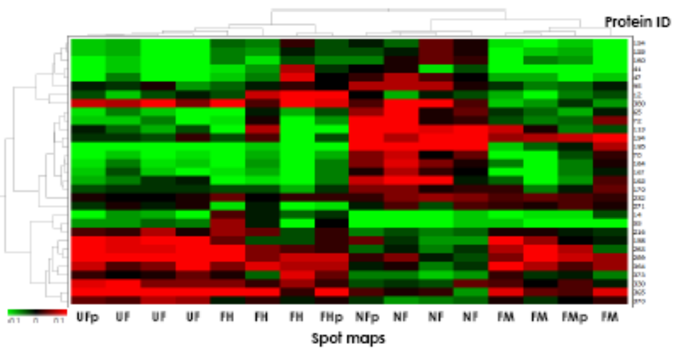
# Growth and muscle proteome response to fish protein hydrolysates in the diet of zebrafish

**Mahaut de Vareilles 2,…, Katerina Kousoulaki 3,…, IvarRønnestad 1**

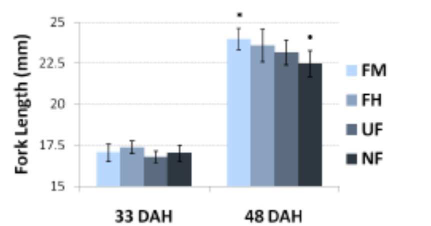
1 **Department of Biology** University of Bergen
2 **CCMAR** Universidade do Algarve
3 **NOFIMA Ingrediens**



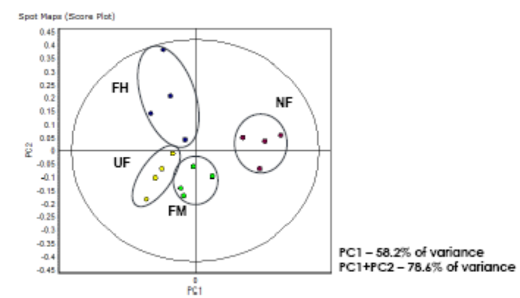

**Low-salt soluble proteins (sarcoplasmic fraction) of *D. rerio* white muscle, stained with CBB G-250.** Spots being sequenced are shown in red.




**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 DeCyder 2-D software V7.0 .



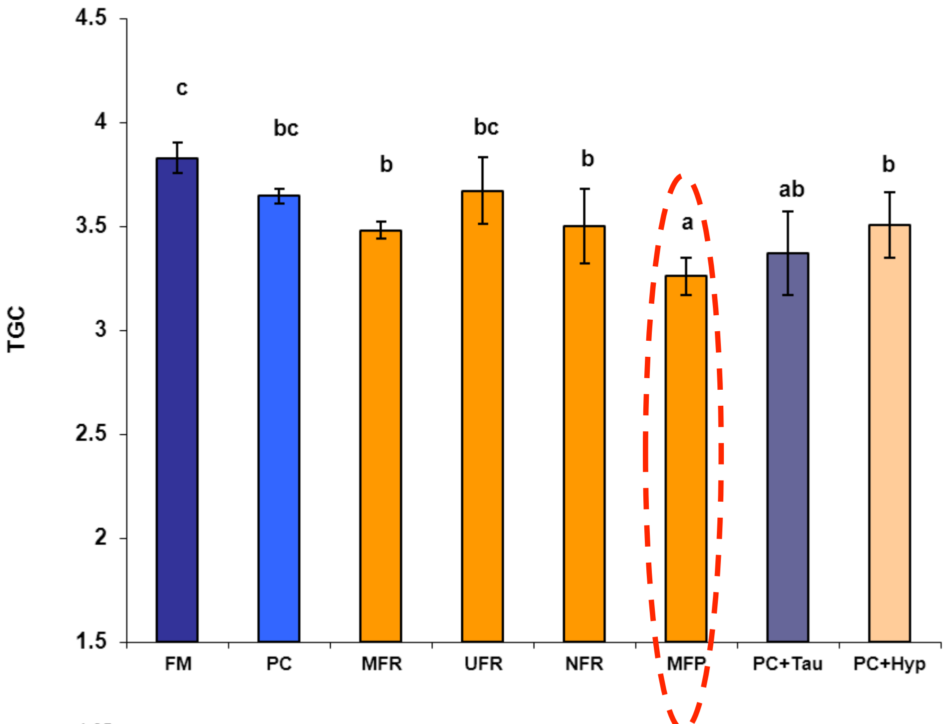

**Growth of juvenile zebrafish during 15 day feeding trial,** expressed as initial and final fork length (mm ±S.D.)
* indicates significantly different values (One-Way ANOVA; p-value<0.05)

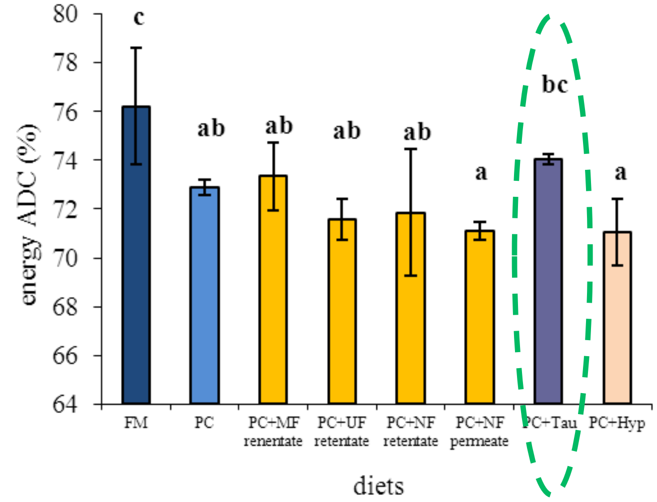


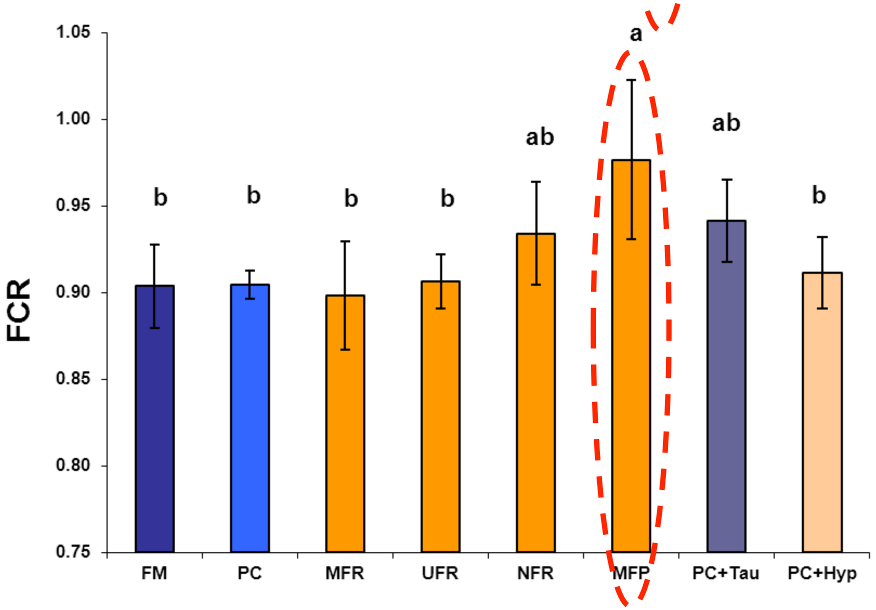

**PCA on differentially expressed proteins (One-way ANOVA; p-value<0.05);** DeCyder 2-D software V.7.0 in-built function used, with mean centering and standard deviation scaling, on protein.

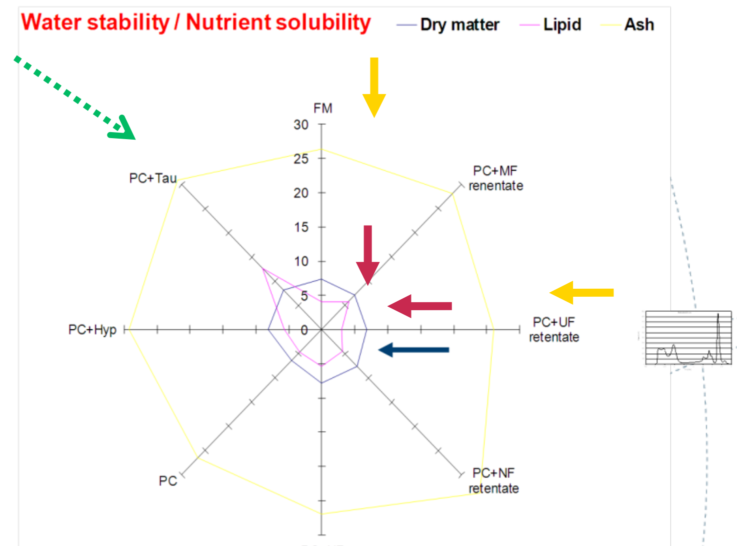
## CONCLUSIONS

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 be 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.

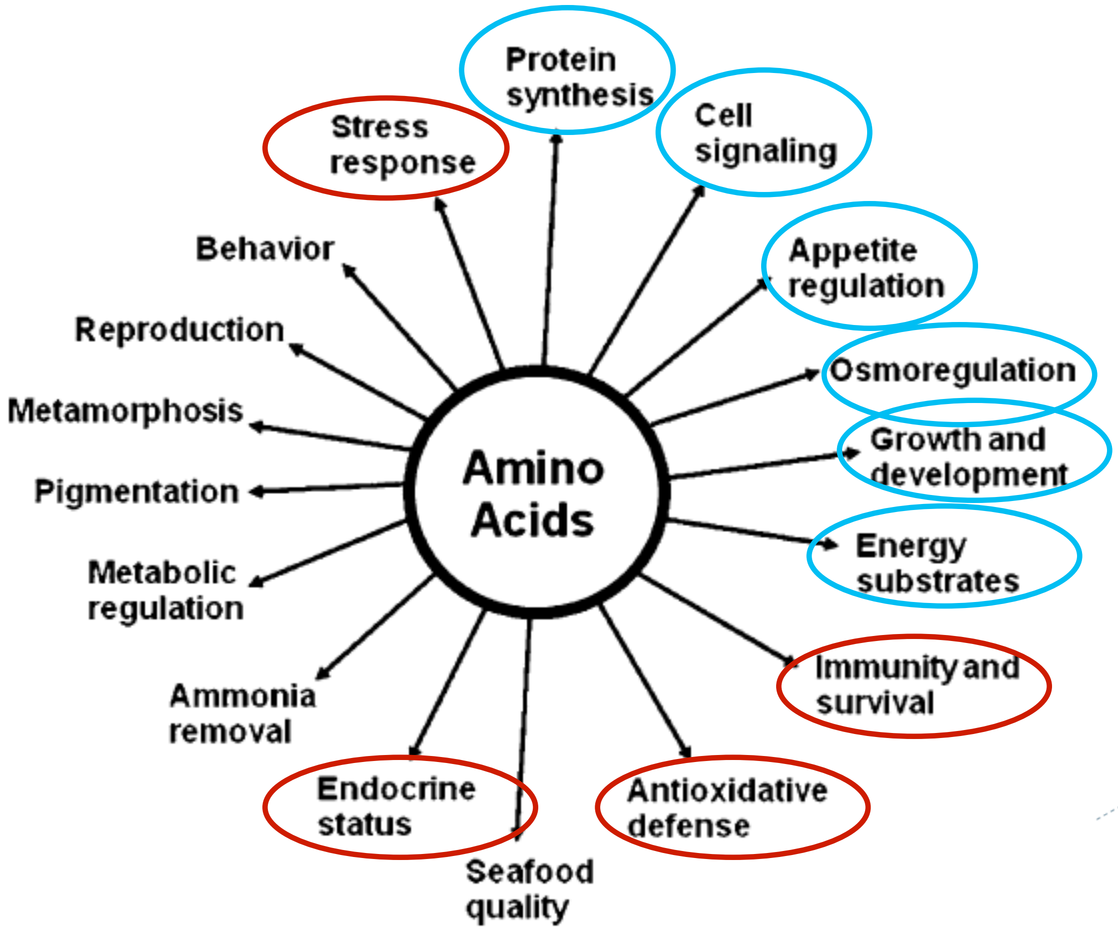

TGC
4.5
4
3.5
3
2.5
2
1.5
c
bc
b
bc
b
a
ab
b
FM
PC
MFR
UFR
NFR
MFP
PC+Tau
PC+Hyp


energy ADC (%)
80
78
76
74
72
70
68
66
64
c
ab
ab
ab
ab
a
bc
a
FM
PC
PC+MF renentate
PC+UF retentate
PC+NF retentate
PC+NF permeate
PC+Tau
PC+Hyp
diets


FCR
1.05
1.00
0.95
0.90
0.85
0.80
0.75
b
b
b
b
ab
a
ab
b
FM
PC
MFR
UFR
NFR
MFP
PC+Tau
PC+Hyp


Water stability / Nutrient solubility
Dry matter
Lipid
Ash
FM
PC+MF renentate
PC+UF retentate
PC+NF retentate
PC+NF
PC
PC+Hyp
PC+Tau
30
25
20
15
10
5
0

# Amino acid regulation of metabolic pathways



Comparative Biochemistry and Physiology, Part D 6 (2011) 178–186

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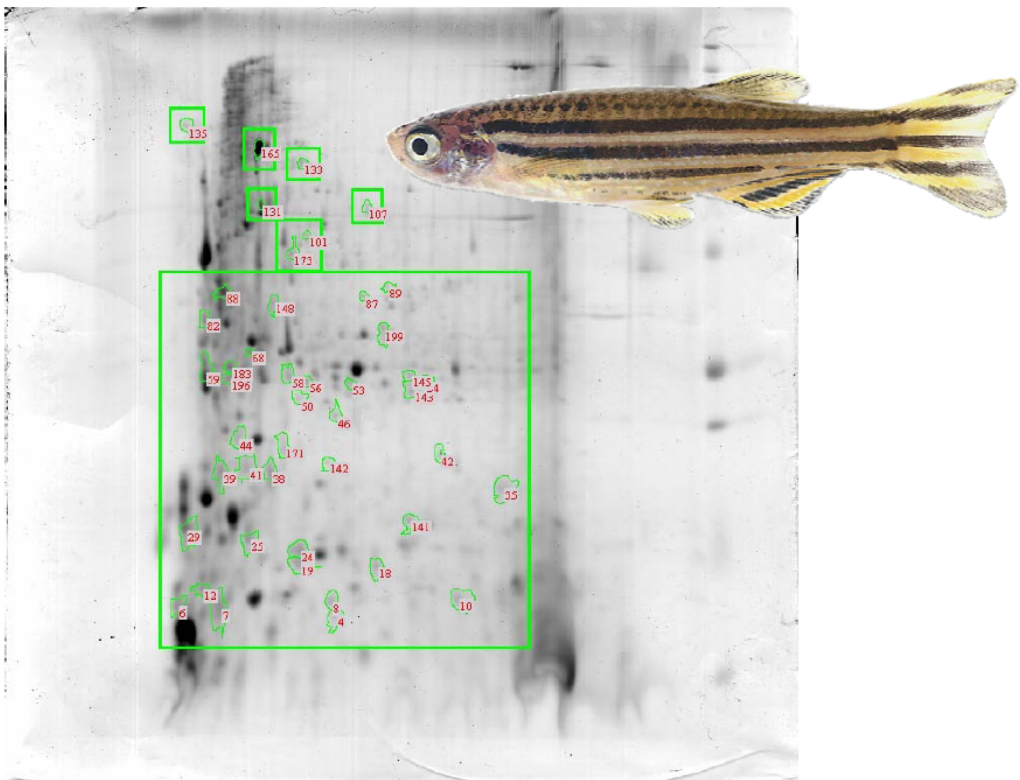
Comparative Biochemistry and Physiology, Part D

journal homepage: www.elsevier.com/locate/cbpd

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

Pedro Gómez-Requeni [a,*], Mahaut de Vareilles [a,b], Katerina Kousoulaki [c], Ann-Elise O. Jordal [a], Luis E.C. Conceição [b], Ivar Rønnestad [a]



## Lysine metabolism

- **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.

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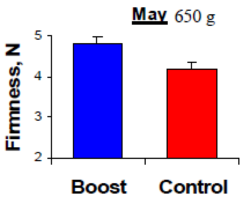
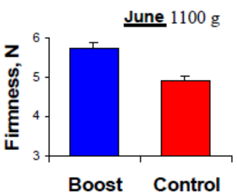
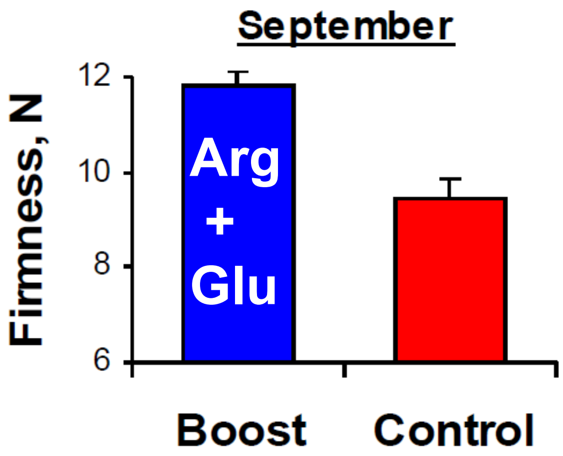
Turid Mørkøre, Nofima Marin, BioMarint Industrie Seminar 2009




BOOST = fôr tilsatt en blanding av arginin & glutamat


Teksturen kan forbedres gjennom blant annet fôret. Foto: iStockphoto.

**+ Hydroxyproline**

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

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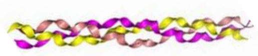
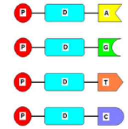
# WOUND HEALING

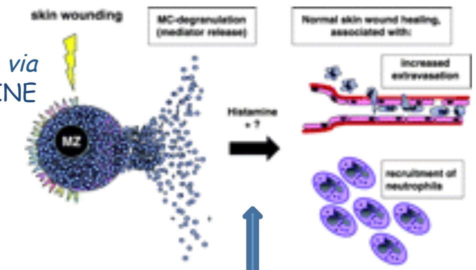
COLLAGEN

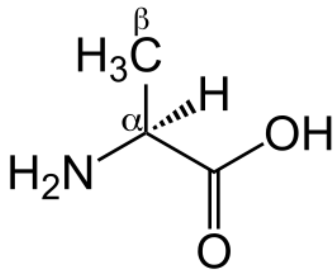




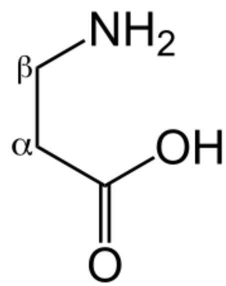
NUC
LEO
TID
ES

WOUND
HEALING *via*
HISTAMINE



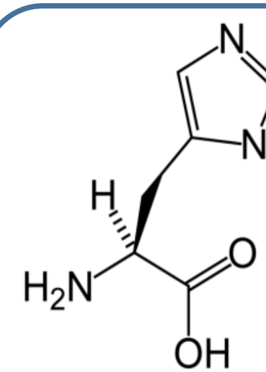




L-alanine



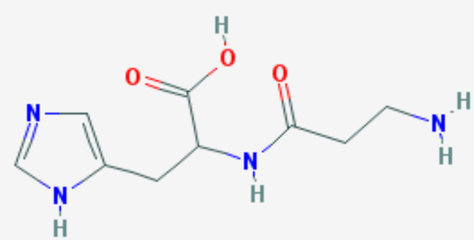

beta-alanine

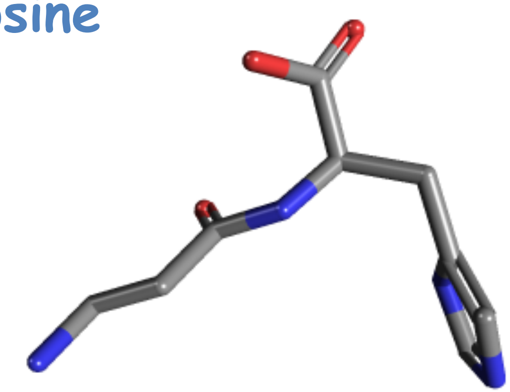
IUPAC name: 3-Aminopropanoic acid




histidine

carnosine



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.**



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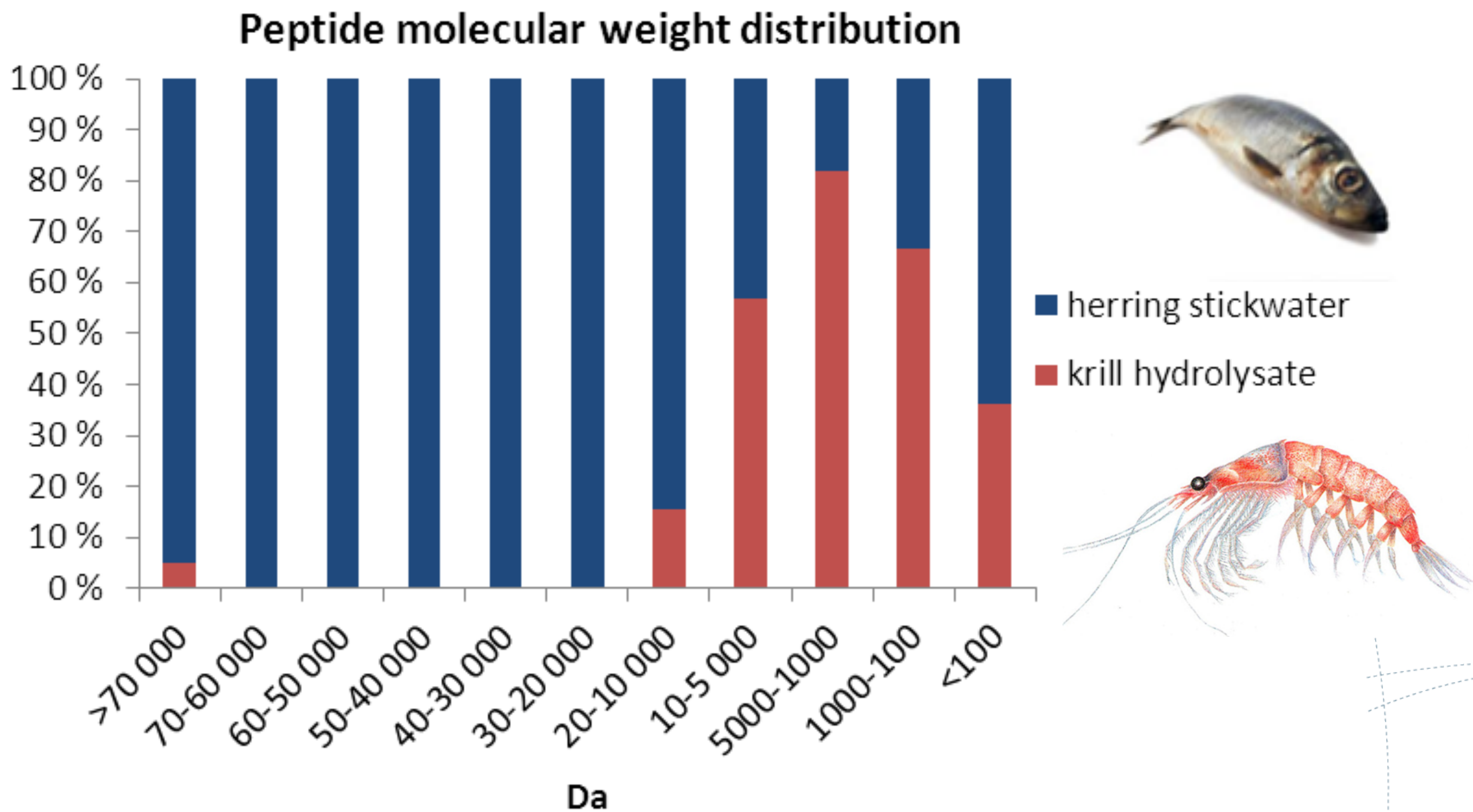
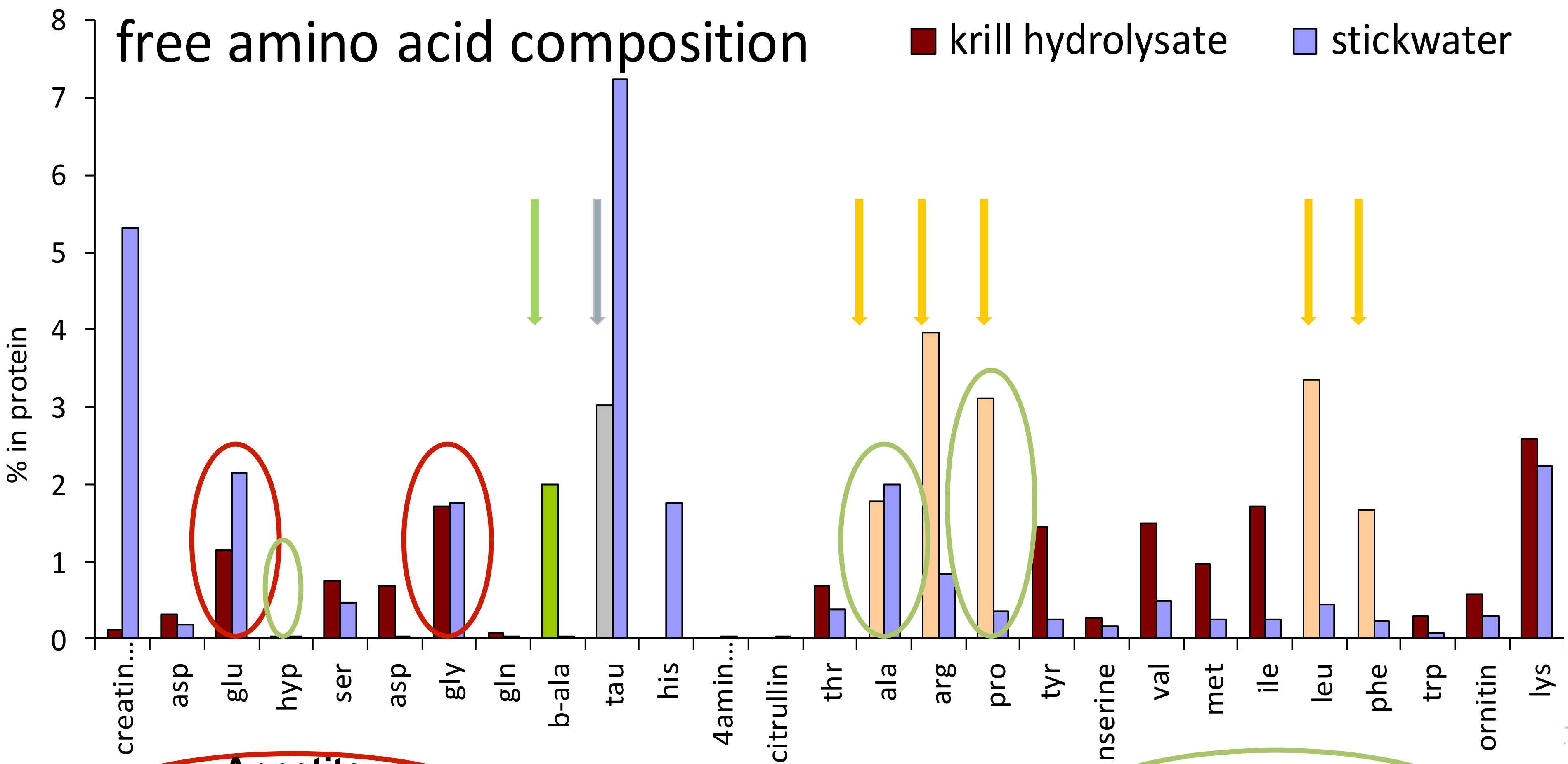
# Soluble marine proteins



# Feed intake stimulating raw materials




**Appetite stimulation in fish**

**Appetite stimulation in salmonids**

**Appetite stimulation in Atlantic salmon**

**Collagen & nucleotide formation**

**Osmoregulation & gut formation**

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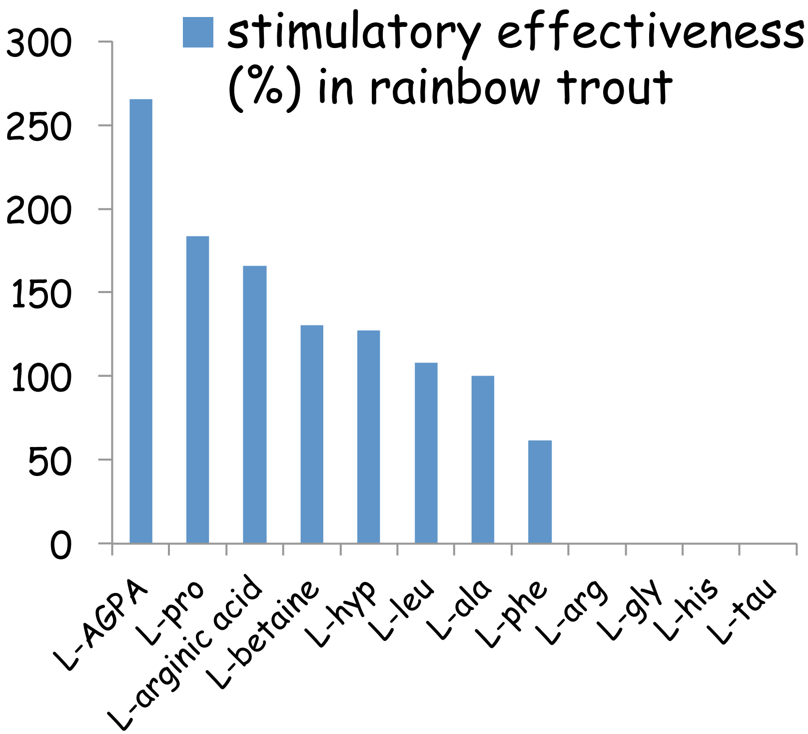
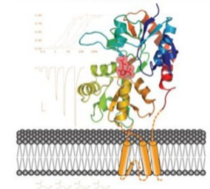
# TASTE STIMULATION IN SALMONIDS




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

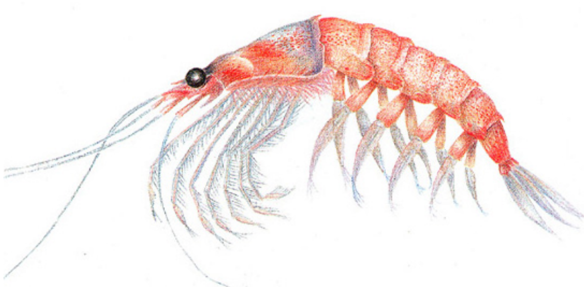
Hara et al. 1994. Journal of Fish Biology 45, 453–465.

L-AGPA is an arginine (arg) metabolite.

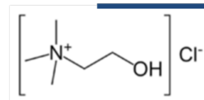
Choline is oxidised to betaine.

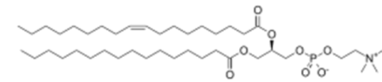
# Salmon feed attractants

## 1. Krill hydrolysate (LAK & HAK)



## 2. Choline chloride ⟷ Phosphatidylcholine

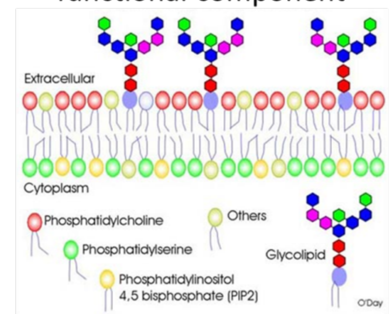


Accelerates growth in:

Attractant for:

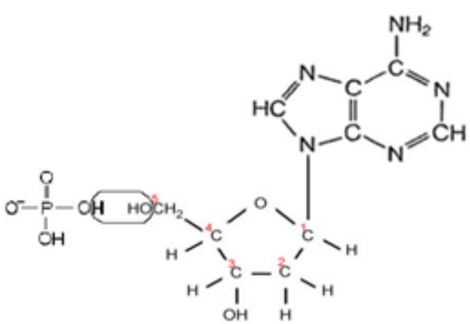
Cell membrane structural and functional component




Health benefits in humans suffering from hepatitis

## 3. Nucleotide AMP

Adenosine monophosphate (AMP)



0.15% AMP improved grouper feed intake growth and immune response (Lin et al., 2009)

## 4. Free AA (as in LAK)

## 5. AMP+CC+AA

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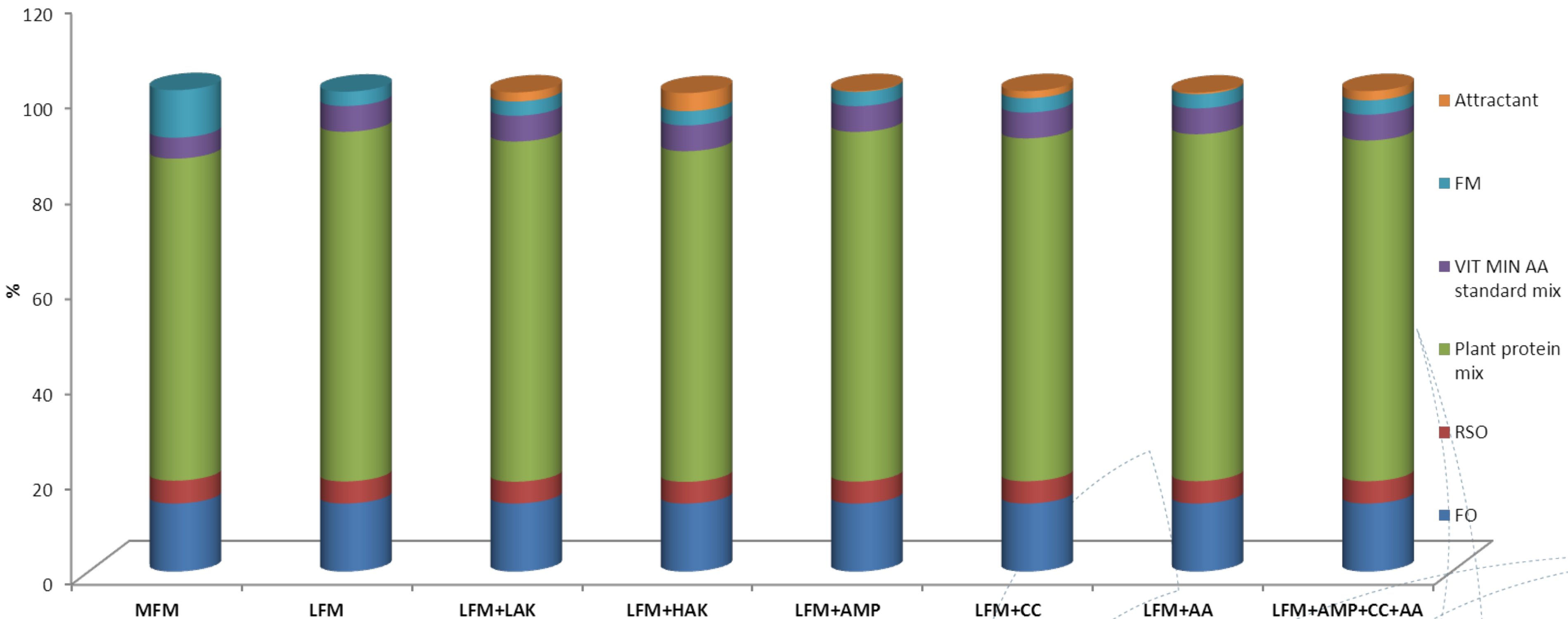
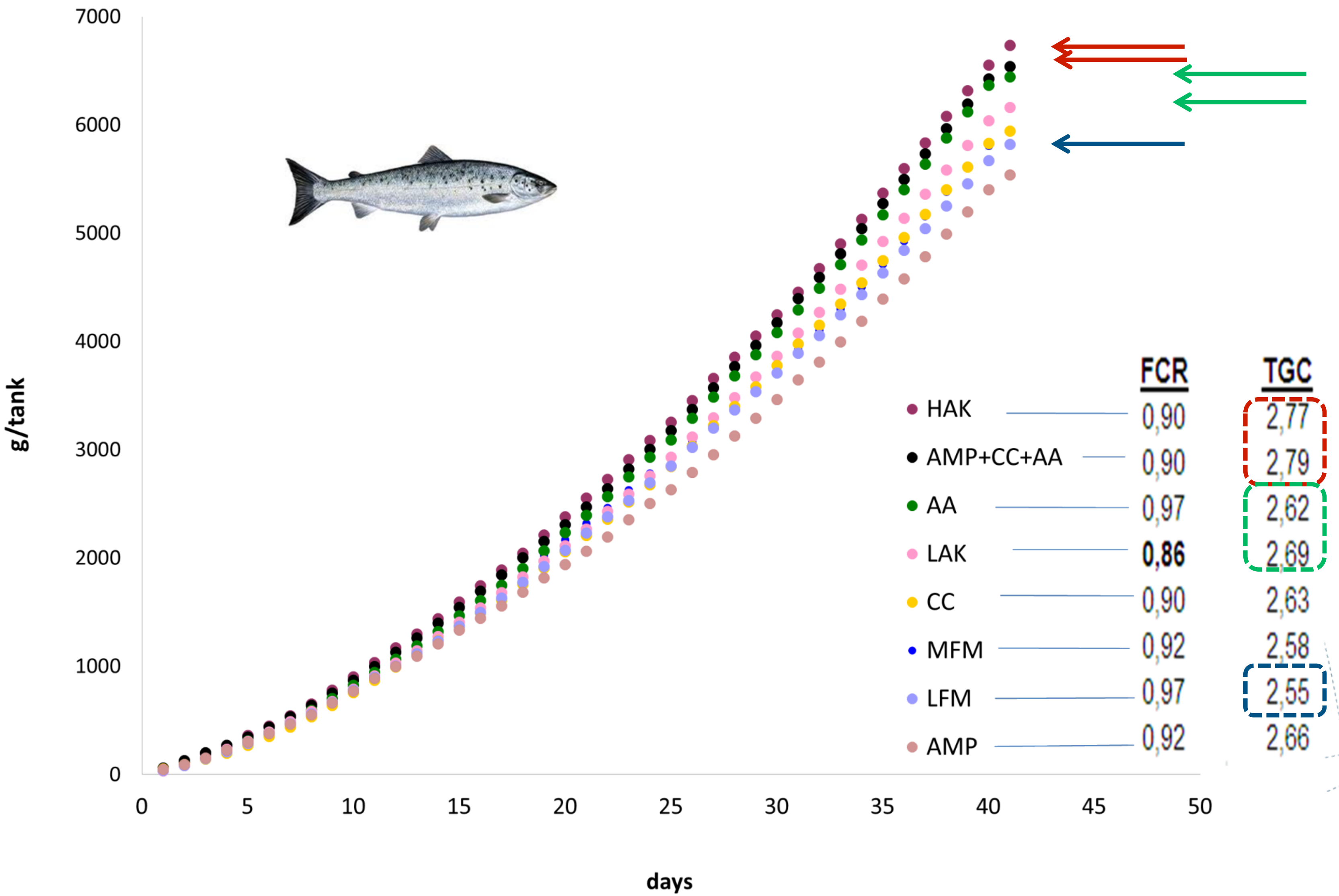
# Feed formulation

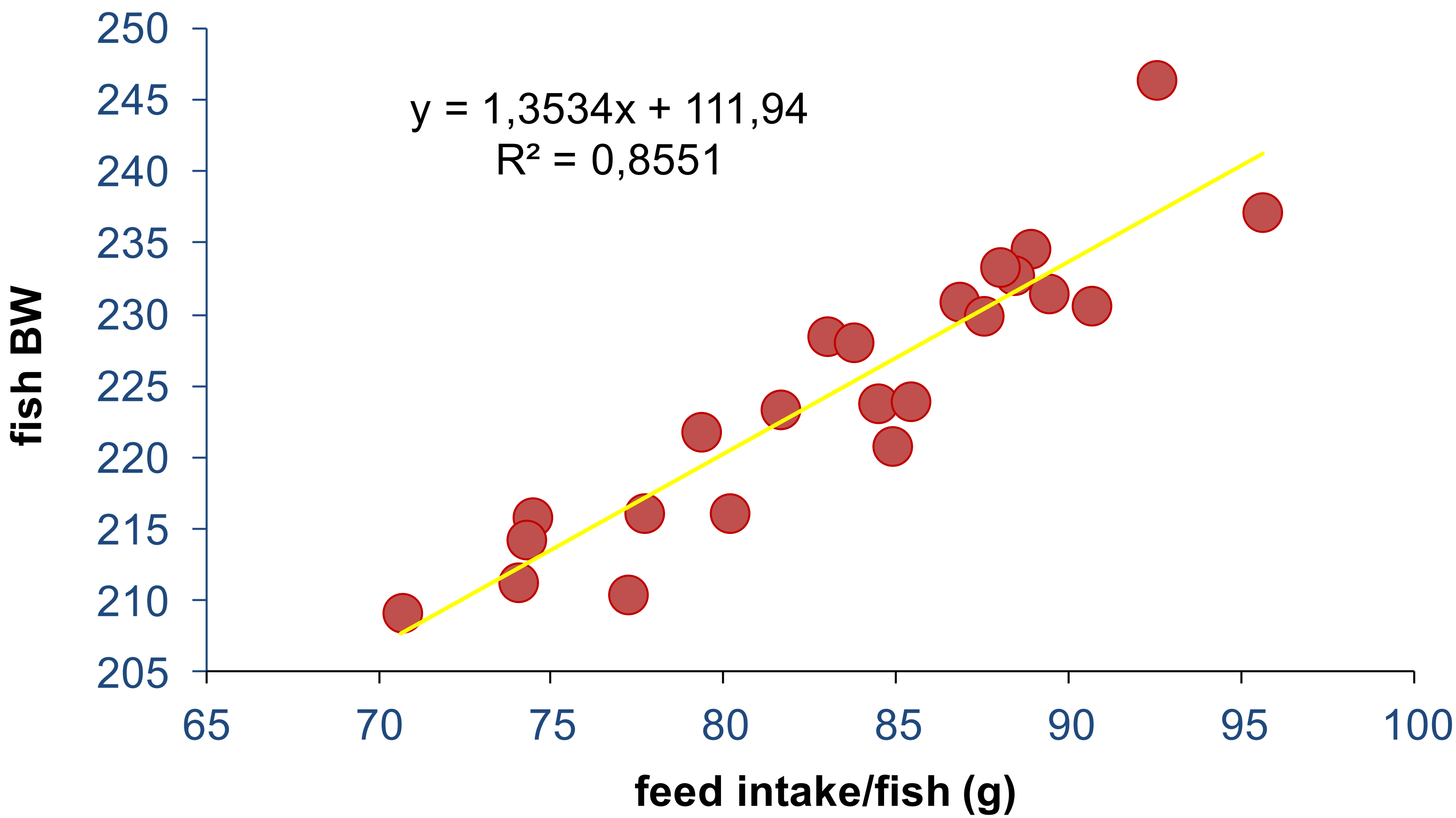


# Results: Cumulative Feed Intake



# Results: Feed Intake *vs* Growth

P<0.01



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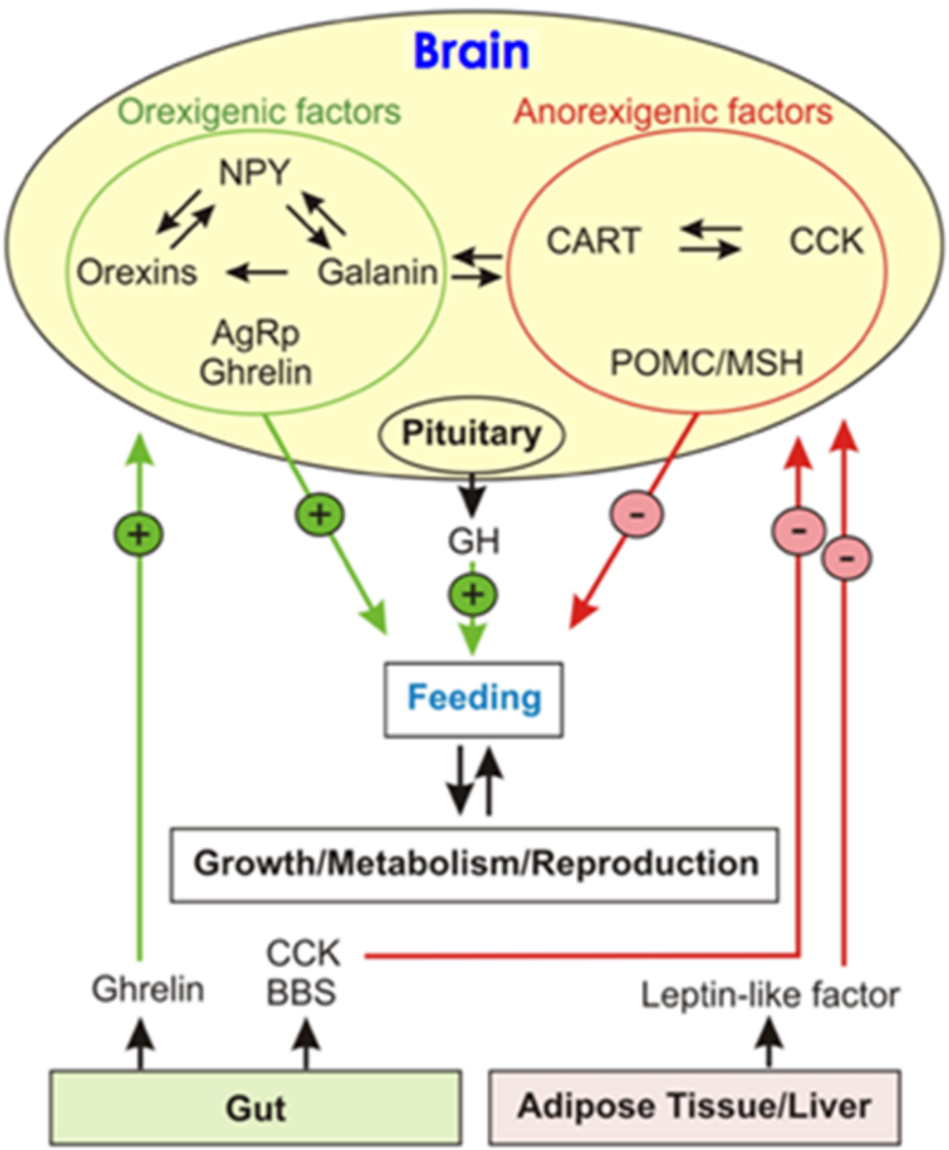
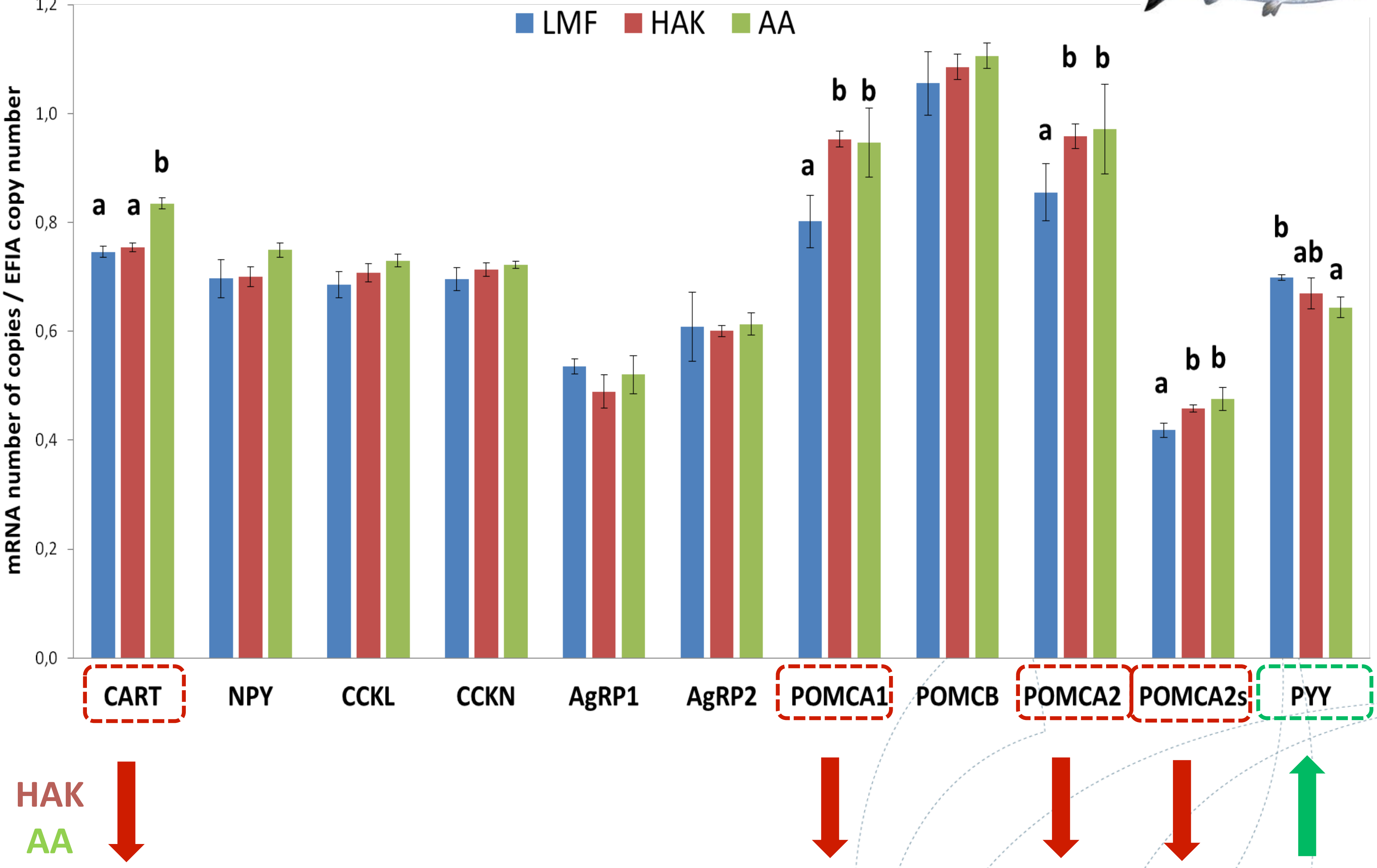
# Appetite regulation in the brain and gut

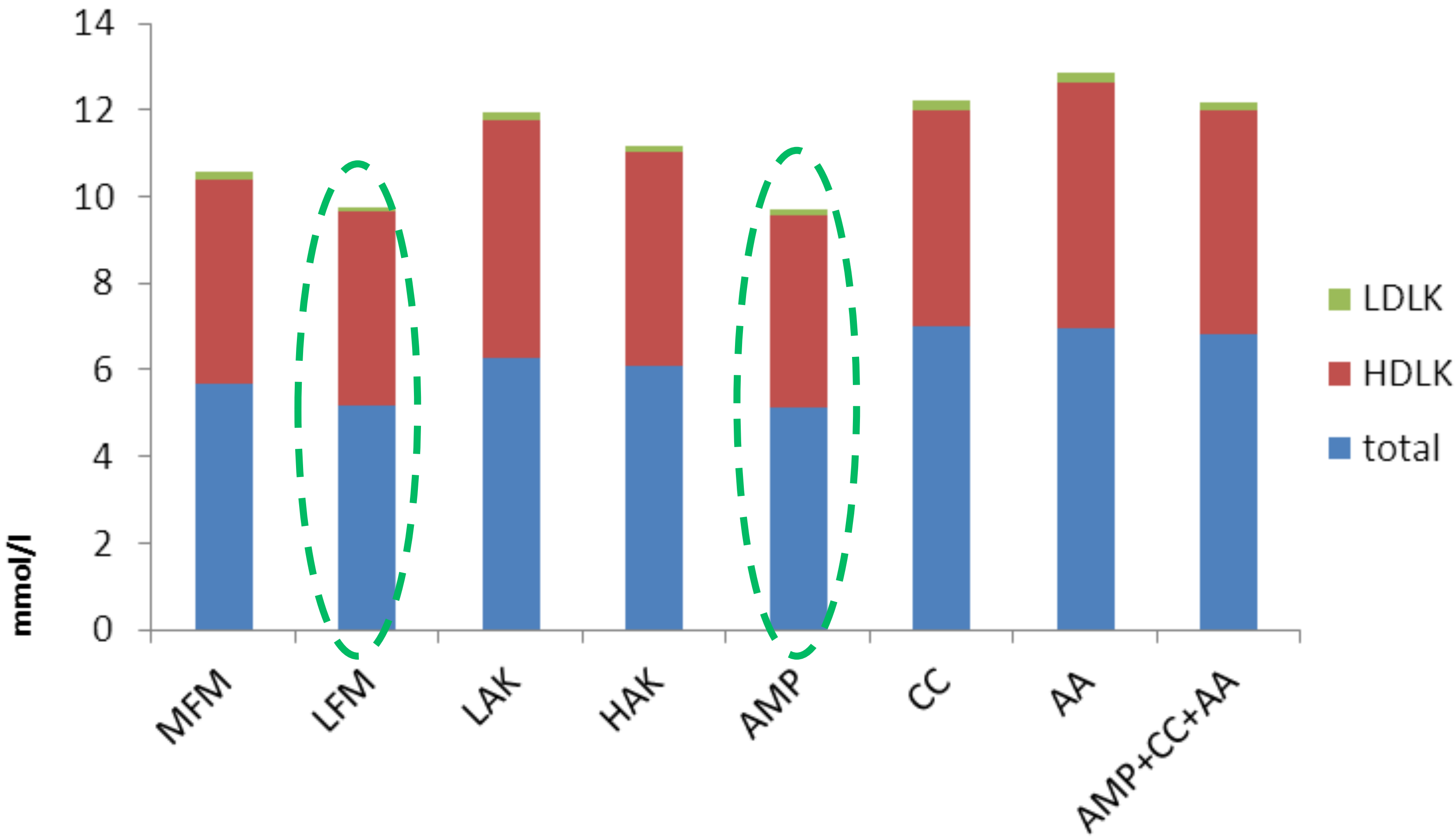



Appetite (Model in adult teleosts, based on Volkoff et al., 2007- Rønnestad 2010)

# Apeptite regulating genes' expression



# Plasma lipids & cholesterol

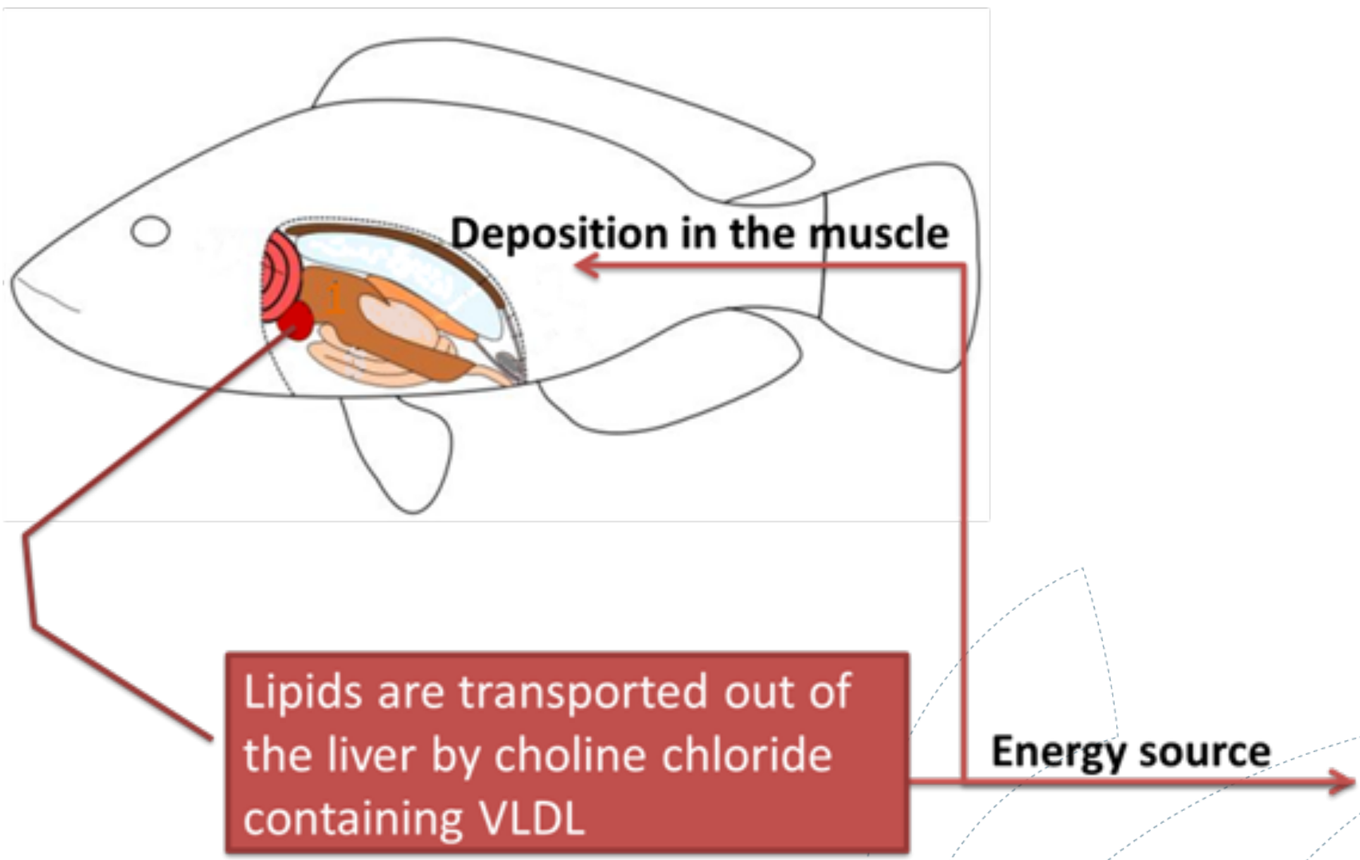



0

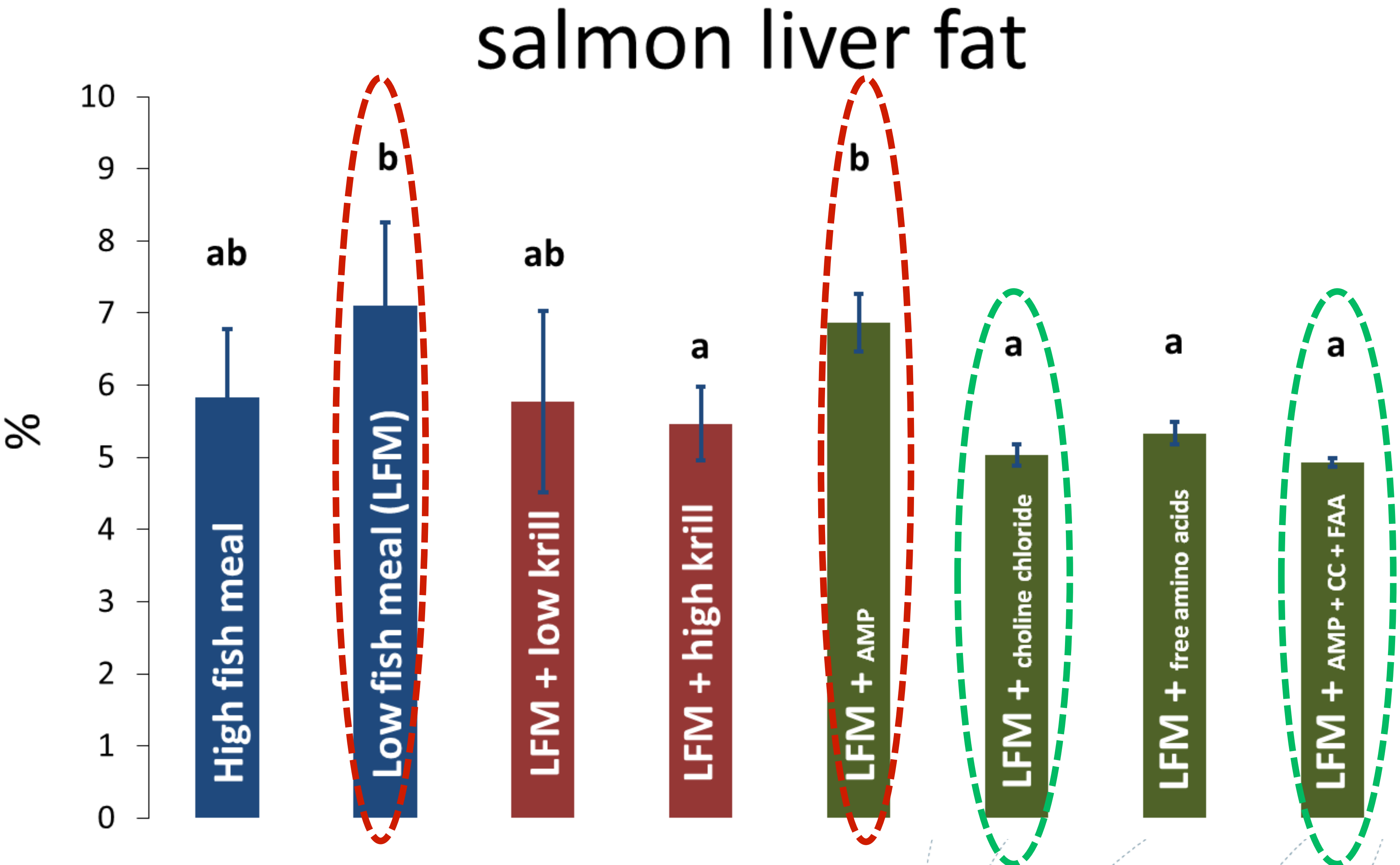
AMP+CC+AA

# Choline function

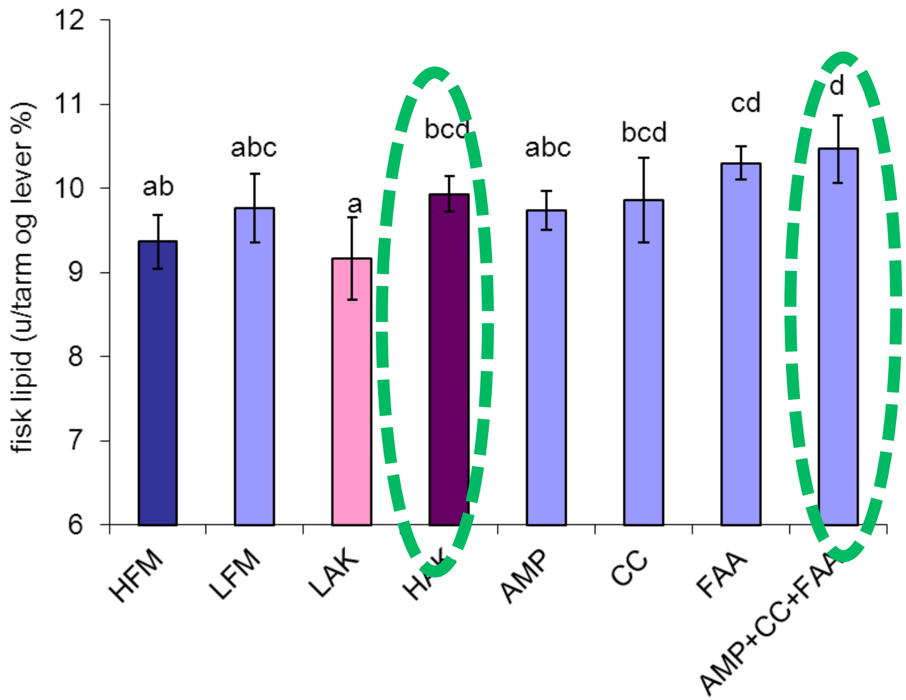
## Lipid stores & transport

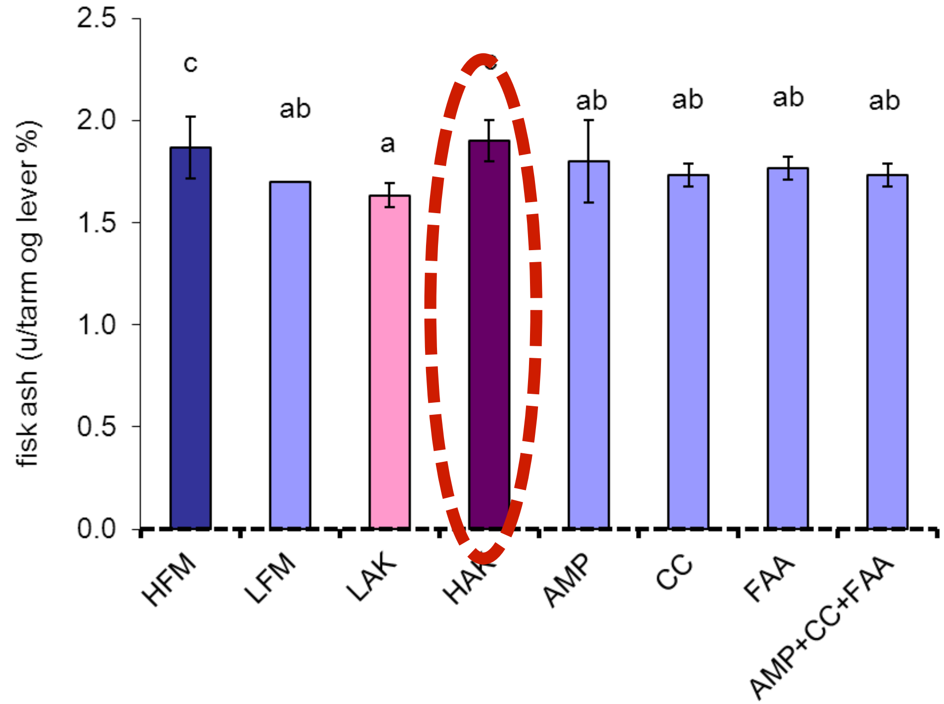


# Liver status

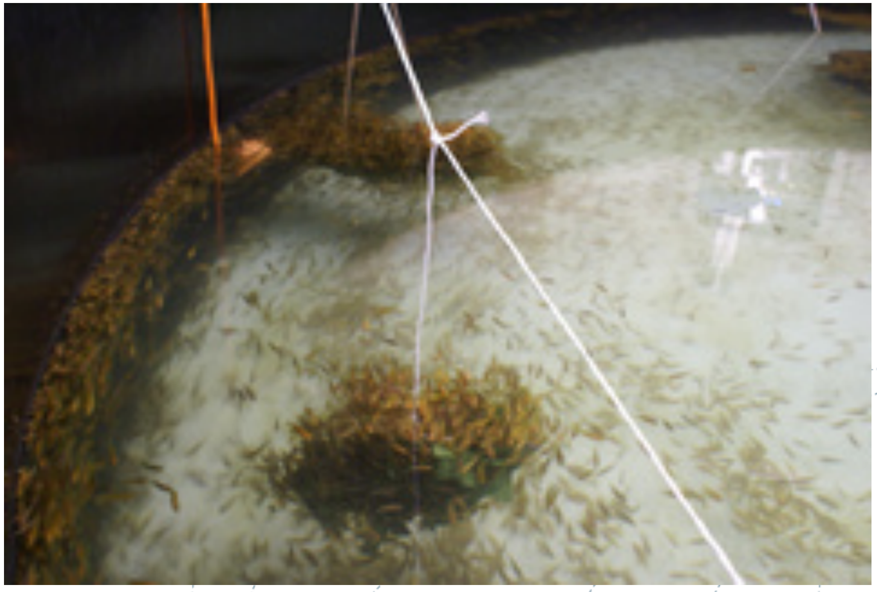


# Gutted body composition



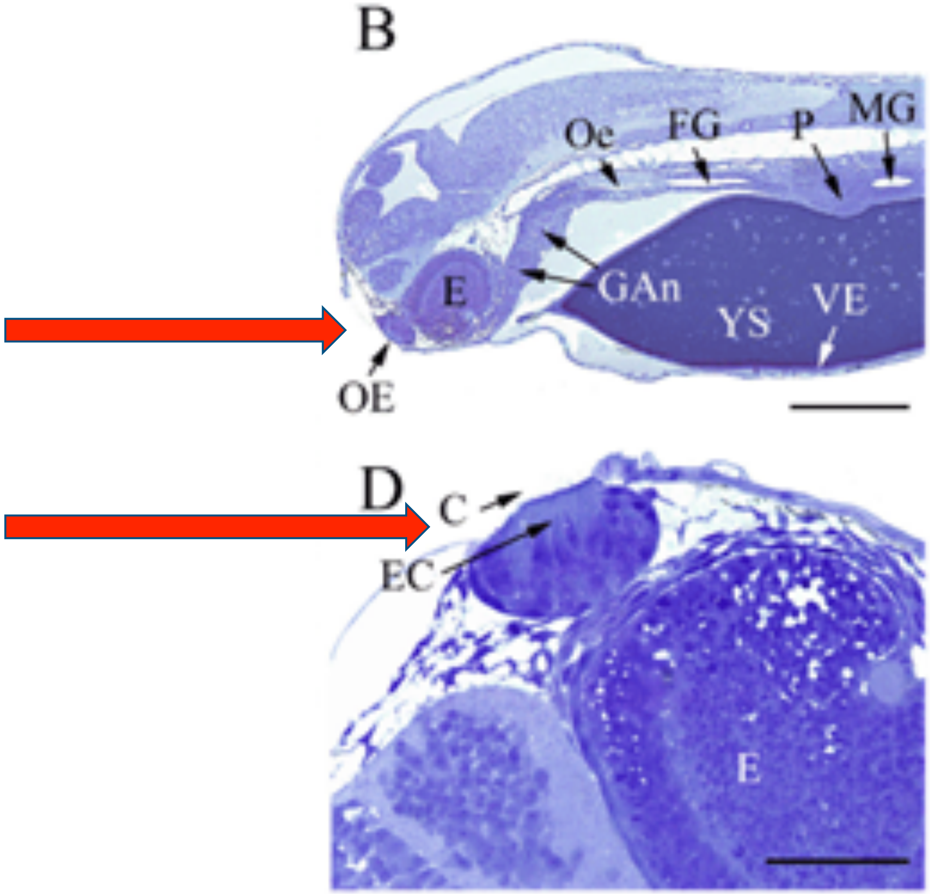



Ballan wrasse, *Labrus bergylta*



# Ballan wrasse *Labrus bergylta* larvae

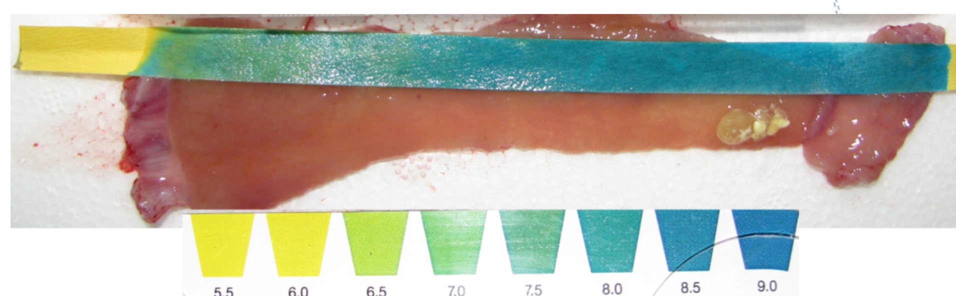
(B) Larva at hatching (D) Olfactory organ at hatching.




Dunaevskaya et al. (2012)

Figueiredo et al., 2005

| 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

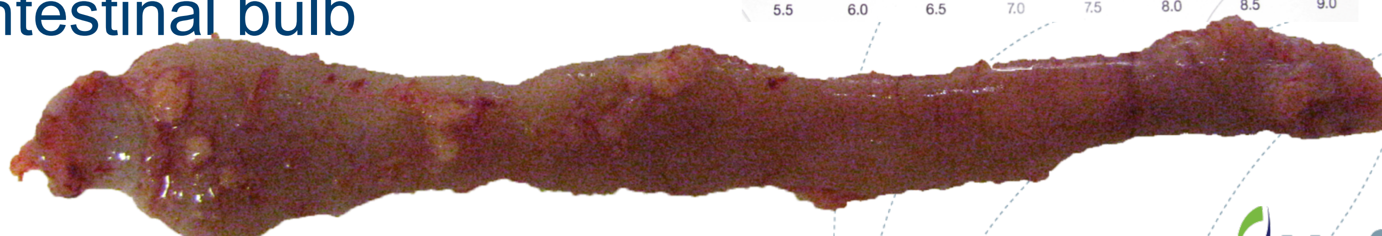
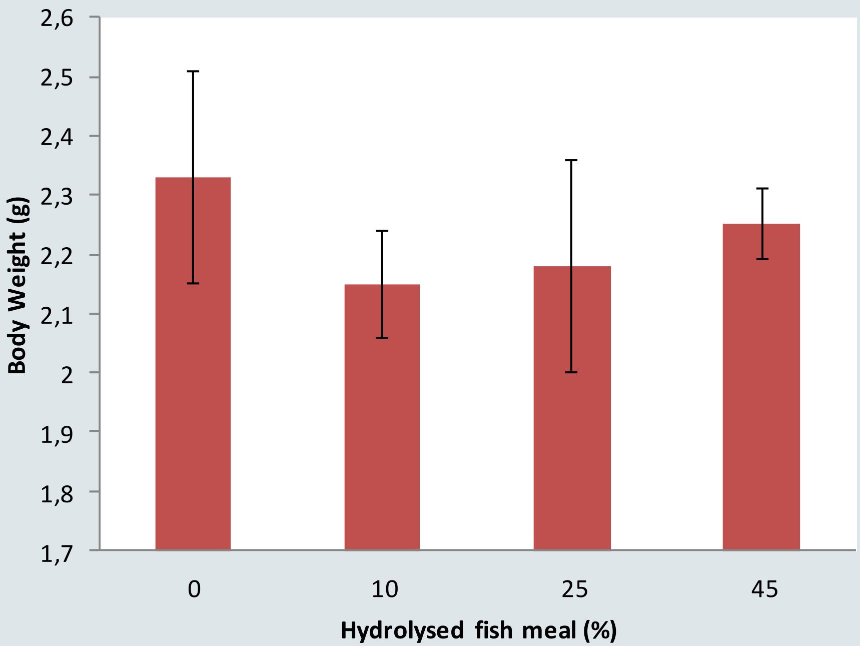
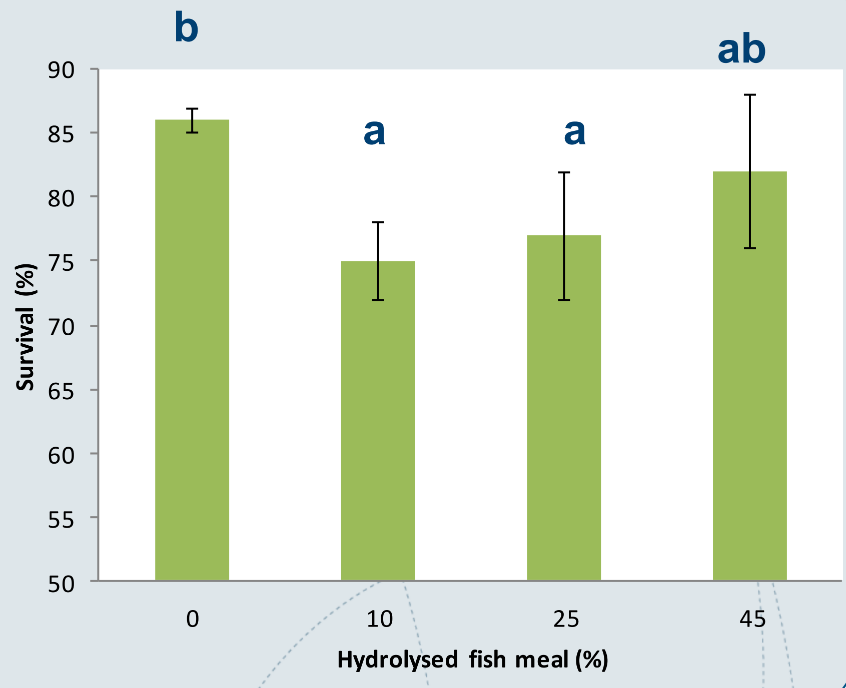


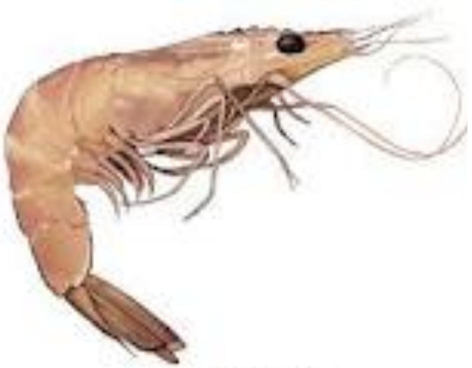
Photo: Saele

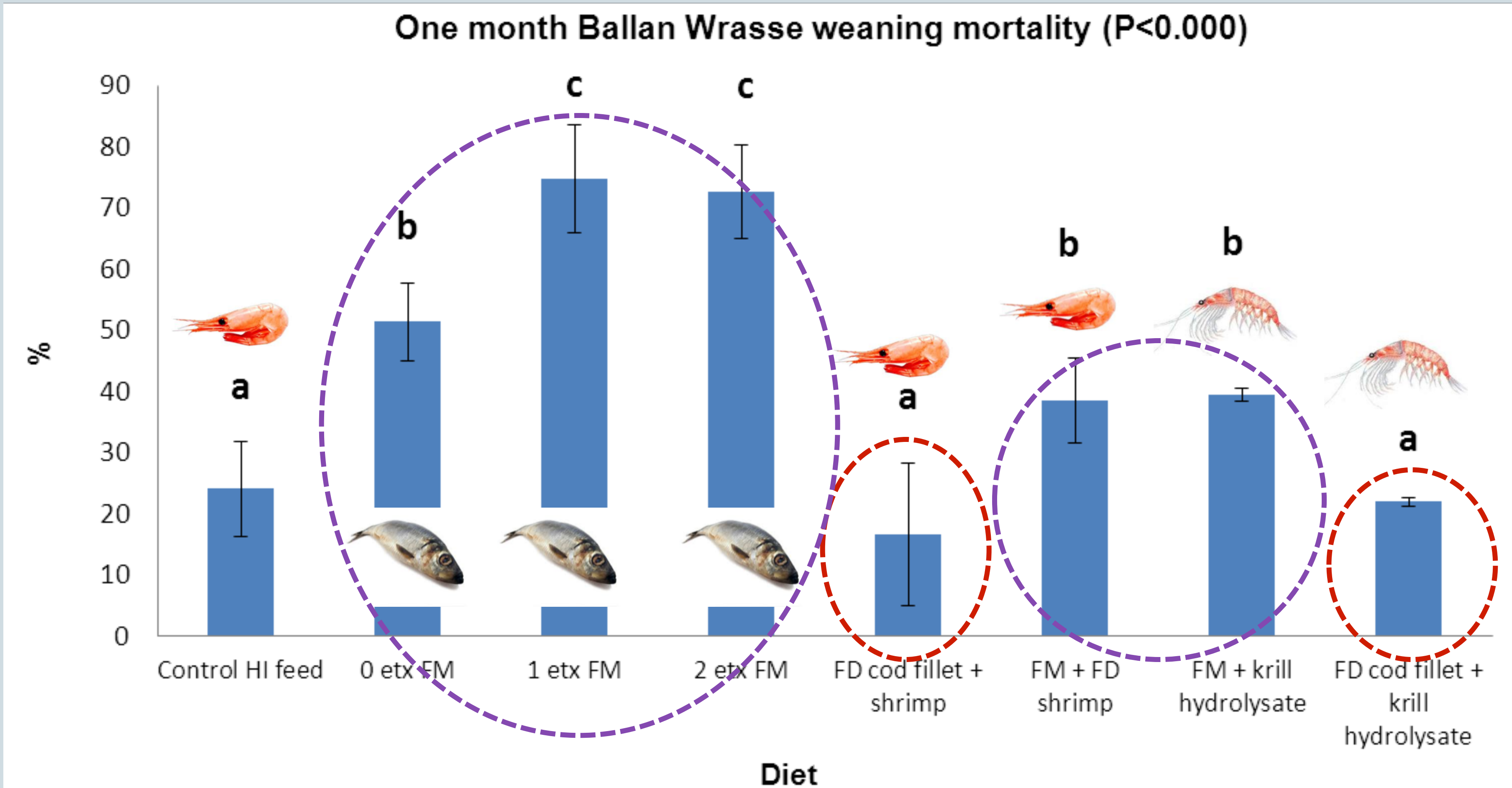
# Increasing amounts of hydrolysed protein



# Raw materials - Attraktant studies

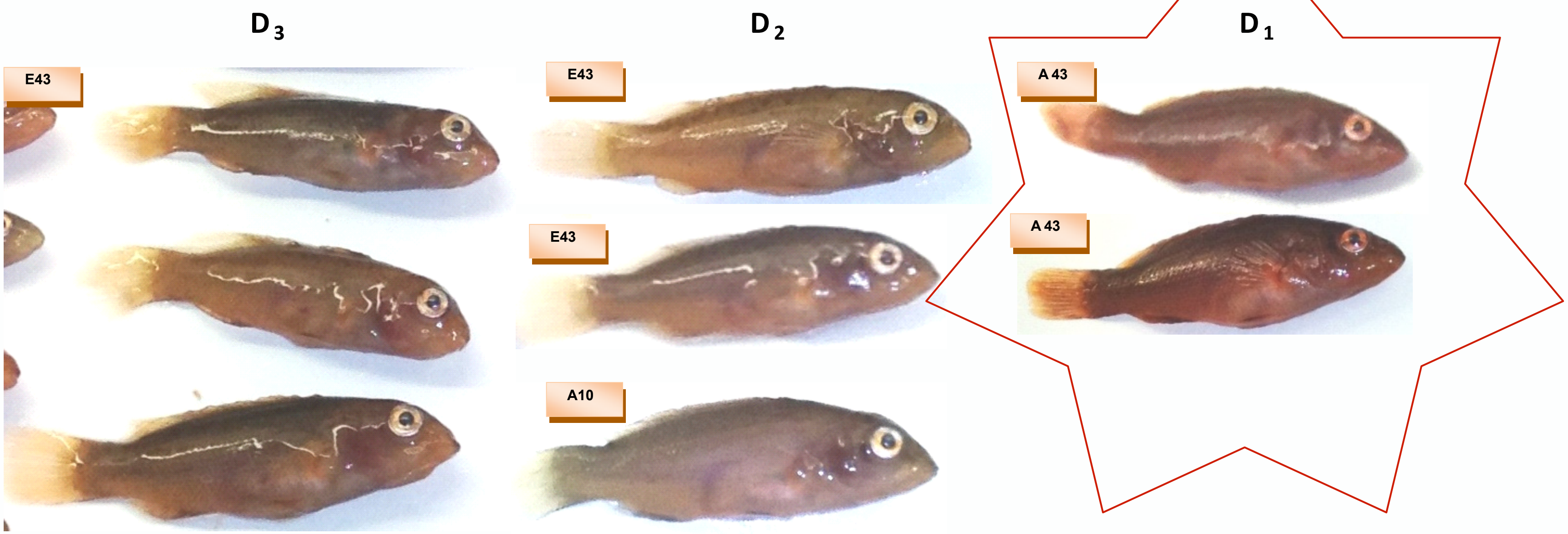




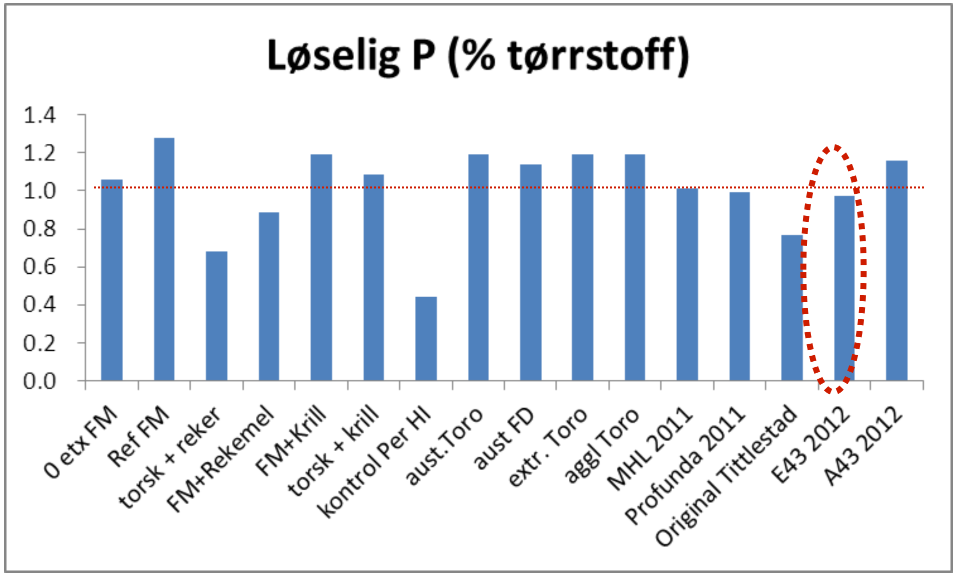
One month Ballan Wrasse weaning mortality (P<0.000)
%
90
80
70
60
50
40
30
20
10
0
a
b
c
c
a
b
b
a
Control HI feed
0 etx FM
1 etx FM
2 etx FM
FD cod fillet + shrimp
FM + FD shrimp
FM + krill hydrolysate
FD cod fillet + krill hydrolysate
Diet

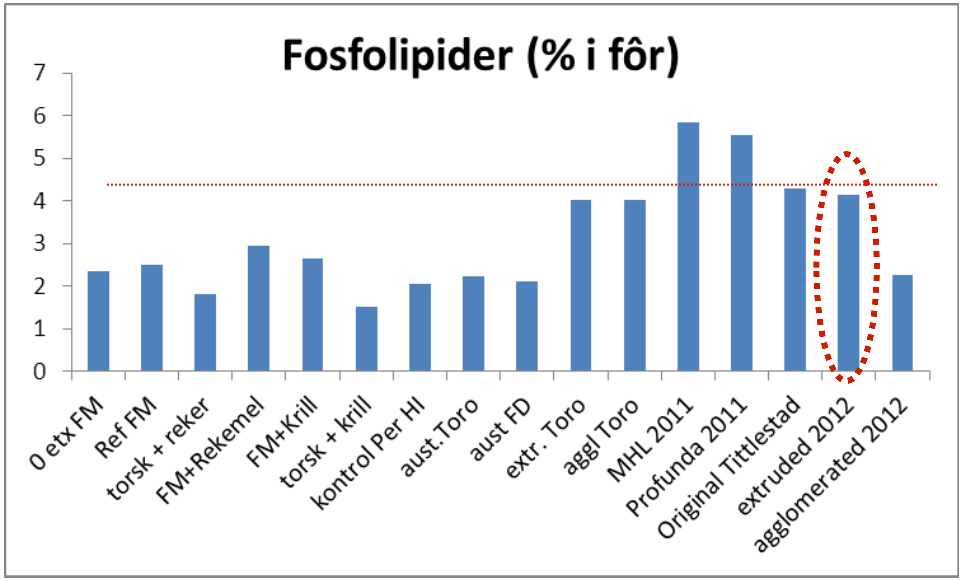

# Head deformities

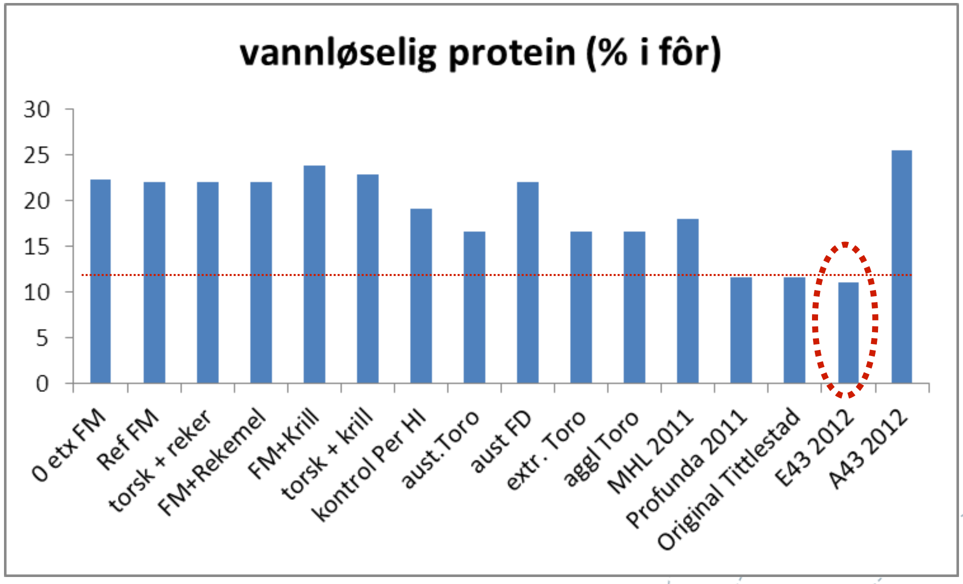
| | Cod muscle + shrimp | Cod Muscle + Stick Water + shrimp |
|---|---|---|
| Survival % | 62 | 35 |
| SGR | 5.1 | 4.9 |
| Good fish % | 3 | 32 |



# Weaning diets history



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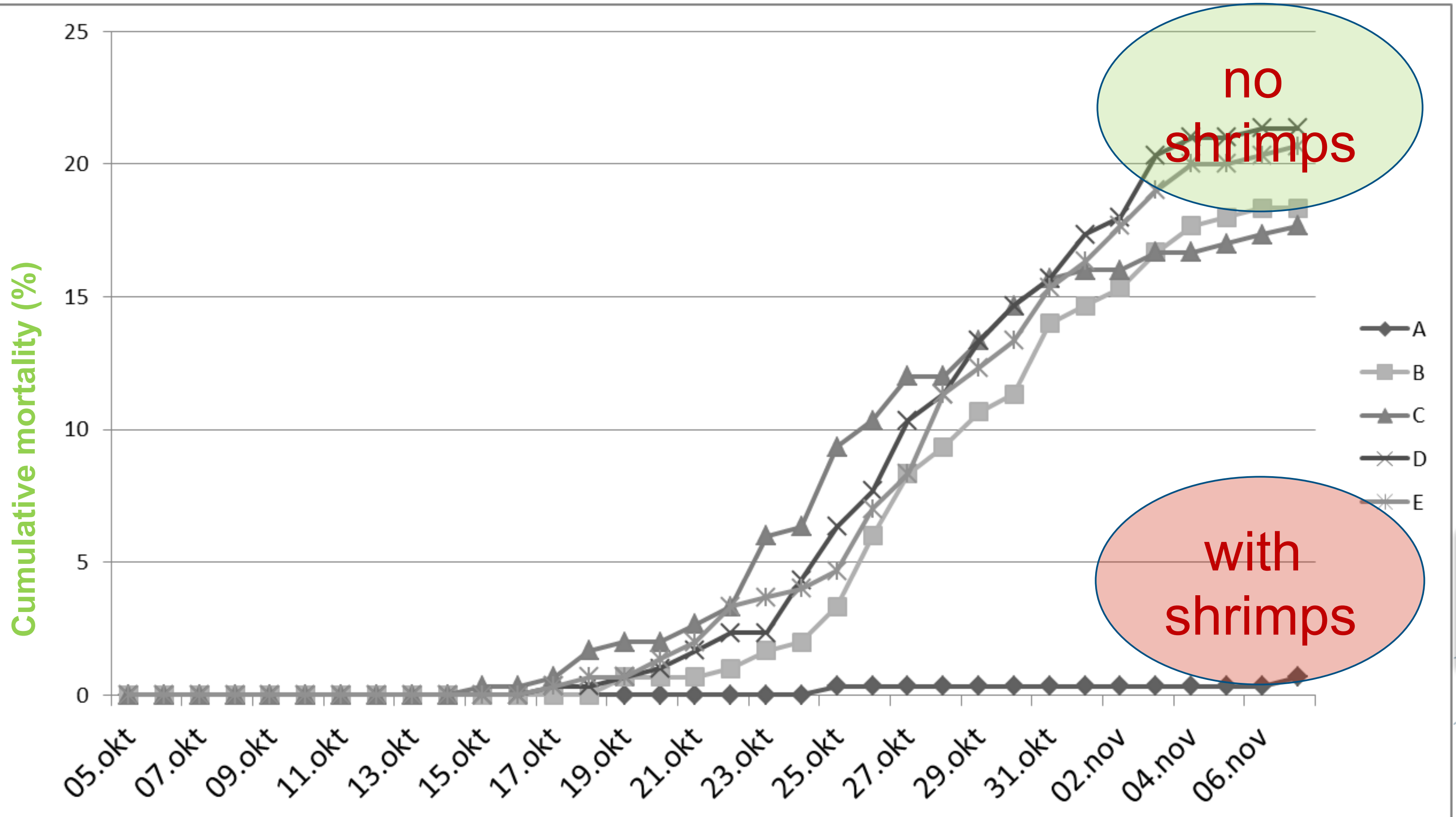
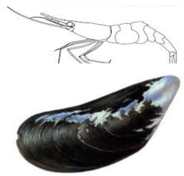
| | Shrimp meal<br>A | Shrimp shell meal<br>B | Blue mussle<br>C | Squid meal<br>D | Fishmeal only<br>E |
|---|---|---|---|---|---|
| Ingredient cost NOK/kg | 61.0 | 15.0 | 19.5 | 16.0 | 15.0 |





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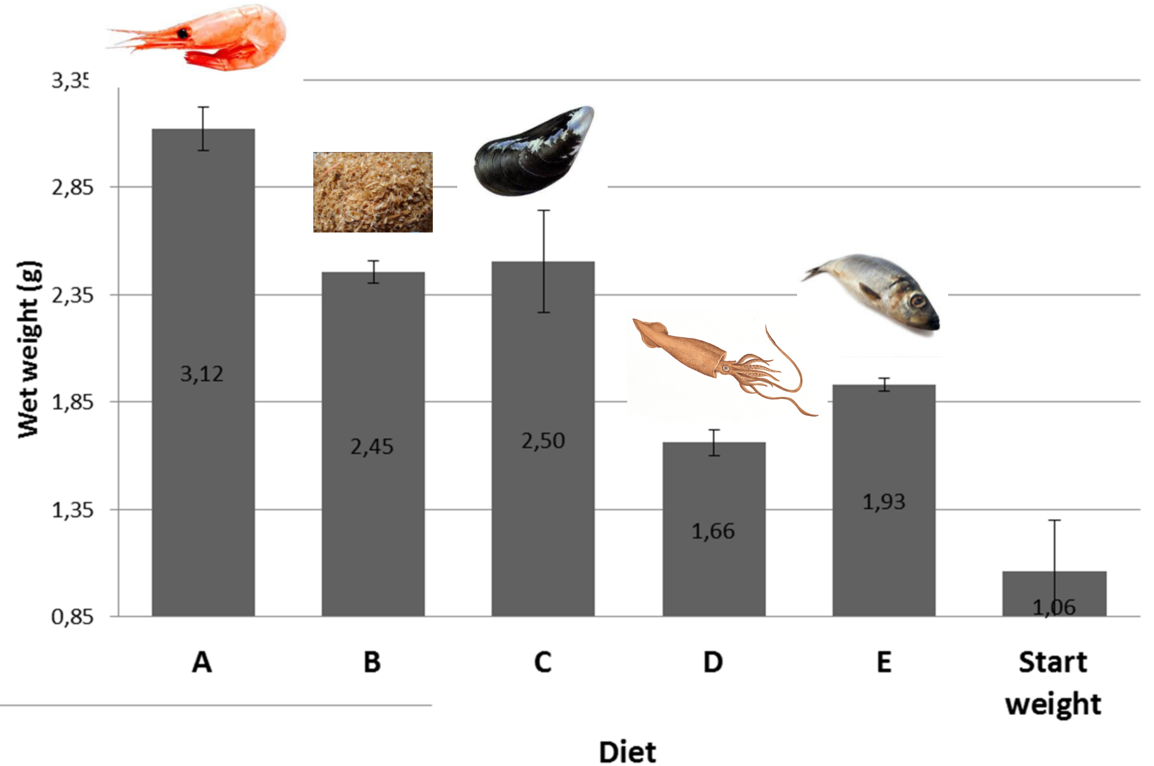
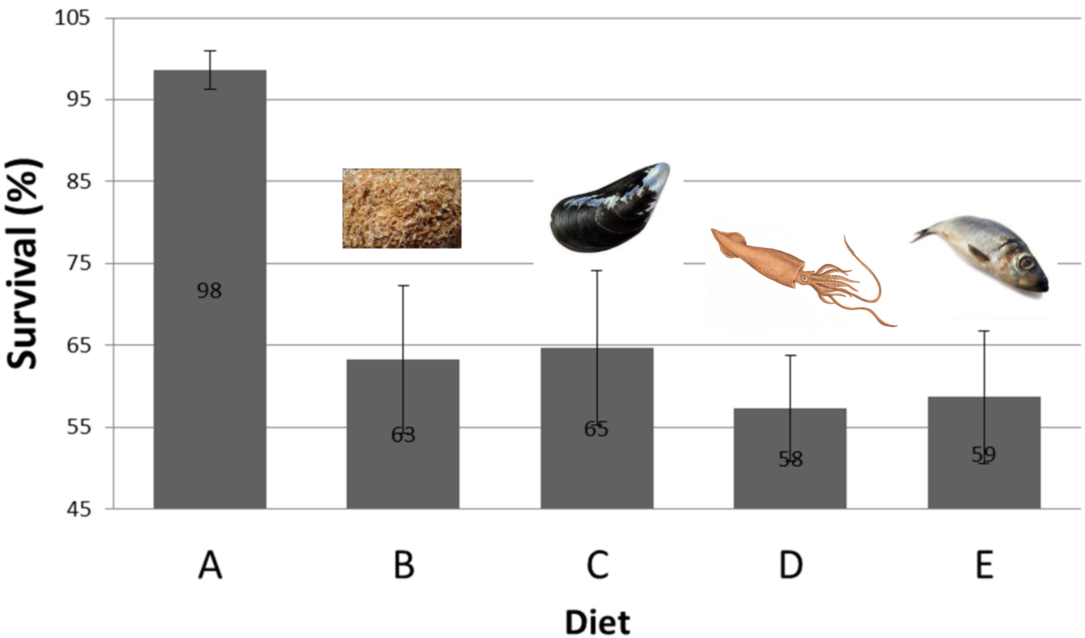
# On-growing



Wet weight (g)
3,35
2,85
2,35
1,85
1,35
0,85
3,12
2,45
2,50
1,66
1,93
1,06
A
B
C
D
E
Start weight
Diet

Survival (%)
105
95
85
75
65
55
45
98
63
65
58
59
A
B
C
D
E
Diet


# What does Ballan wrasse need?





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Thank you for your attention