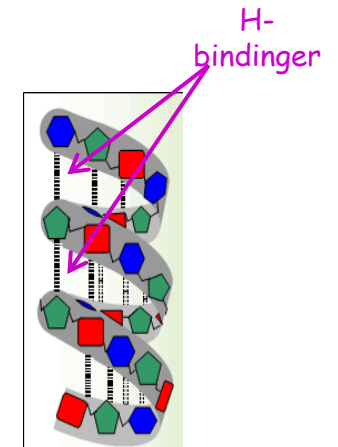
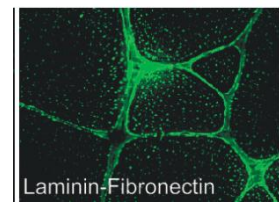
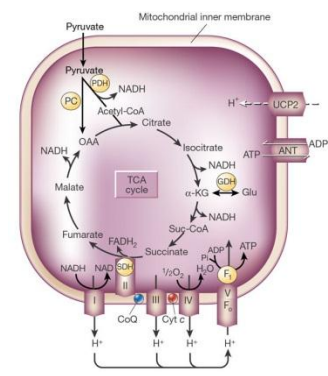
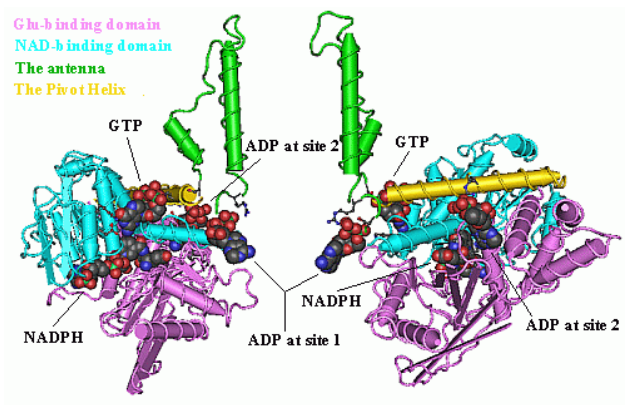


Tekstur i laks

Turid Mørkøre



Participants, contributors



Salmobreed

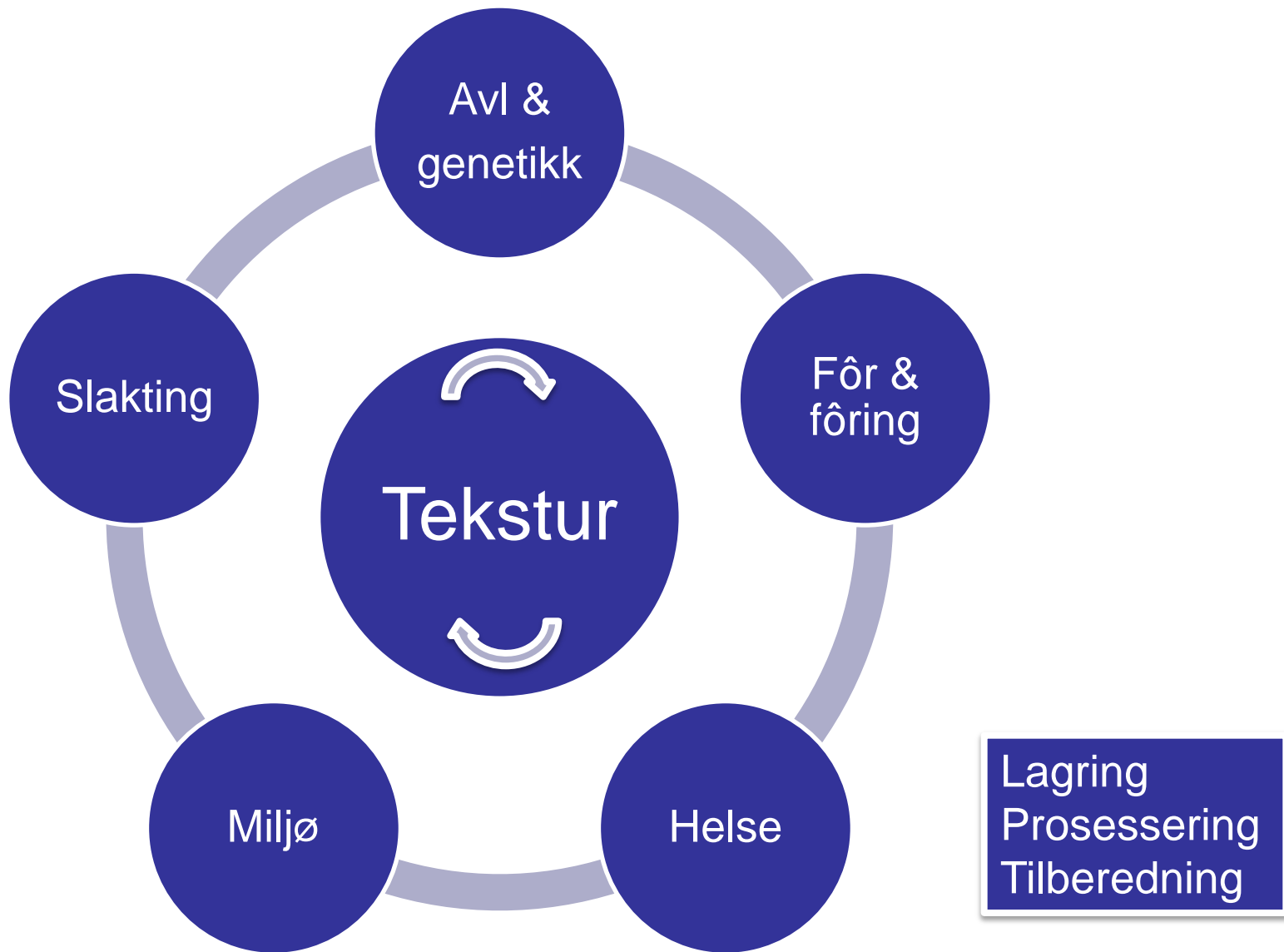


EWOS

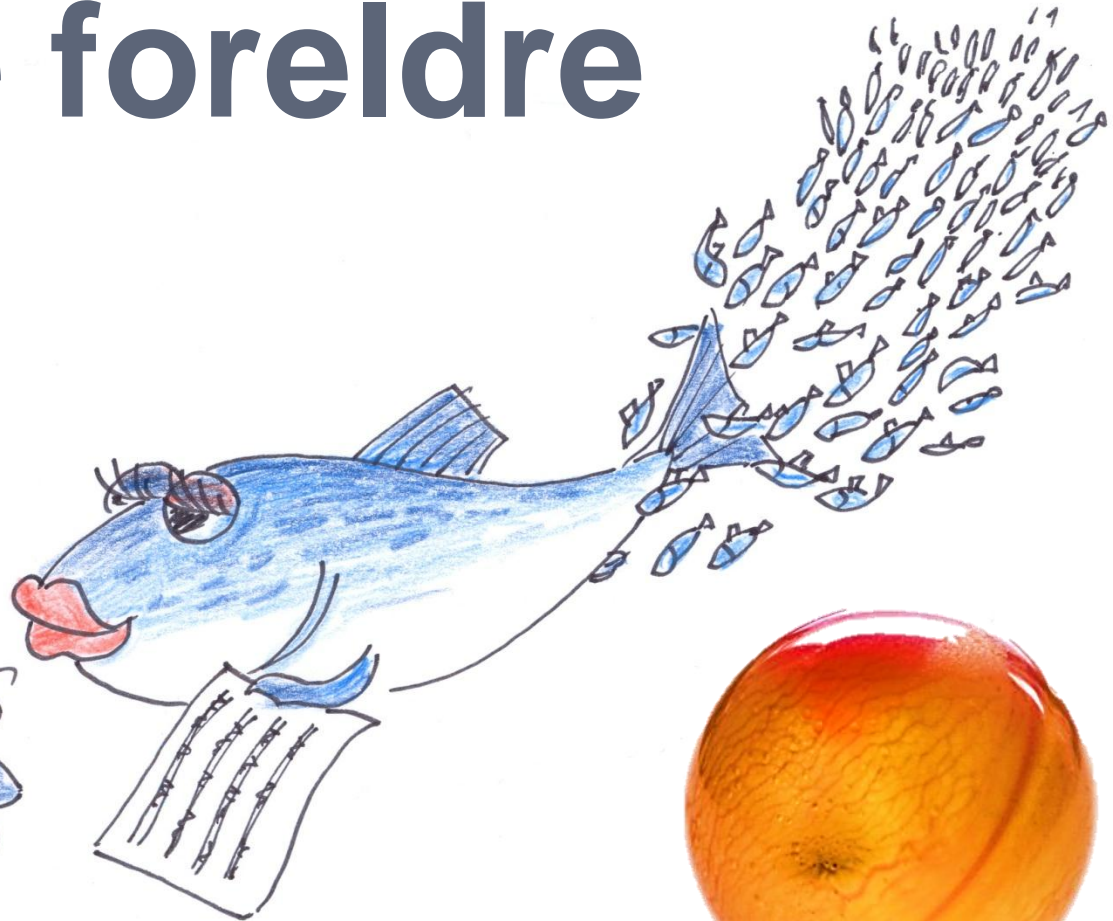
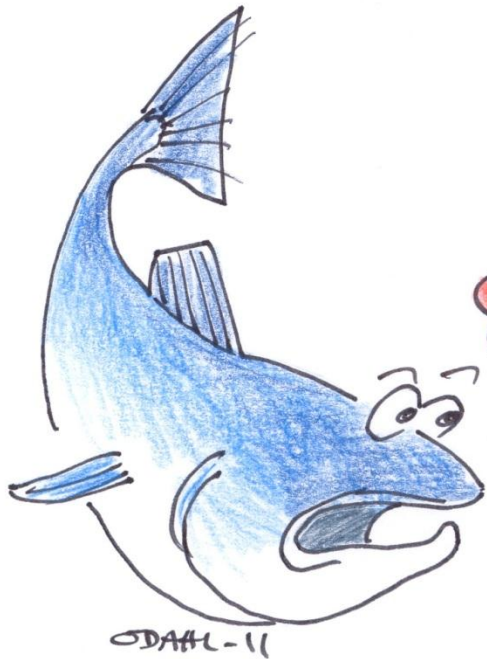


Norges veterinærhøgskole





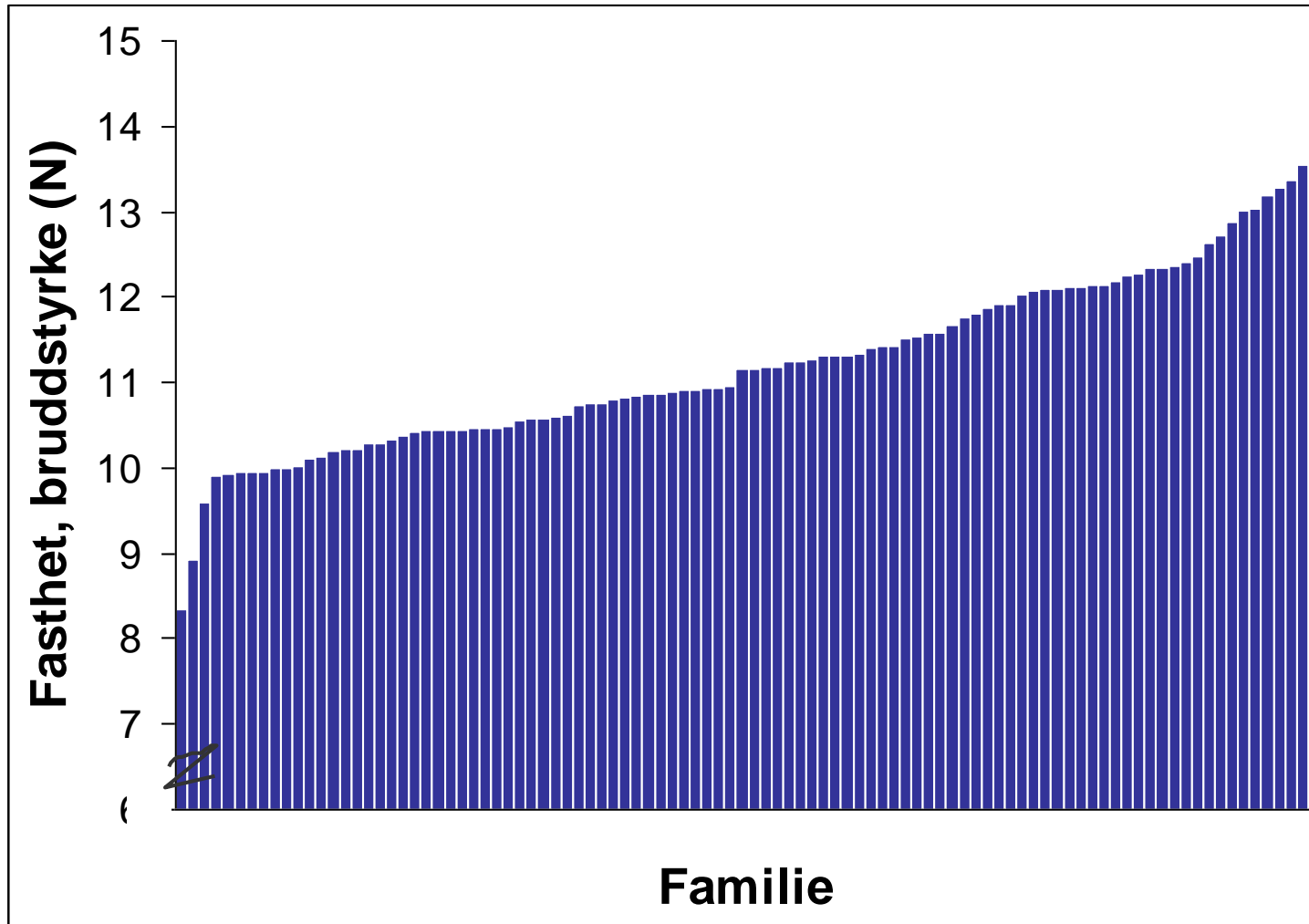
Gode foreldre



$$h^2 = 0.16 - 0.31$$

Betydelig variasjon i tekstur mellom familier

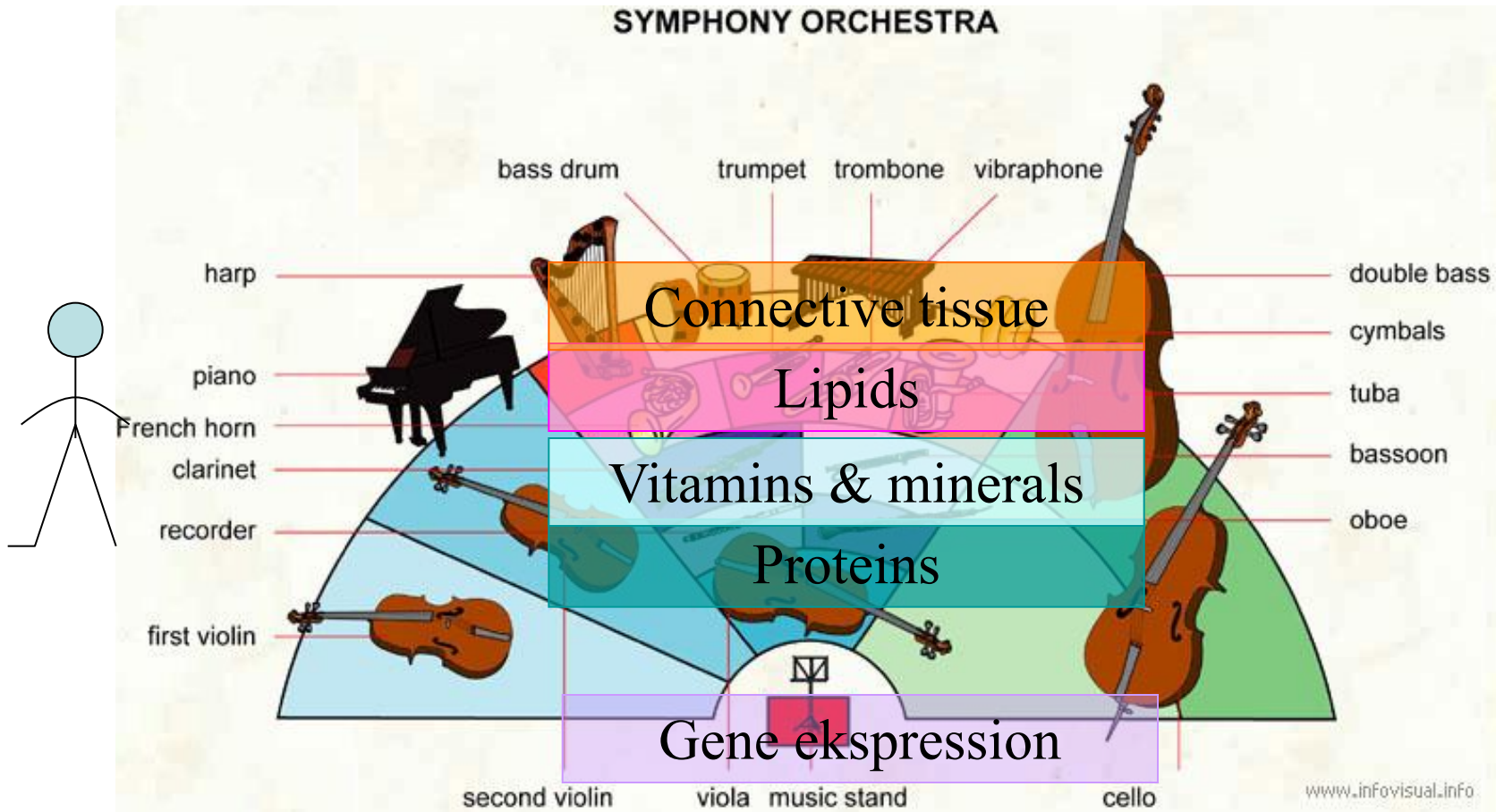
⇒ Fastheten kan forbedres gjennom avlsarbeid



Hvilke gener er aktive i hhv bløt og fast laks

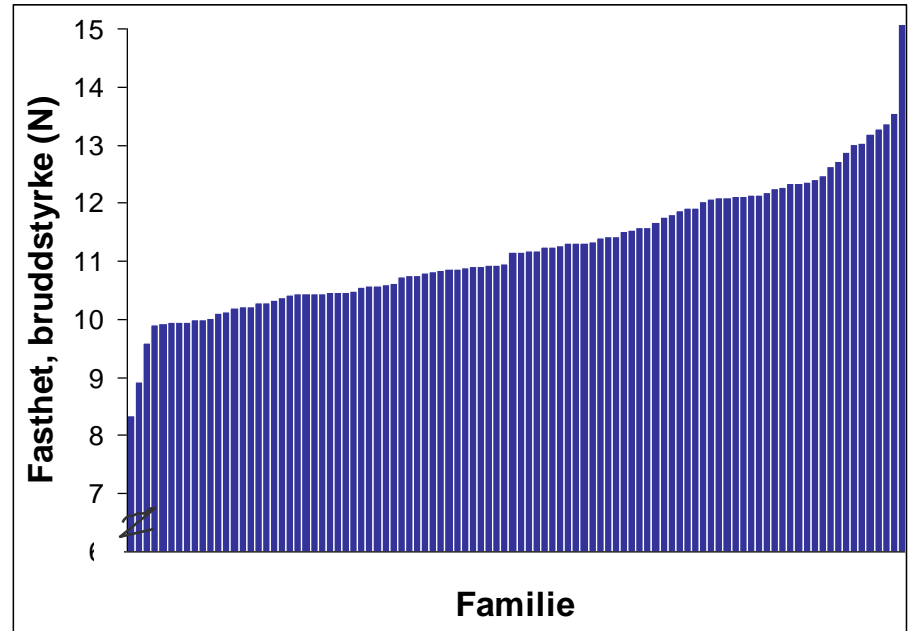


Physical components



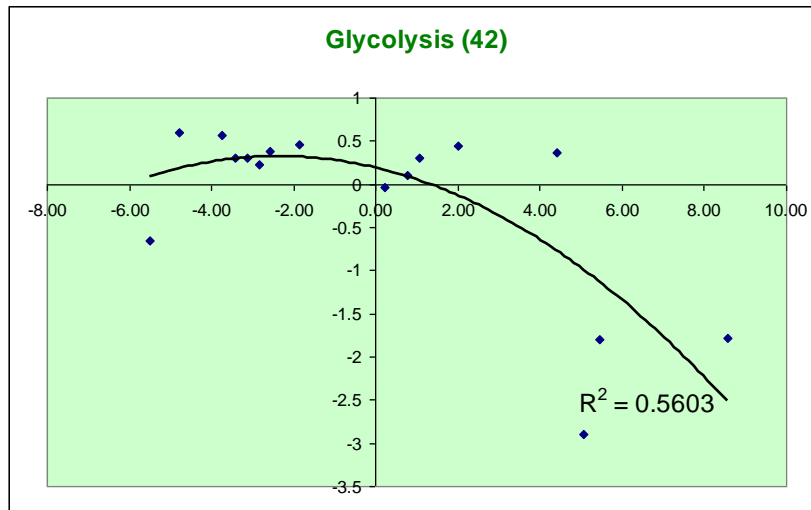
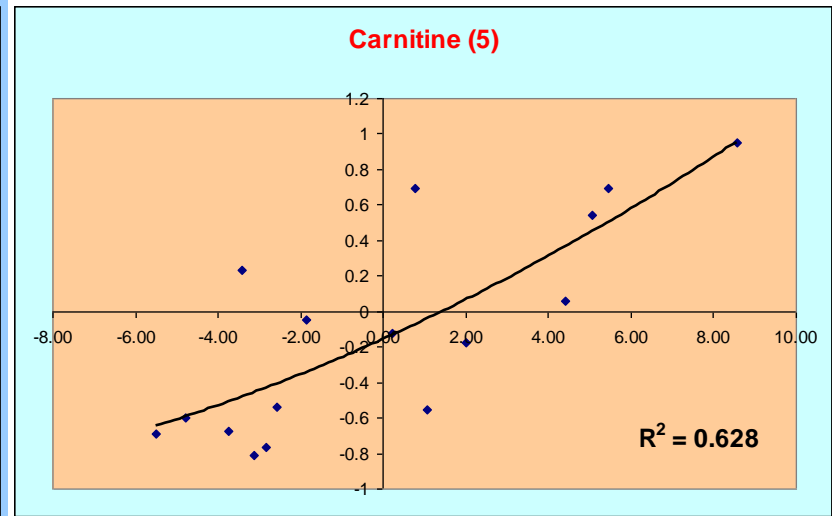
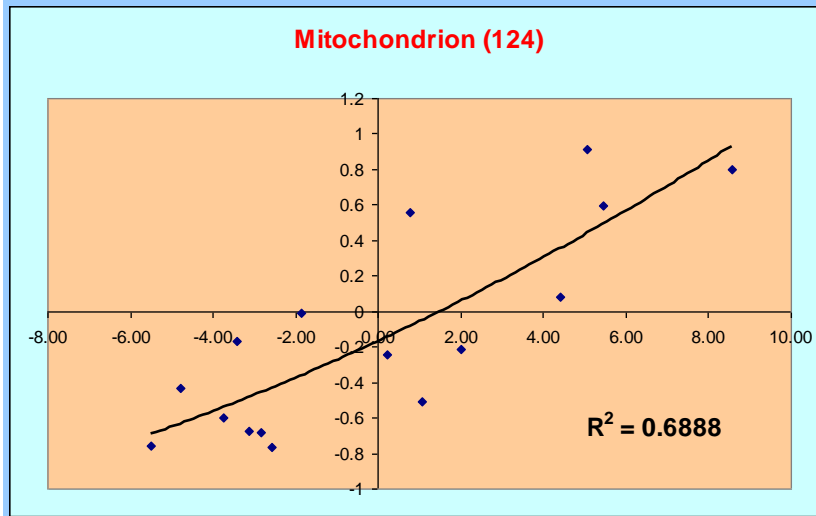
Avlspopulasjon på 1000 laks

- Valgt ut 16 individer
 - Bløt \Rightarrow fast
- Analysert ved Microarray
- 21 K oligonucleotide microarray
- Veldig bra sorteringsverktøy
- Undersøkt ca 2000 gener



- Utviklet i prosjektet

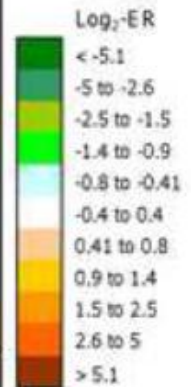
Correlations with firmness – Microarray



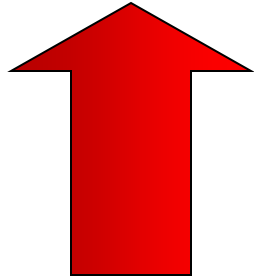
- Salmon with soft muscle suffered from hypoxia
- Low expression of carnitine
- Low expression of mitochondria proteins
- High expression of immune genes
- Texture problems appear to originate from problems inside the muscle cells

Aminosyre metabolisme

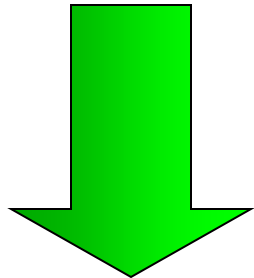
Genes	Firmness (N)														r	S		
	-5.5	-3.7	-4.8	-3.4	-3.1	-2.8	-2.6	-1.9	0.2	1.1	2.0	4.4	0.8	5.0			5.5	8.6
Amino acids metabolism																		
Aspartate aminotransferase, cytoplasmic	-0.9	-0.5	-0.7	0.0	-0.5	-0.4	-0.7	-0.1	0.0	-0.3	-0.2	-0.1	1.2	0.9	1.3	1.0	0.80	0.13
Aspartate aminotransferase, mitochondrial	-1.2	-0.6	-0.3	0.5	-0.7	-0.8	-1.0	0.7	0.0	-0.4	0.2	0.6	0.1	1.0	0.8	1.1	0.77	0.14
Pyroline-5-carboxylate reductase 2	-0.4	-0.8	0.0	0.4	-0.5	-0.5	-0.8	-0.1	0.4	-0.5	0.1	0.5	-0.2	0.4	0.8	1.1	0.77	0.10
Cystathionine gamma-lyase	-0.8	-0.8	-0.1	0.4	-0.2	-0.9	-0.9	0.1	0.0	-0.4	0.2	0.3	0.7	0.5	0.9	1.0	0.75	0.11
Glutamate decarboxylase-like	1.1	1.9	0.5	-0.8	2.2	2.7	1.6	0.8	-0.7	0.9	-0.5	0.7	-3.2	-1.5	-3.3	-2.0	-0.65	-0.29
S-adenosylmethionine synthetase isoform	2.1	1.9	0.4	-0.2	1.7	1.5	0.5	0.0	-1.0	0.9	-0.8	1.3	-2.3	-1.9	-1.8	-2.2	-0.69	-0.25
Branched-chain-amino-acid aminotransferase	0.6	0.8	0.7	0.7	1.4	1.1	0.8	0.7	0.1	0.5	0.0	-0.1	-2.3	-1.2	-1.8	-2.0	-0.79	-0.22
Cysteine dioxygenase type 1	0.9	1.1	1.0	0.1	0.9	0.5	0.3	0.9	0.3	1.1	0.5	-0.6	-1.6	-2.4	-1.3	-1.8	-0.78	-0.22
L-arginine:glycine amidinotransferase	1.5	1.0	1.4	-0.3	0.8	1.3	1.3	-0.4	-0.3	0.3	-0.4	-0.6	-1.5	-1.6	-1.4	-1.2	-0.82	-0.22
L-pipecolic acid oxidase	1.0	0.8	0.2	0.1	0.9	0.7	1.0	-0.1	0.2	0.3	-0.3	0.3	-1.5	-1.4	-1.1	-1.1	-0.74	-0.16
Sodium-coupled neutral amino acid transporter 3	0.7	0.5	0.5	0.4	0.7	-0.1	1.0	-0.1	0.4	-0.2	0.0	-0.4	-1.1	-1.0	-0.5	-0.8	-0.78	-0.12
Argininosuccinate synthase	0.7	0.7	0.5	0.3	0.6	0.0	0.3	0.3	-0.2	0.3	-0.2	-0.1	-1.0	-0.3	-0.9	-0.9	-0.81	-0.11
Intracellular proteases																		
Cathepsin H	-1.0	-0.3	-0.7	0.1	-0.3	-0.4	-0.6	0.6	-0.4	-0.6	-0.7	-0.1	1.2	0.7	1.4	1.2	0.66	0.12
Cathepsin L1	-0.8	-0.5	-0.4	-0.2	-0.6	-0.4	-0.5	-0.3	0.0	-0.3	-0.2	0.0	1.7	1.0	0.8	0.9	0.72	0.12
Dipeptidyl-peptidase 1	-0.5	-0.9	-0.3	-0.1	-0.7	-0.5	-0.7	-0.3	0.0	-0.3	0.1	-0.1	1.5	1.5	0.9	0.5	0.67	0.12
Dipeptidyl-peptidase 3	-1.2	-0.6	-0.6	0.1	-0.6	-0.8	-0.7	0.2	-0.1	-0.3	0.4	-0.2	1.1	1.1	1.2	1.0	0.80	0.15
Cysteine protease ATG4B	-1.7	-0.6	-0.6	0.3	-0.9	-0.8	-1.3	0.3	0.3	-1.2	0.8	0.1	0.8	0.9	2.0	1.7	0.78	0.20



Firm meat



Aerobic metabolism
Fatty acids as main fuel
Rapid degradation of damaged
protein via proteasomes



Anaerobic metabolism
Amino acids as main fuel

Soft meat

What is inherited: structure of muscle?
Interactions with environment?
Resistance to hypoxia?

Bindevevet, hvilken betydning har det ?



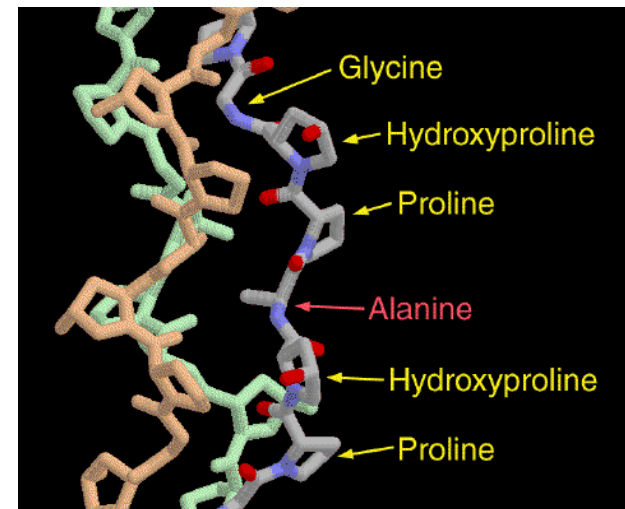
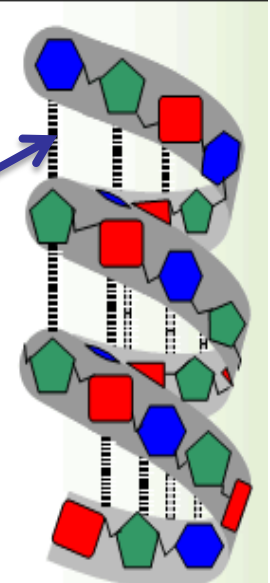
Fast vs. bløt laks

Samme mengde bindevev

Høyere nivå av aminosyrene alanin og glycin ($r = 0.7 - 0.9$)

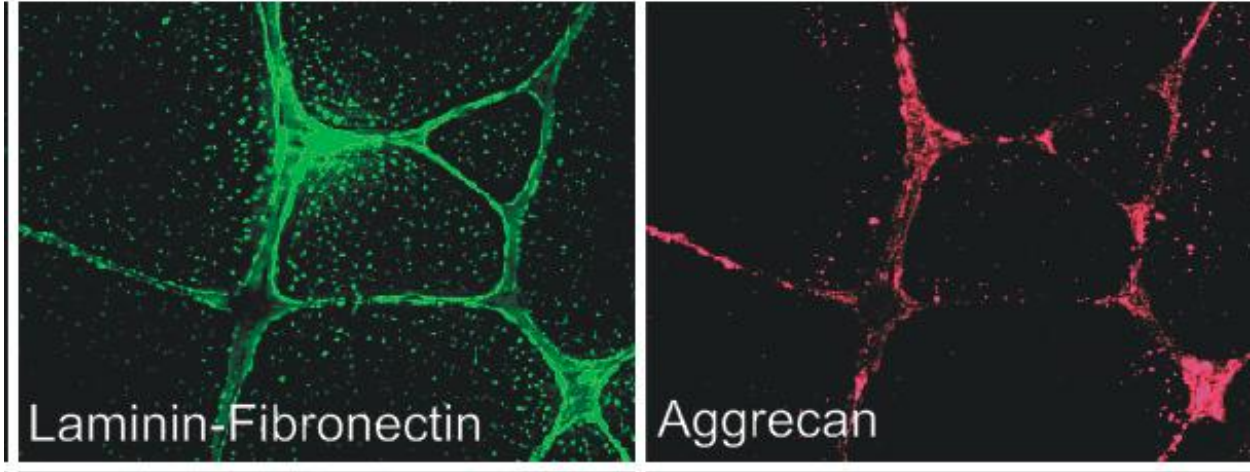
Mer stabilt bindevev

Hydrogen bindinger



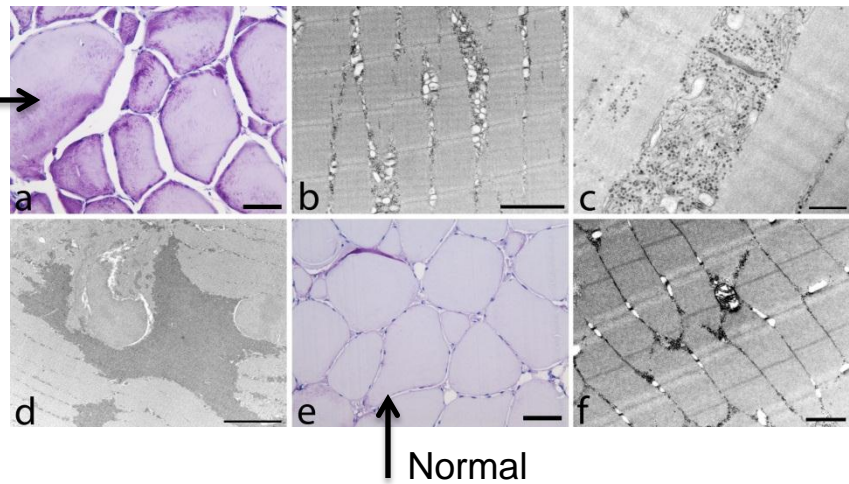
Mikroskopi-bilder av laksemuskel

Fast



Sukkerlaks/ bløt laks

Opphopning glykogen →



Viktig årsak hos andre dyr:

- **Udekket ernæringsmessig behov i spesifikke perioder?**
- **Genetikk ?**

Opphopning av glykogen medfører

- Nedbrytning av muskelprotein
- Bløt (bleik?) muskel
- Takler ikke stress
- Hjerterproblemer (kan gi plutselig død hos voksne dyr)
- Diagnostiseres ved blodanalyser og histologi

Bløt laks hadde også forstørrede mitokondrier

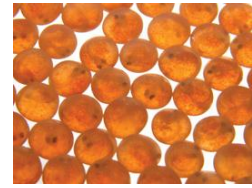
Vi vet ikke når problemet oppstår

Mulige årsaker

- Lavt nivå vitamin E i fôret (evt tidlig i livet)
- Proteinfattig fôr/ lite karnitin & taurin
- Metabolsk syndrom (problemer med omsetning av næringsstoffer)

Quality downgrading due to cartilage

– associated with production conditions and nutrition during early life stages

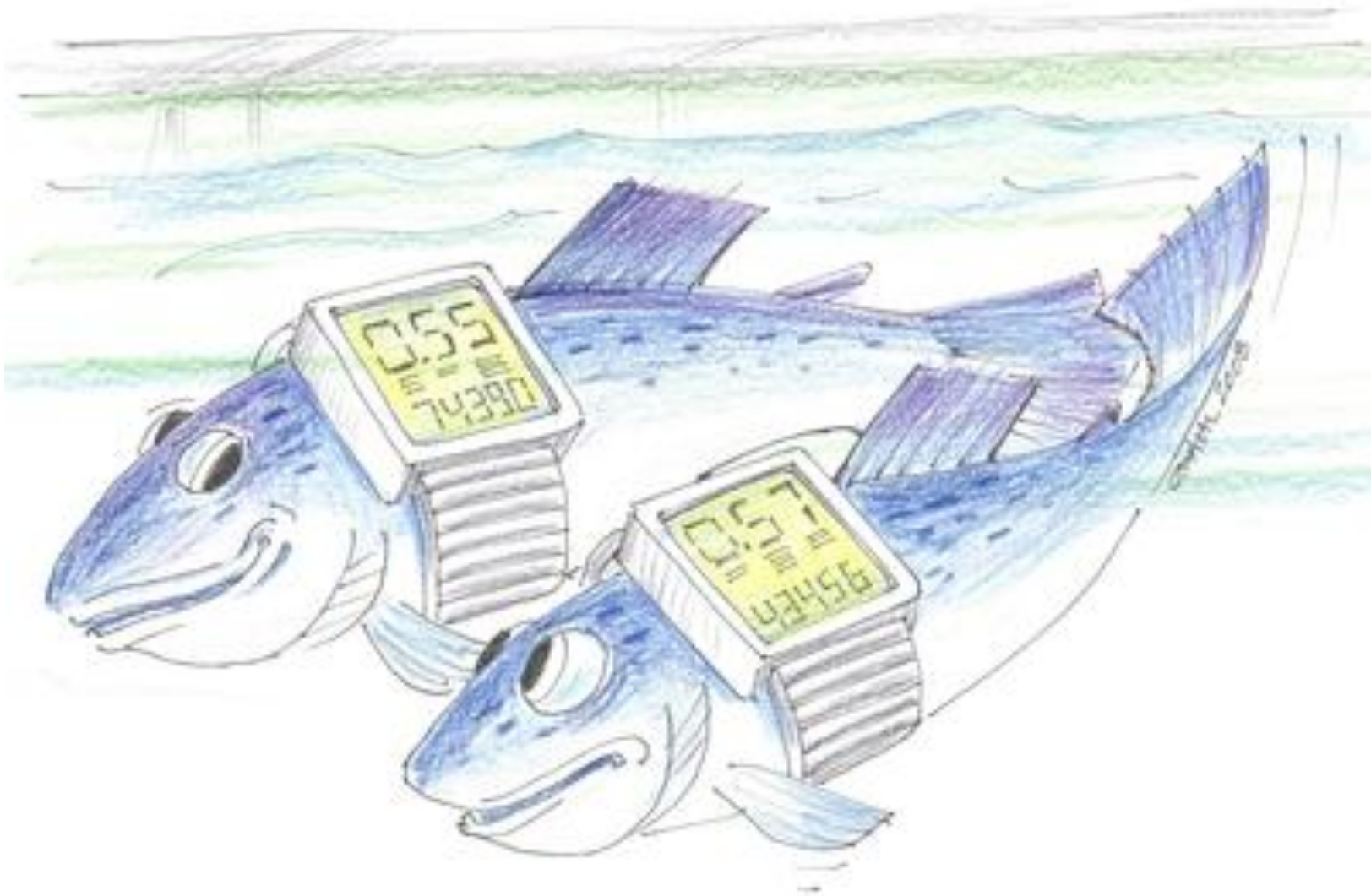


- Vertebrae damages
- develop over time



Source: Bæverfjord, Lein & Helland

Trening er sunt – også for laks



”The pure and simple truth is rarely pure and never simple”

Oscar Fingal O'Flahertie Wills Wilde

men :



- Det ser ut til av vi har funnet en viktig årsak til bløt tekstur
- Er årsak(er) til bløt/bleik filet og hjerteproblemer i «familie» ?

Åsted identifisert, ikke underliggende årsak

- Hva er frekvensen av fenomenet ?

Hovedmistenkte

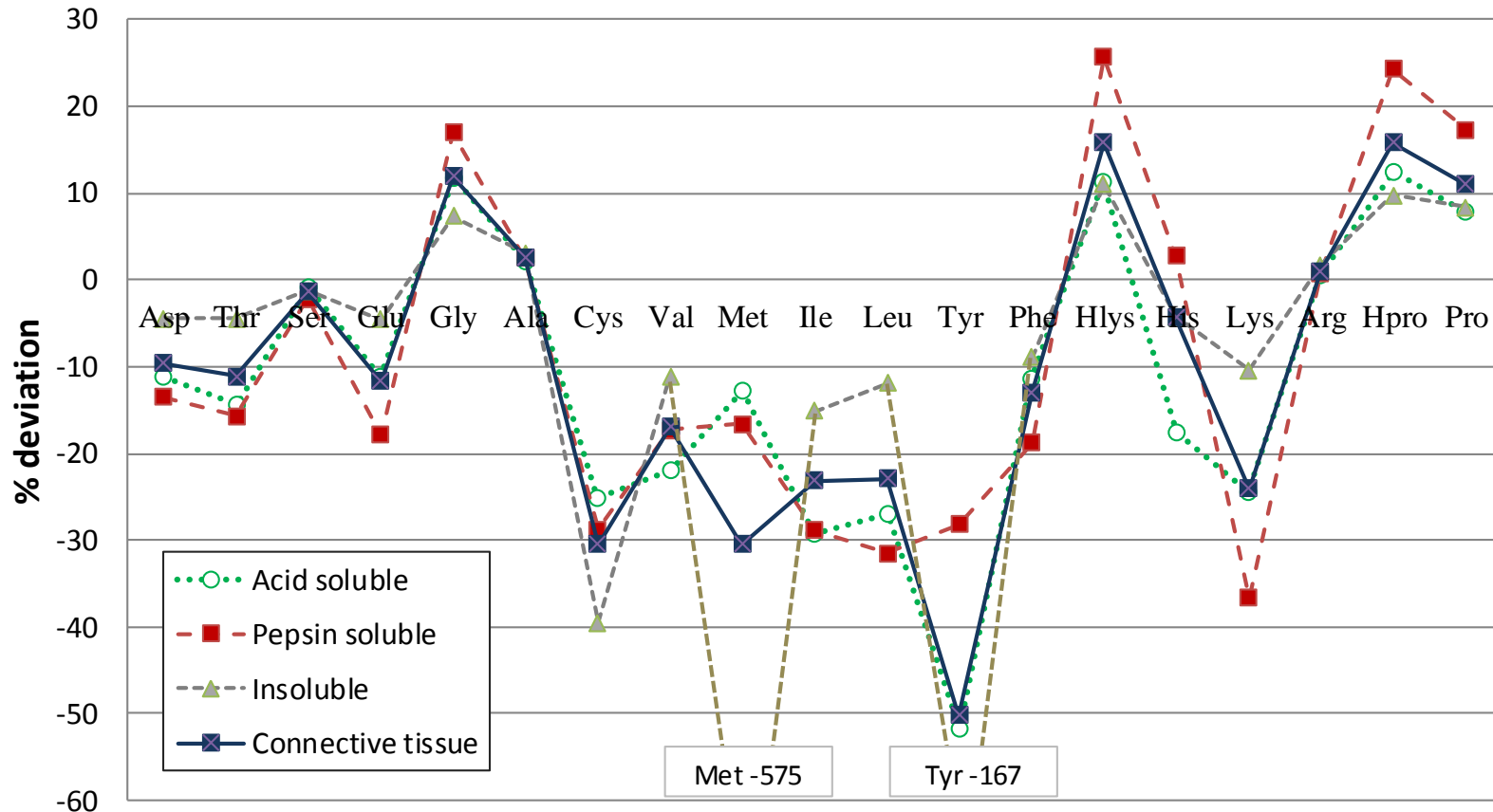
- Underdekning av visse fôringredienser / når i livet, tidlig ?
- Mulig å reparere med fôr evt. slaktefôr?
- «Genfeil»?

Store forskjeller i bindevevssammensetningen mellom vill og oppdrettslaks

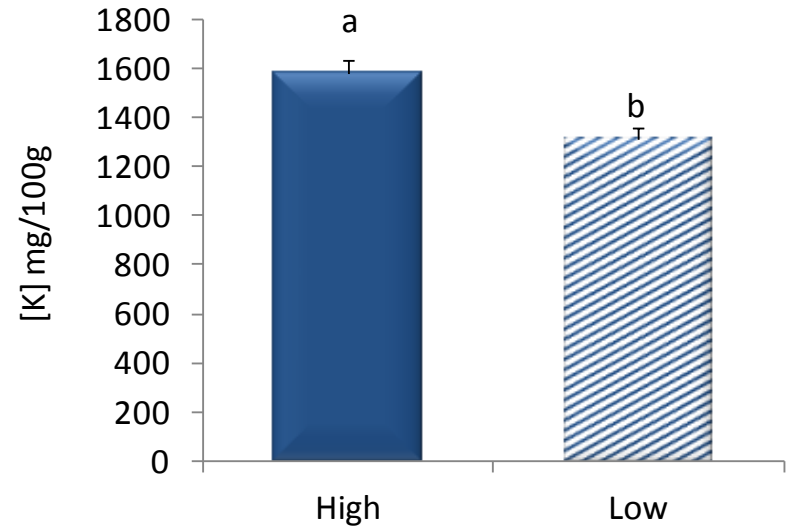
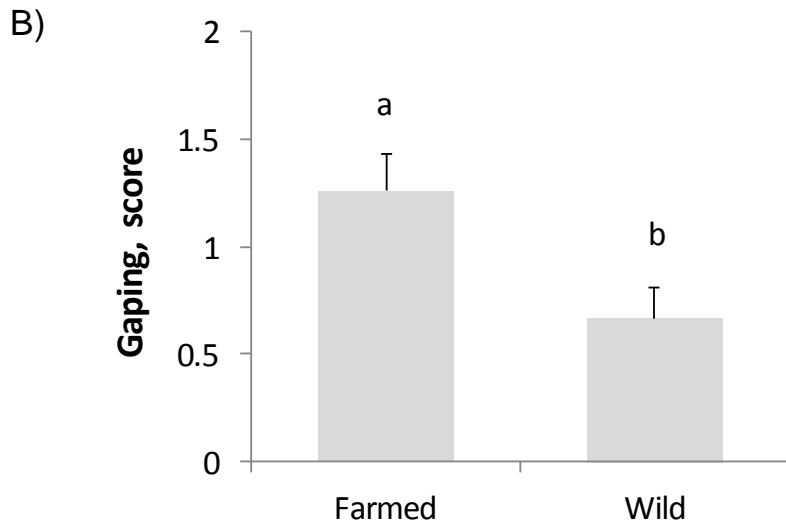
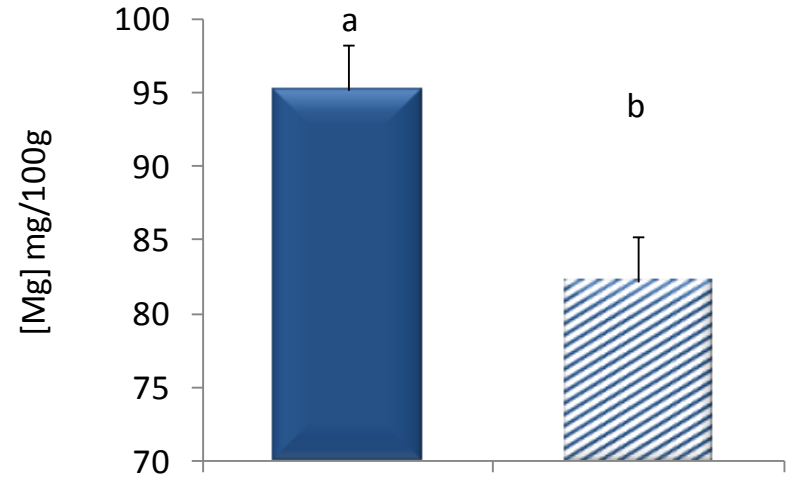
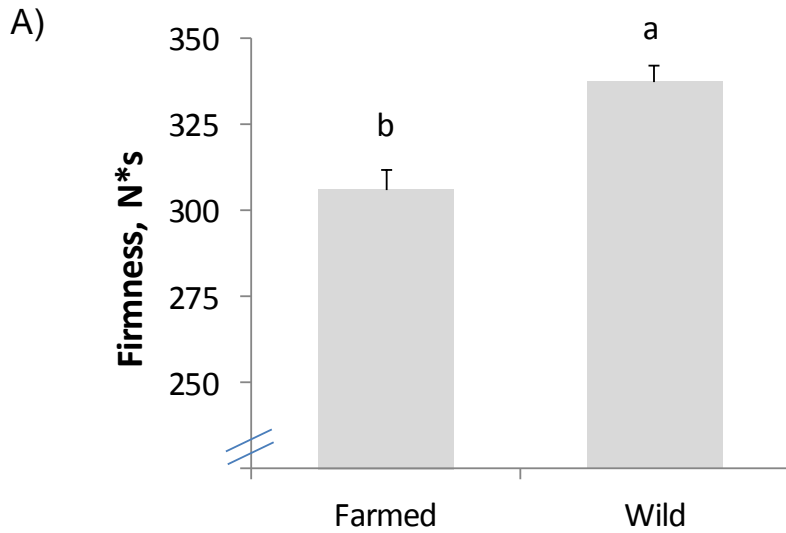
	<i>Acid soluble</i>		<i>Pepsin soluble</i>		<i>Insoluble</i>		<i>Connective tissue</i>	
	Unselected	Selected	Unselected	Selected	Unselected	Selected	Unselected	Selected
Aspartic acid	65.5	58.8***	76.2	67.1***	70.3	67.3***	70.6	64.4***
Threonine	31.7	27.7***	38.2	33.0***	38.3	36.7**	36.1	32.5***
Serine	49.6	49.2 ^{ns}	52.4	51.3**	60.5	59.8 ^{ns}	54.1	53.4 ^{ns}
Glutamic acid	94.0	84.5***	108.7	92.2***	92.5	88.4***	98.8	88.5***
Glycine	265.9	301.2***	209.3	252.2***	248.7	268.4***	241.3	273.9***
Alanine	110.0	112.5**	98.0	100.6***	99.8	103.0**	102.6	105.4***
Cysteine	1.8	1.4**	3.1	2.4***	1.8	1.3**	2.2	1.7***
Valine	26.3	21.6***	35.4	30.2***	38.0	34.2***	33.5	28.6***
Methionine	20.6	18.2***	20.8	17.8***	7.1	1.1***	16.2	12.4***
Isoleucine	18.8	14.5***	24.0	18.6***	28.0	24.3***	23.6	19.2***
Leucine	36.5	28.7***	49.7	37.8***	45.4	40.6***	43.9	35.7***
Tyrosine	9.6	6.3***	14.4	11.2**	5.5	2.0**	9.8	6.5***
Phenylalanine	20.0	18.0***	21.6	18.2 ^{ns}	21.2	19.5***	20.9	18.5*
Hydroxylysine	7.5	8.4**	6.7	9.0***	9.0	10.1**	7.7	9.2***
Histidine	10.7	9.1***	16.0	16.5 ^{ns}	13.9	13.3**	13.5	13.0 ^{ns}
Lysine	40.4	32.4***	52.4	38.3***	40.1	36.3***	44.3	35.7***
Arginine	48.1	48.3 ^{ns}	46.6	45.0 ^{ns}	48.5	49.3**	47.7	48.2 ^{ns}
Hydroxyproline	55.4	63.3***	50.3	66.4***	49.9	55.2**	51.9	61.6***
Proline	88.3	95.8***	75.0	90.7**	81.7	89.2***	81.5	91.6***
Hydrophobic AA	351.7	336.6	362.7	346.4**	359.5	348.5***	358.0	343.8**

Store forskjeller i bindevevssammensetningen mellom vill og oppdrettslaks

Selected vs. unselected salmon

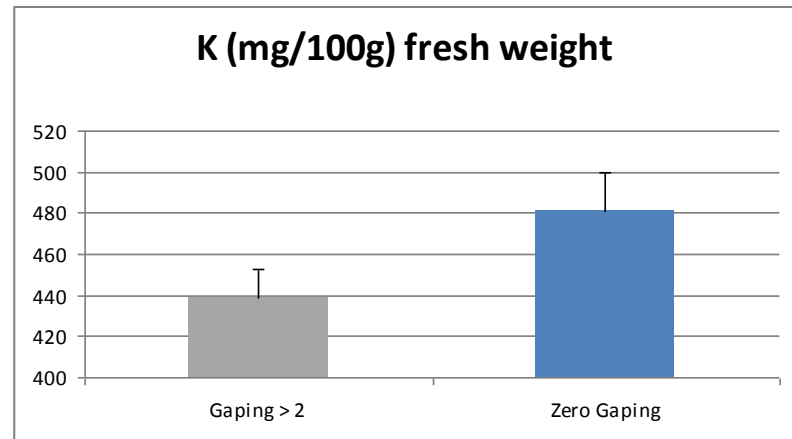
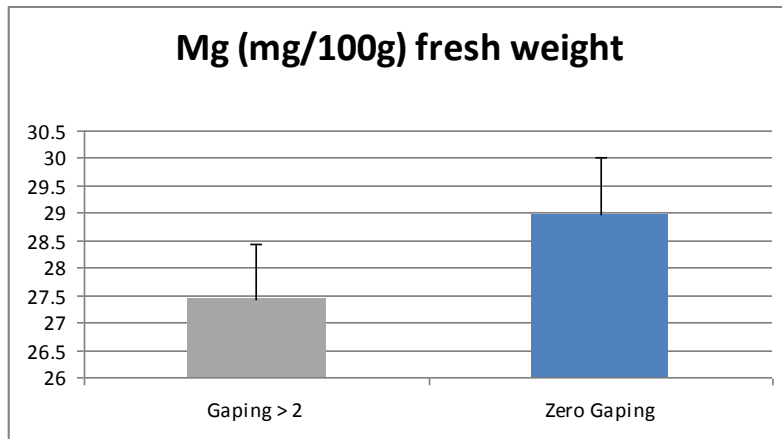


Villaks vs oppdrettslaks



Lavere mineralstatus i laks med gaping

Lavt innhold av vitamin E





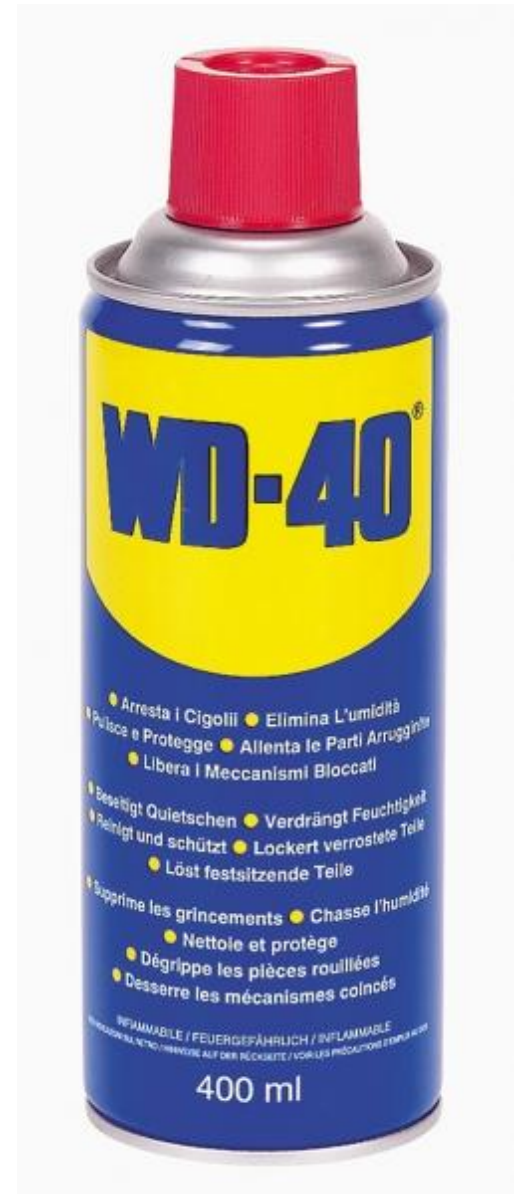
1. Fliser. Fugemasse
2. Flislim.
3. Membran.
4. Påstøp. Støpemørtel
Varmekabler primer.
5. Betong, mur, puss, gips,
våtromsplater
6. Ventilering

Verktøy på boks

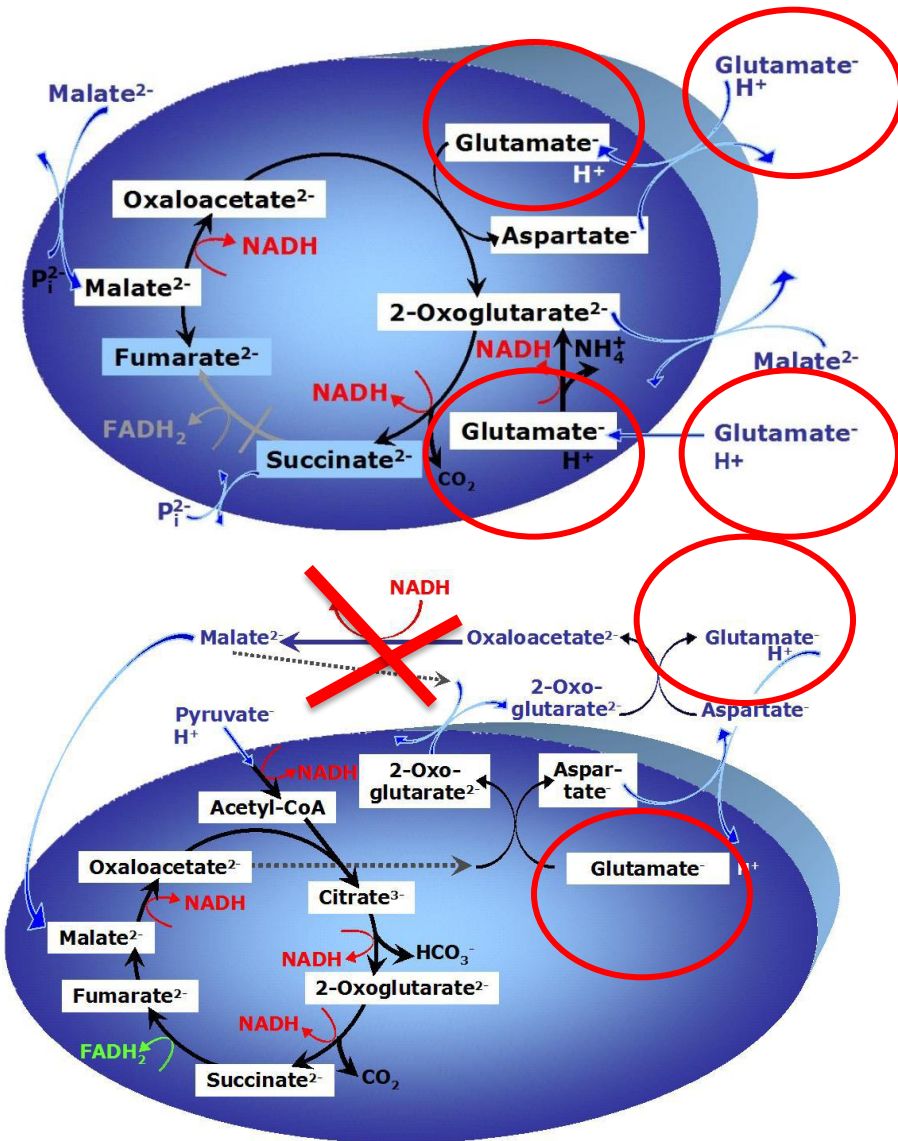
WD40 - For tusen formål på jobben og hjemme

- smører
- driver ut fuktighet
- renser og beskytter
- løsner fastrustedede deler
- løsner fastlåste mekanismer

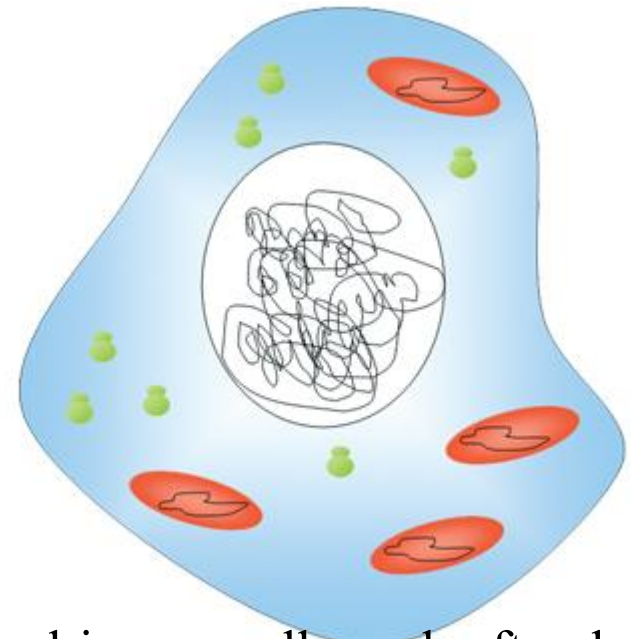
Innehold 400ml



Glutamate

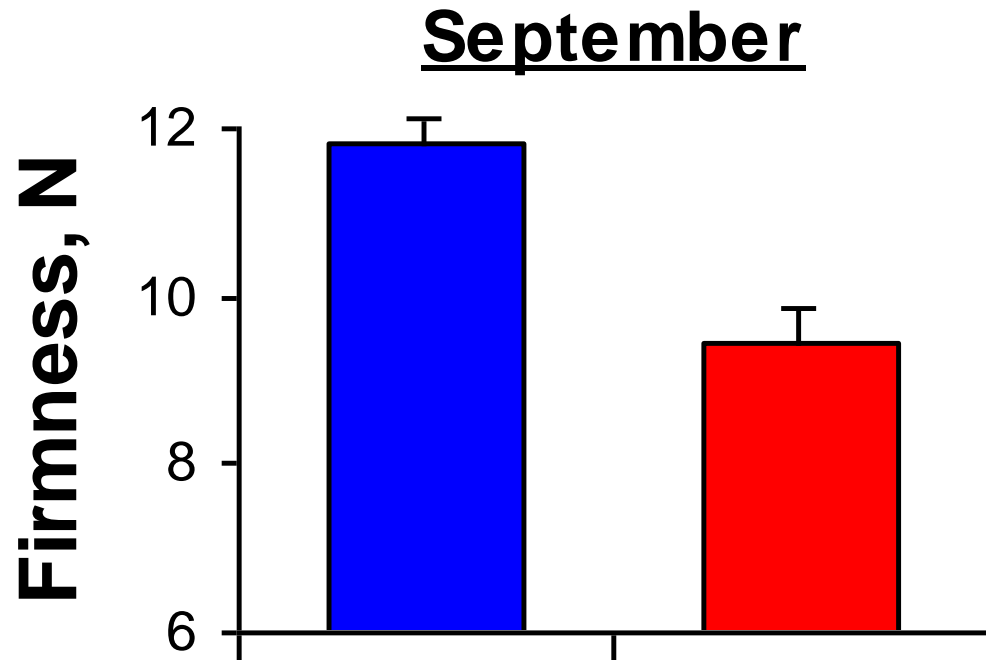
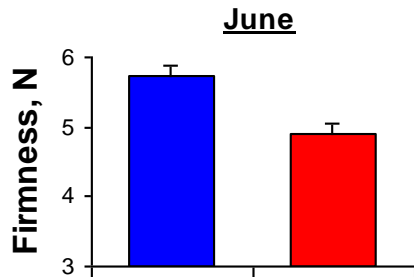
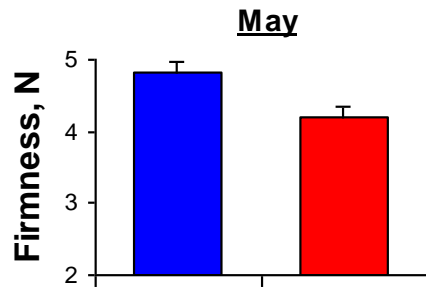


Glutamate derived from hydrolyzation of glutamine is a very important aerobic substrate



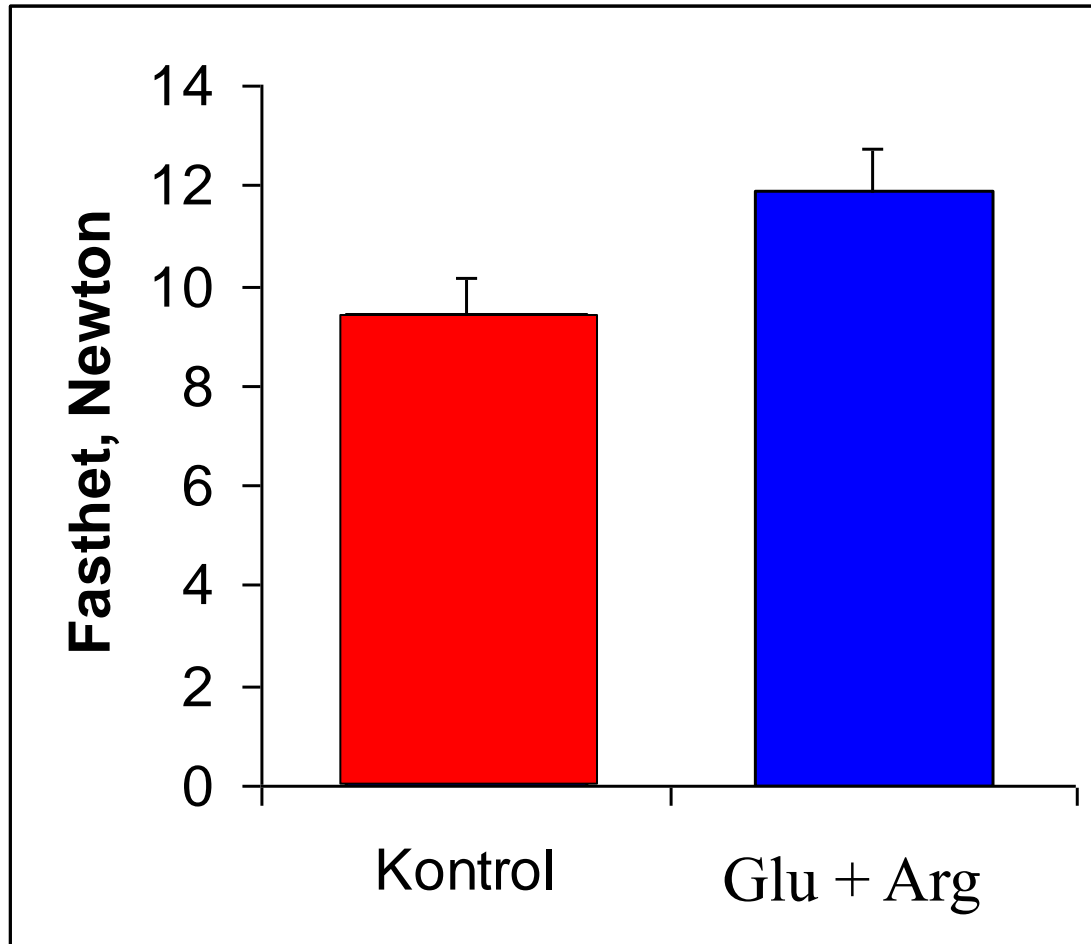
Mitokondriene er cellens «kraftverk» og produserer de energirike forbindelser som cellen trenger til sin virksomhet

Fôr tilsatt glutamat+arginin \Rightarrow fastere filet enn **standardfôr**



Mulig å oppnå fastere filet via fôret

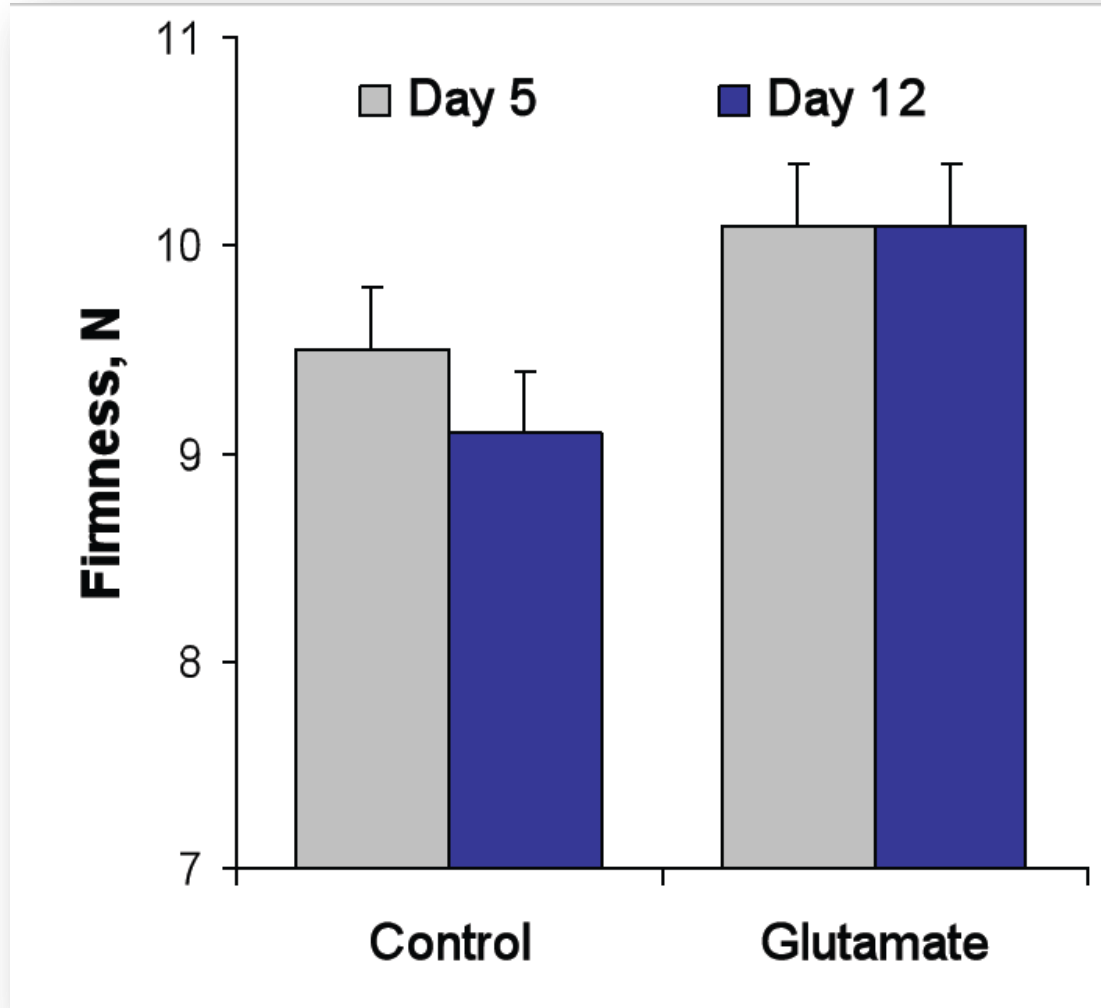
Etter 20 timers stress



Konklusjon

- Teksturen i laksefilet påvirkes av mange faktorer der fôret synes å spille en viktigere rolle enn tidligere antatt
- Det er mulig å forbedre teksturen ved å justere aminosyreprofilen i fôret
- Aminosyrene glutamat og arginin ga helsemessige gevinster, og disse var sammenfallende med fastere tekstur
- Gevinst ved tilskudd av aminosyrer kan variere gjennom året
- Optimalt nivå, varighet og alternative fôrkilder bør vurderes

Firmer salmon fillets

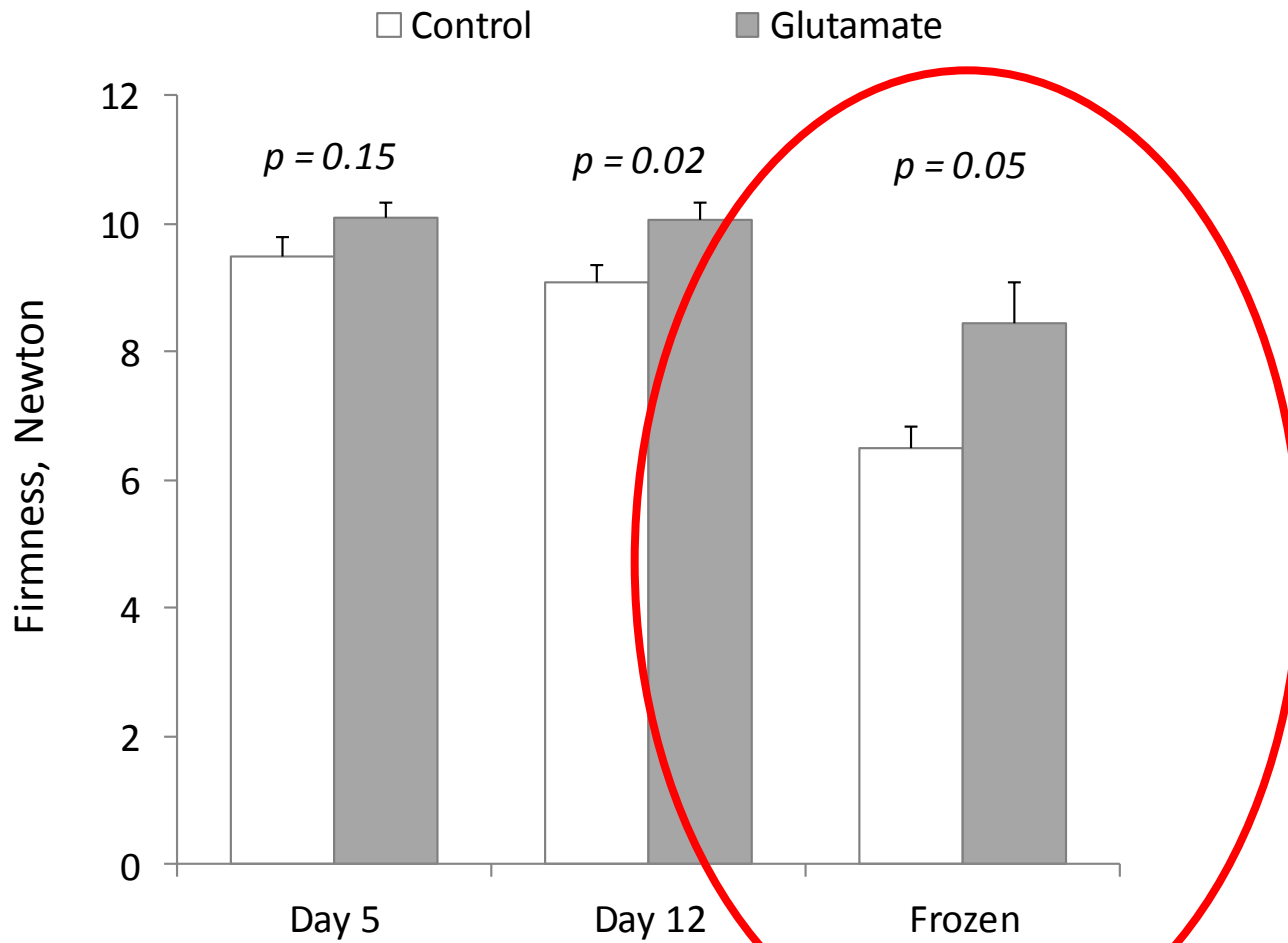


Addition of glutamate and arginine separately improved the

- Fillet firmness
- Health of the fish
- Robustness to stress

In particular **glutamate**

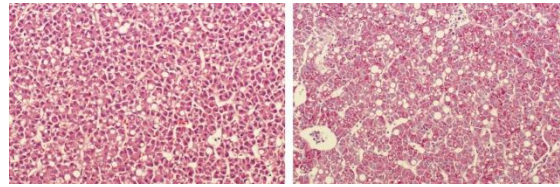
Fruitful interdisciplinary research



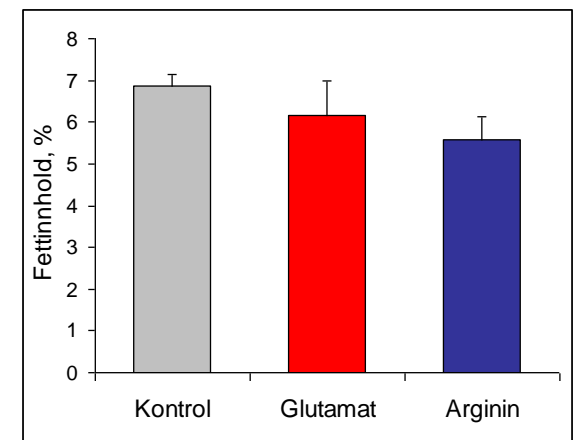
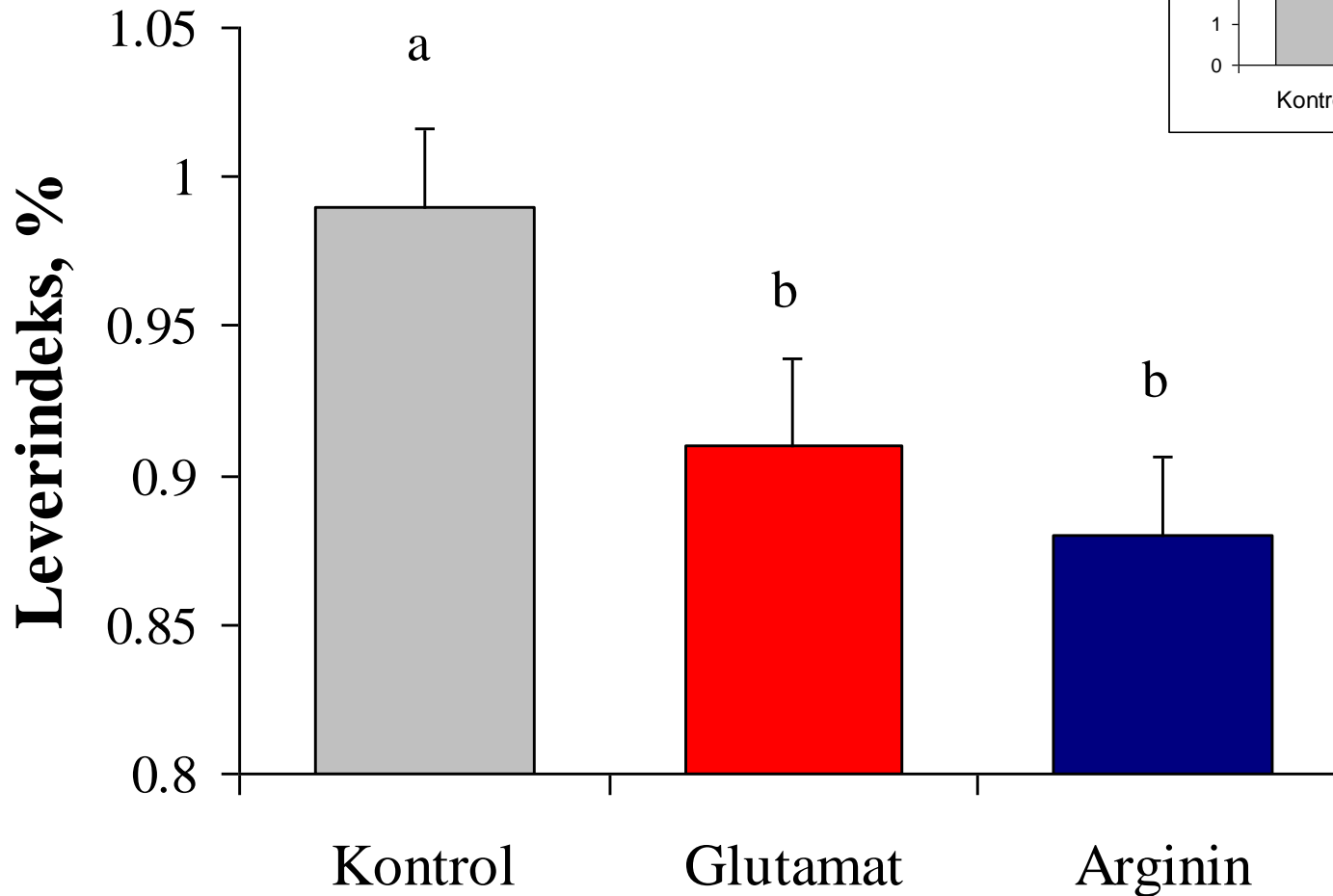
Thomas Larsson, Erling Olaf Koppang, Marit Espe, Aleksei Krasnova, Helena Maria Moreno, Kjell-Arne Rørsvika, Bendik F. Terjesen, Magny Thomassen, Turid Mørkøre

Dietary glutamate level affects fillet texture and health parameters of Atlantic salmon (*Salmo salar* L). *Manuscript*

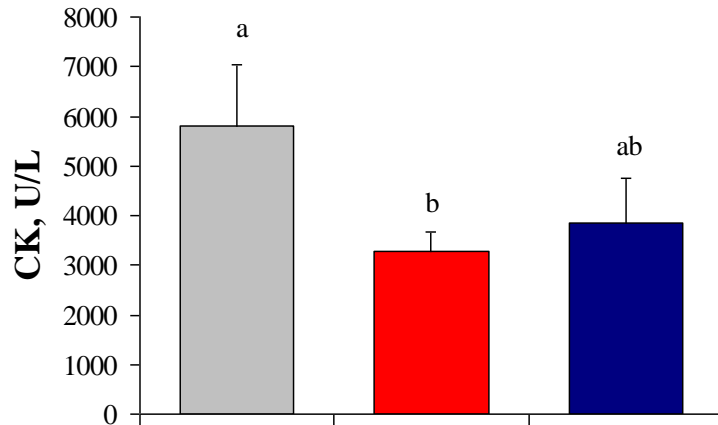
Lever



Avvik fra normalstruktur: Vakuolisering i lever

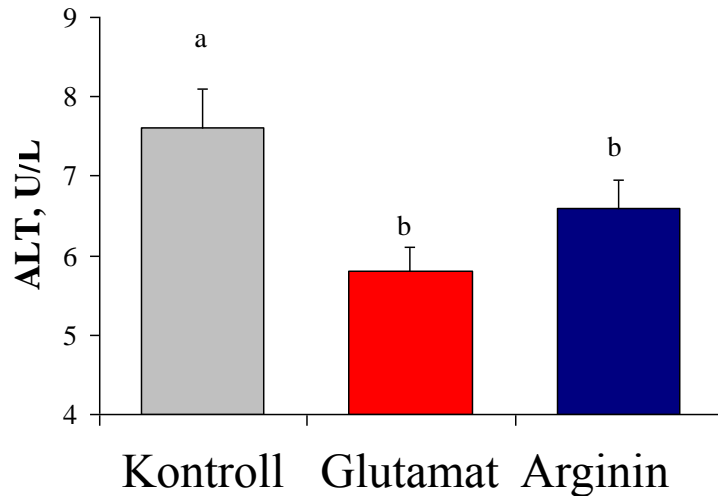


Plasmaanalyser



CK, kreatinkinase

- enzym som finnes i muskelvev og som spalter kreatinfosfat
- **stiger ved muskelskade**



ALAT, alaninaminotransferase

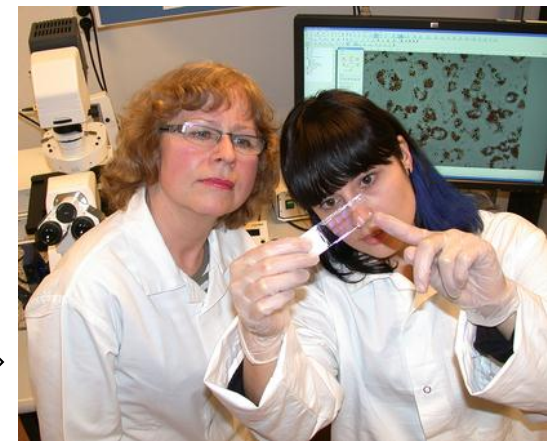
- enzym som er virksomt i aminosyrestoffskiftet
- **stiger ved skade av levervev**



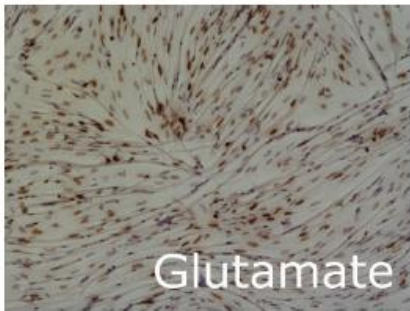
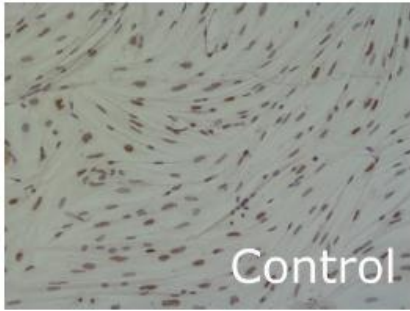
«Hva»



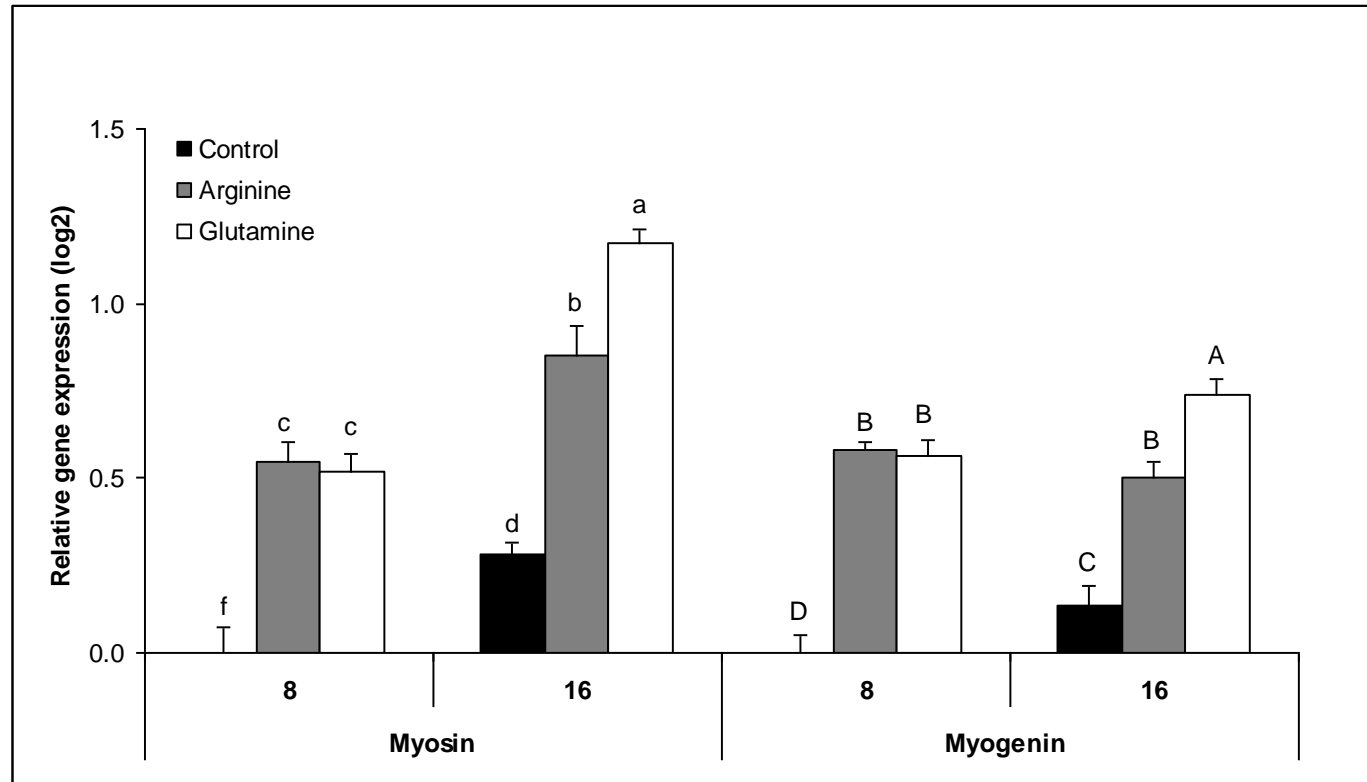
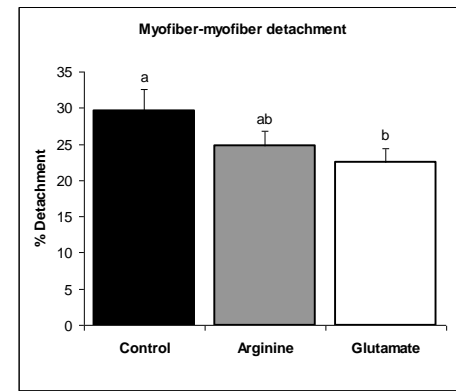
«Hvorfor»



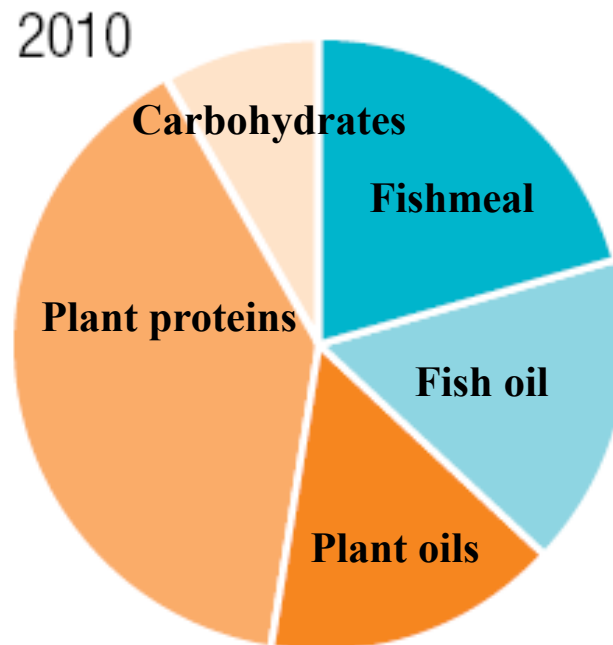
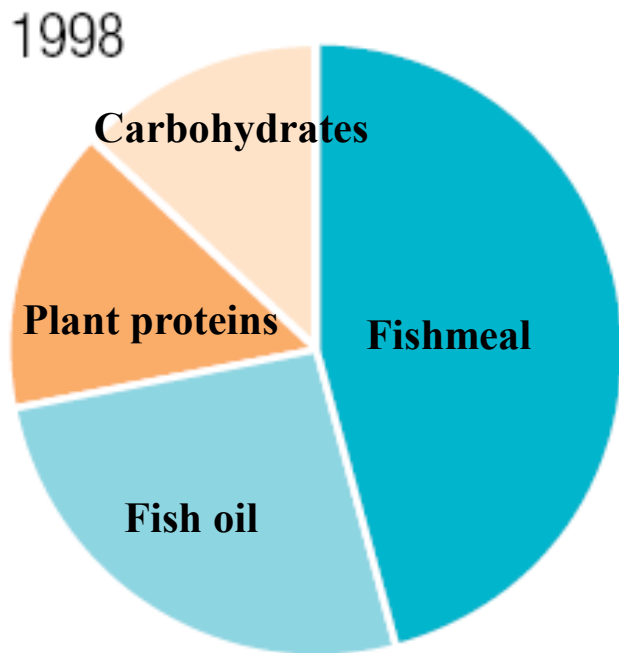
Underlying causes








Muscle precursor cell morphology and proliferating versus non-proliferating cells nuclei



Raw material use

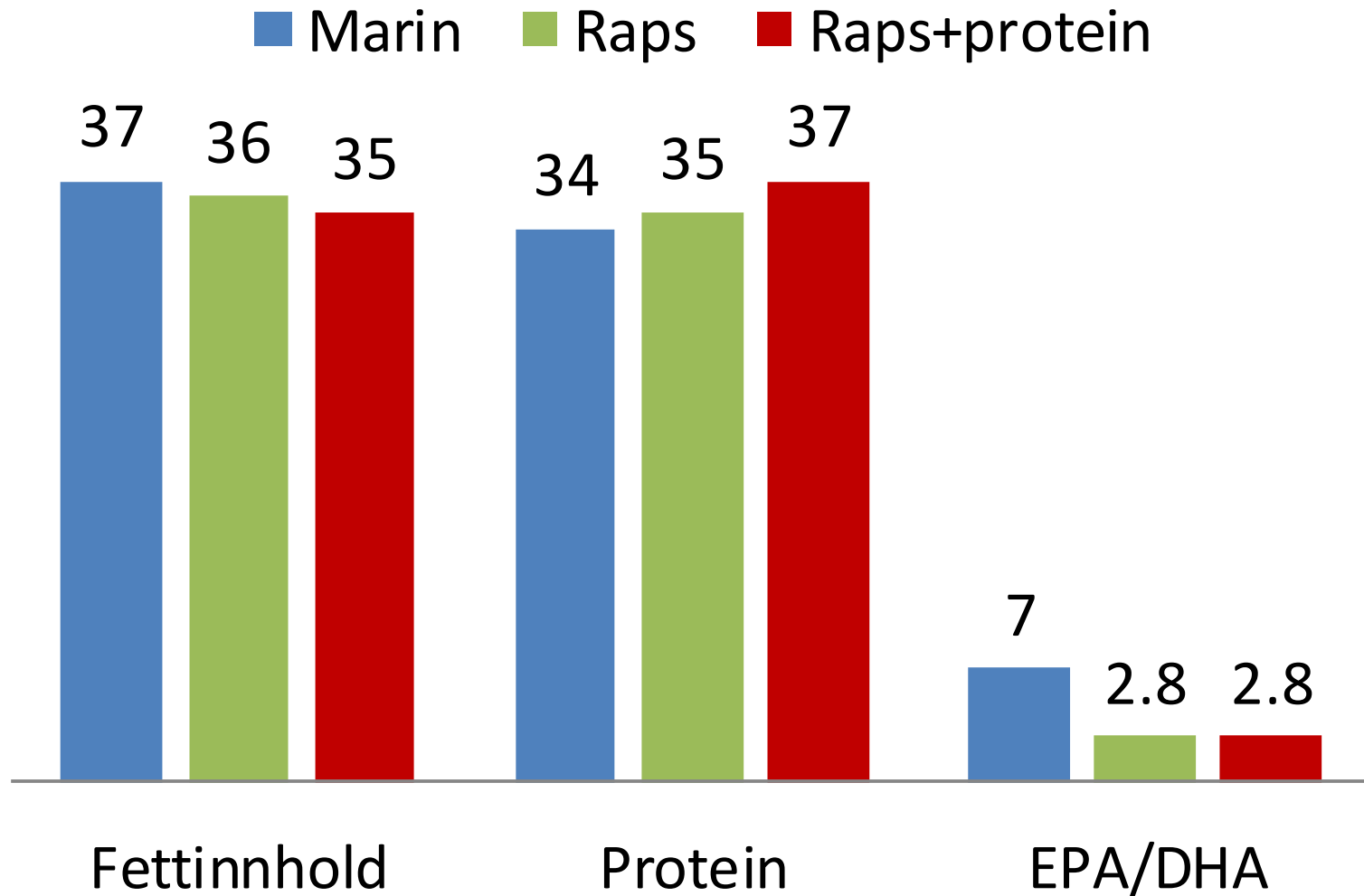


 Fiskemel, *fishmeal*
 Fiskeolje, *fish oil*

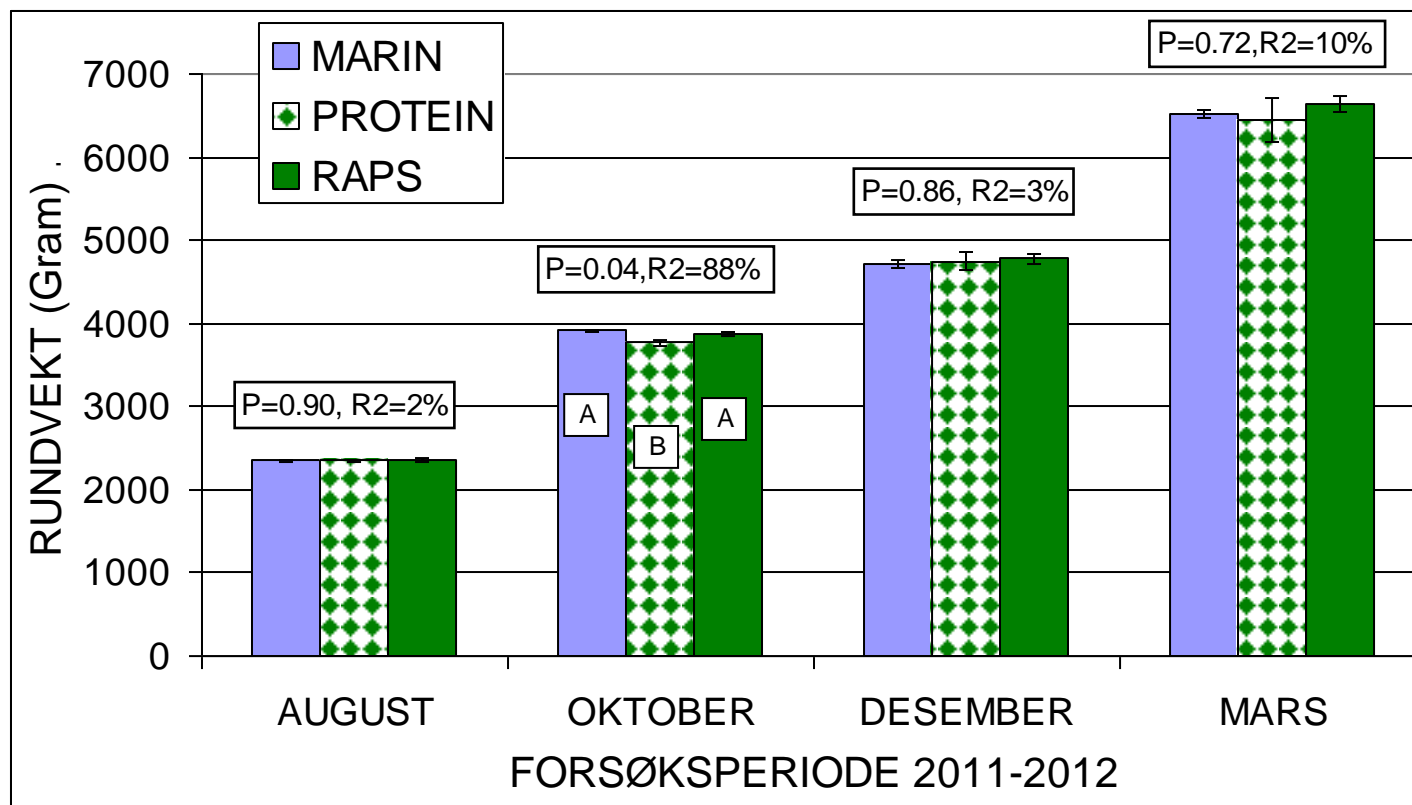
 Vegetabilisk olje, *vegetable oil*
 Vegetabilisk protein, *vegetable protein*
 Karbohydrat, *carbohydrates*

Fôrsammensetning (9mm)

«Rousselot® FG is an edible fish gelatin made of all essential amino-acids except tryptophan. It is particularly rich in glycine, glutamic acid, proline, alanine and arginine. Rousselot® FG»

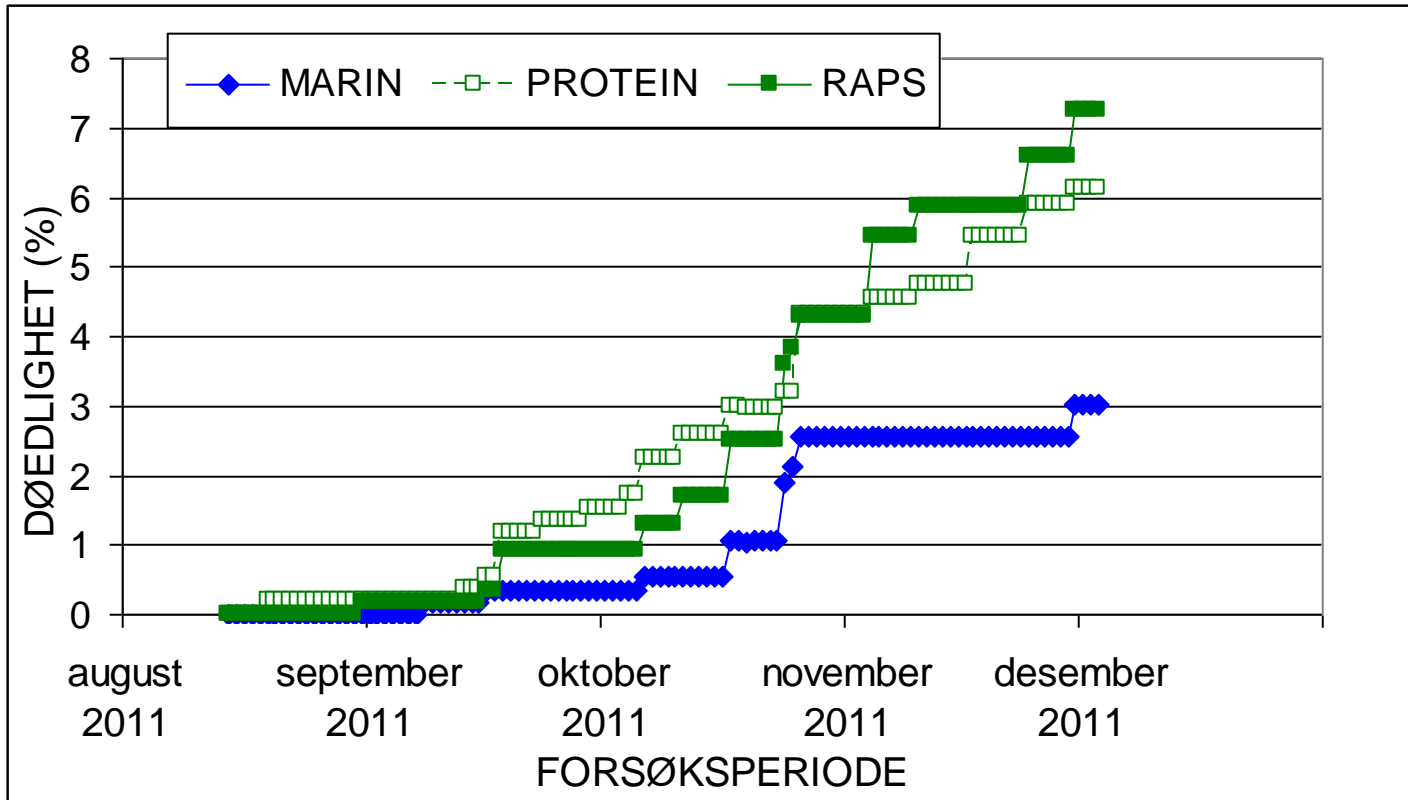


LITEN EFFEKT AV FORSØKSFØRENE PÅ KROPPSVEKT GJENNOM FORSØKSPERIODEN

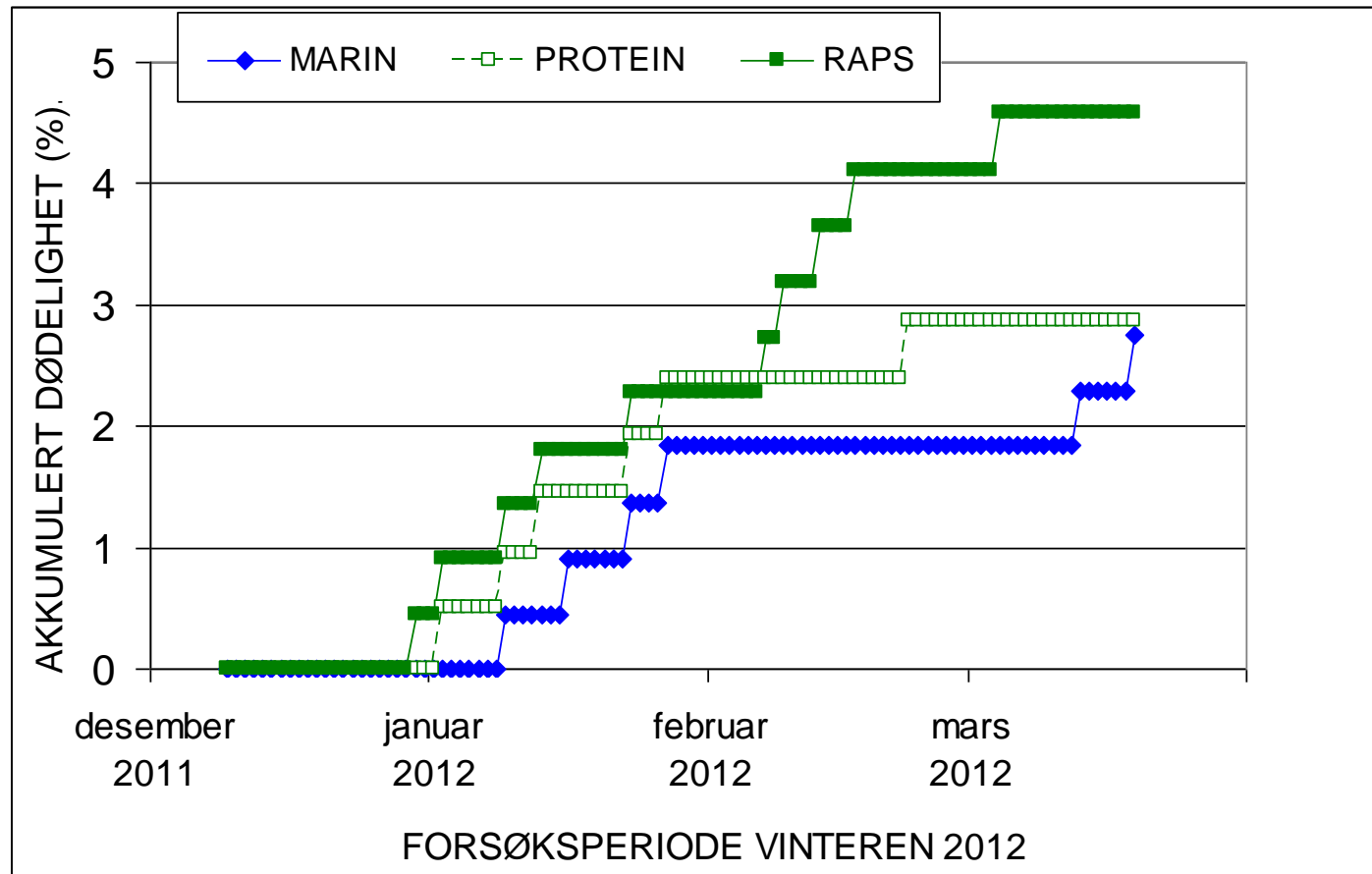


UTVIKLING I USPESIFIKK DØDELIGHET HØSTEN 2011

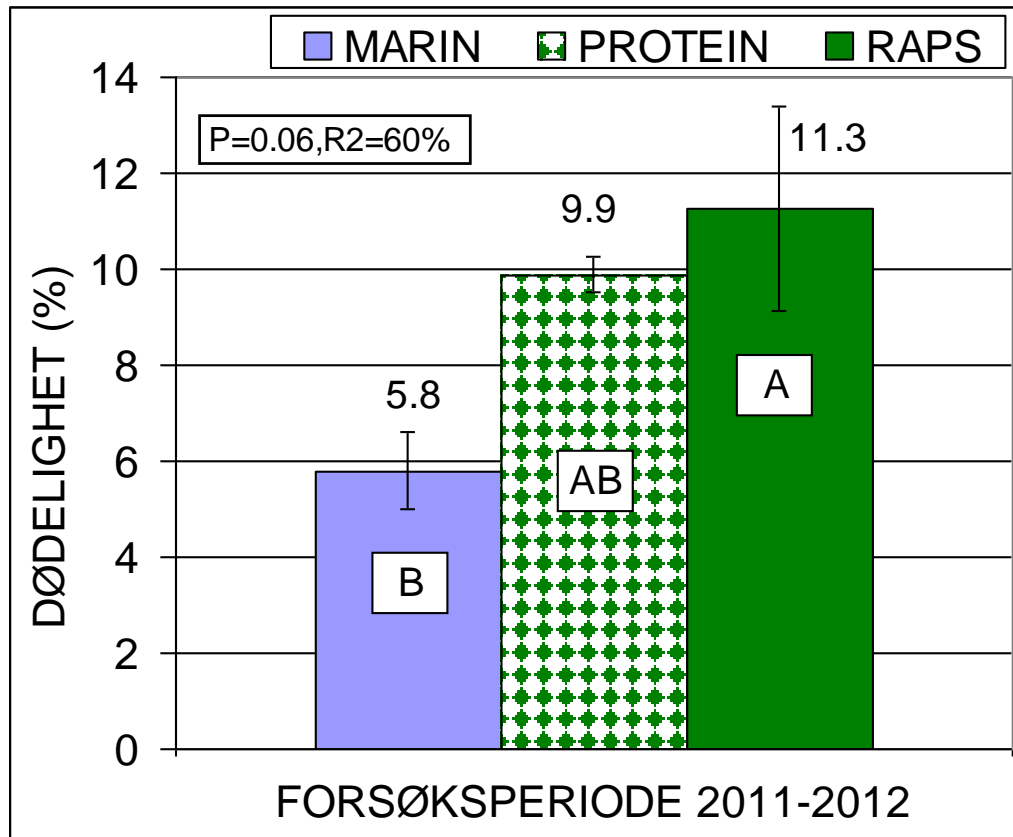
Test for uavhengig dødelighet mellom fôrgrupper gir $P=0.005$



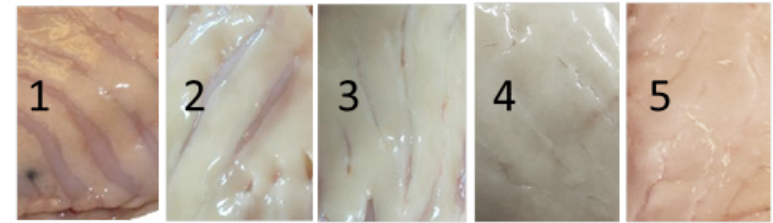
UTVIKLING I USPESIFIKK DØDELIGHET VINTEREN 2011



AKKUMULERT USPESIFIKK DØDELIGHET GJENNOM HELE FORSØKSPERIODEN



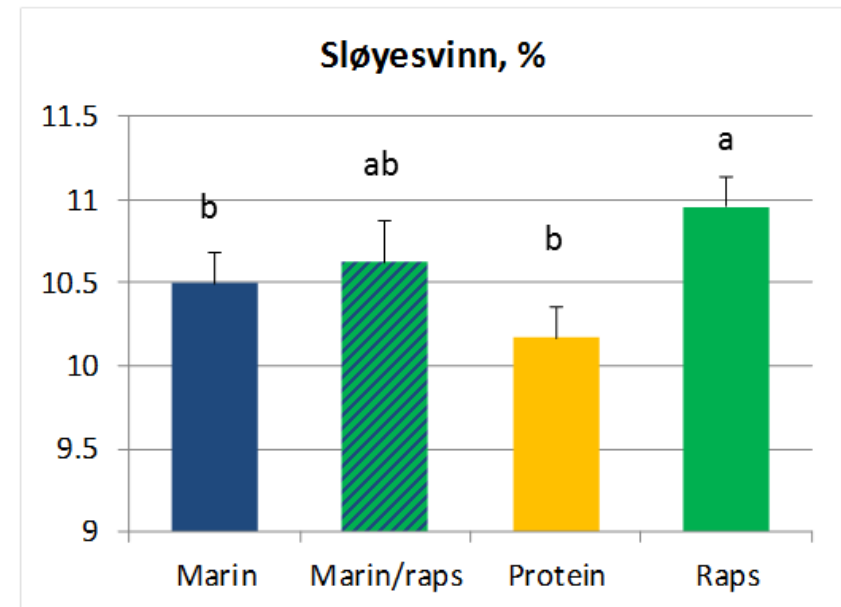
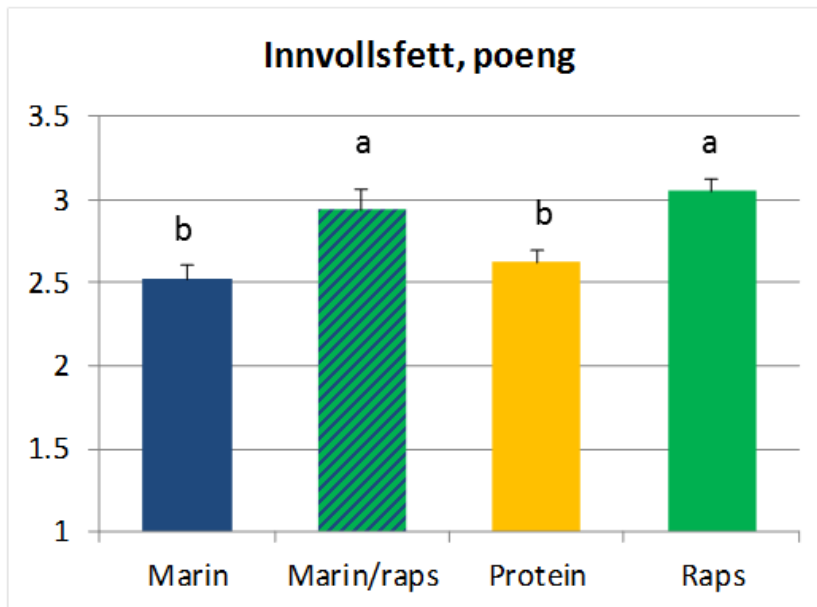
Innvollsfett og sløyvesvinn



Ny skala, utviklet i prosjektet

Blindsekker synlige 1: Godt synlige; 2: synlige, 3: sprekker, 4: synlige gjennom fett, 5: ikke synlige gjennom fett.

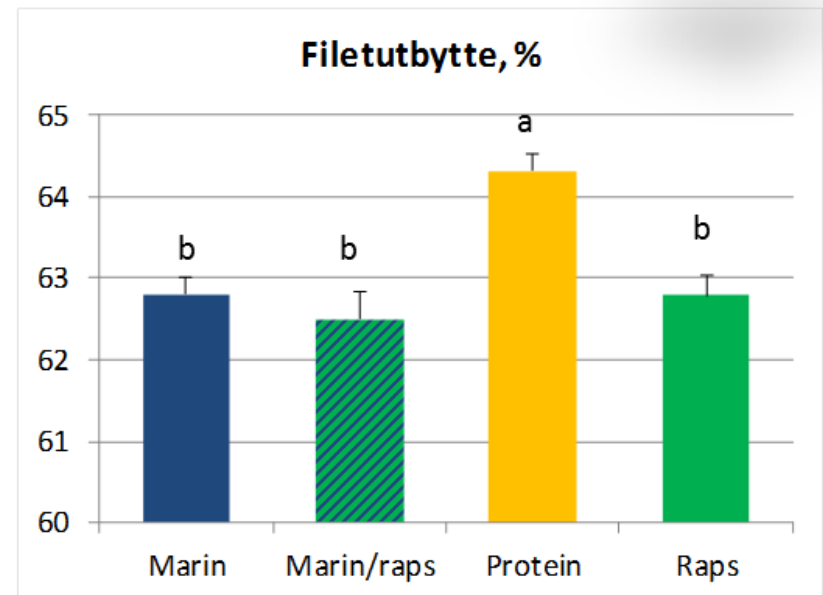
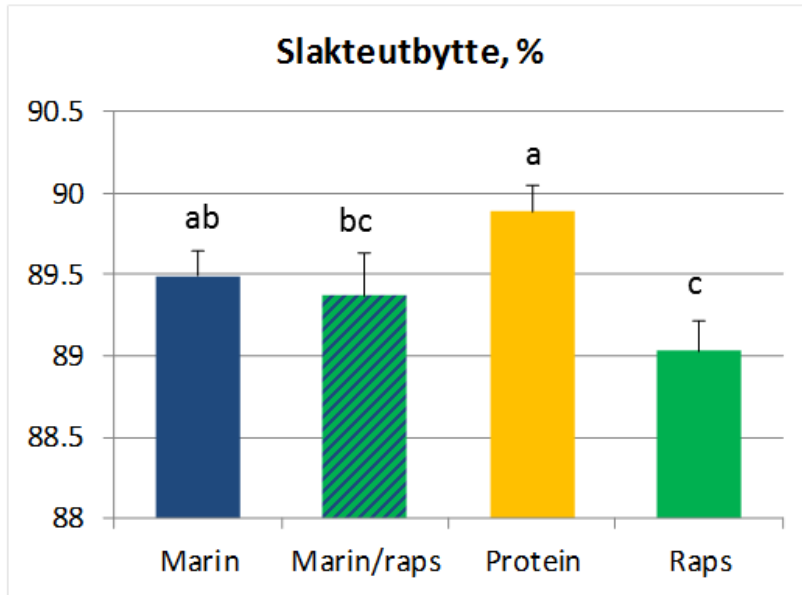
Fungerte bra!



Laksen som fikk rapsfôret hadde mest innvollsfett og høyest sløyvesvinn

Utbytte

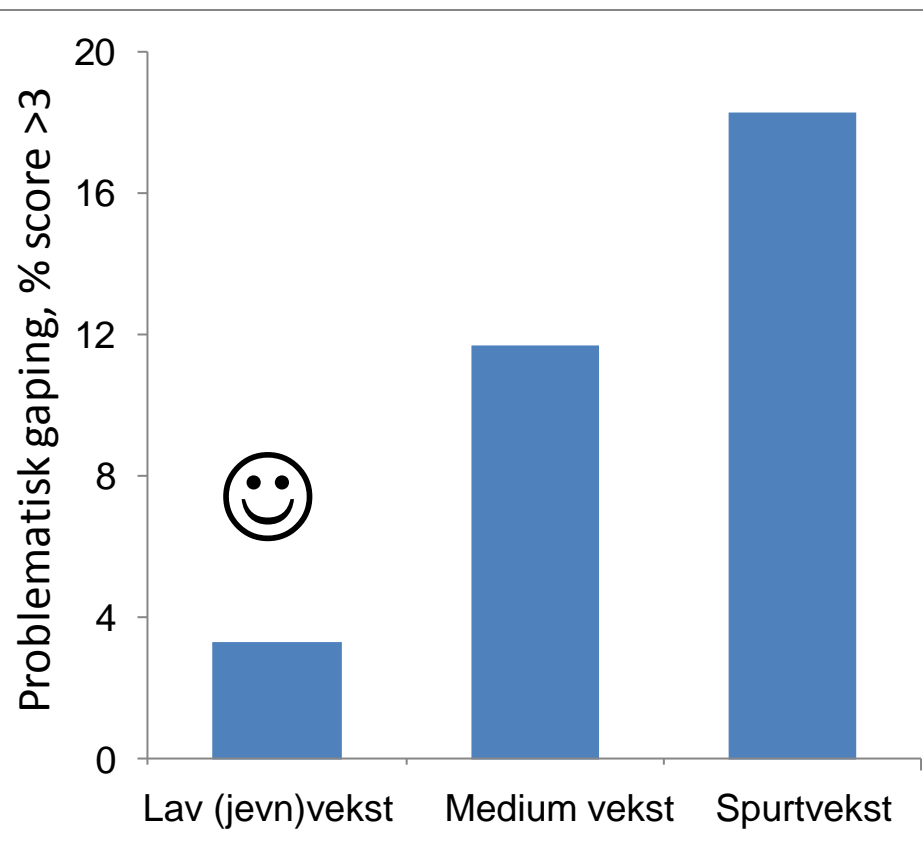
Fin fisk – Omtrent samme vekta for alle fôra - Over 6 kilo i gjennomsnitt og noen over 10 kg



Proteinfôret ga høyest slakteutbytte og høyest filetutbytte. Rapsfôret lavest

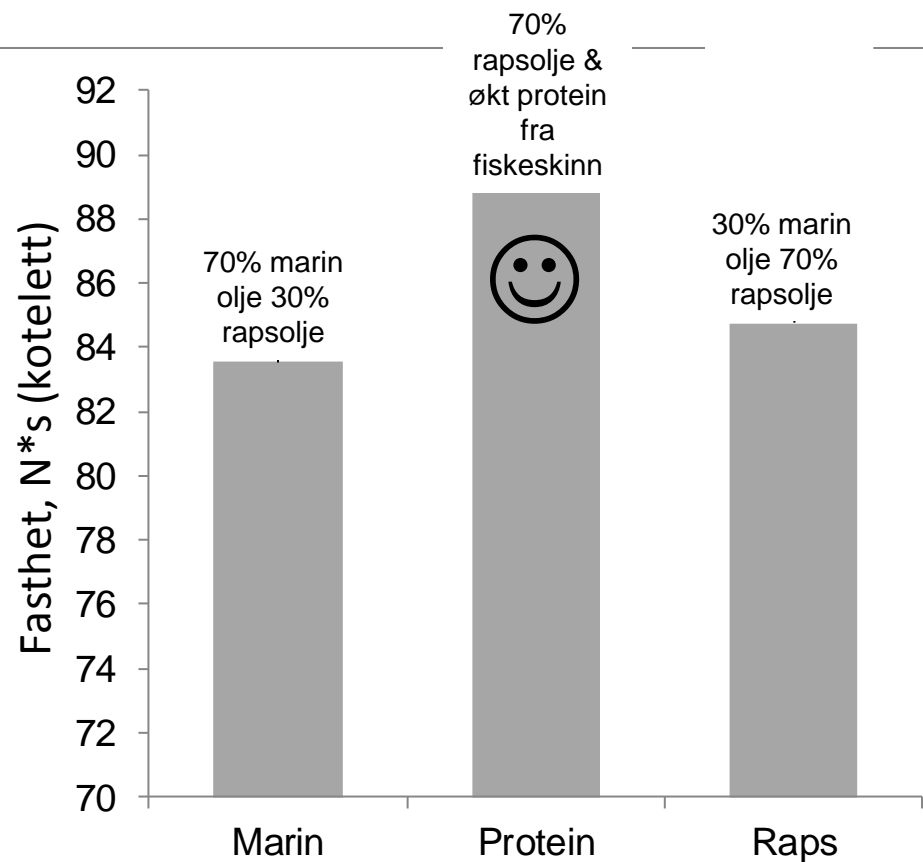
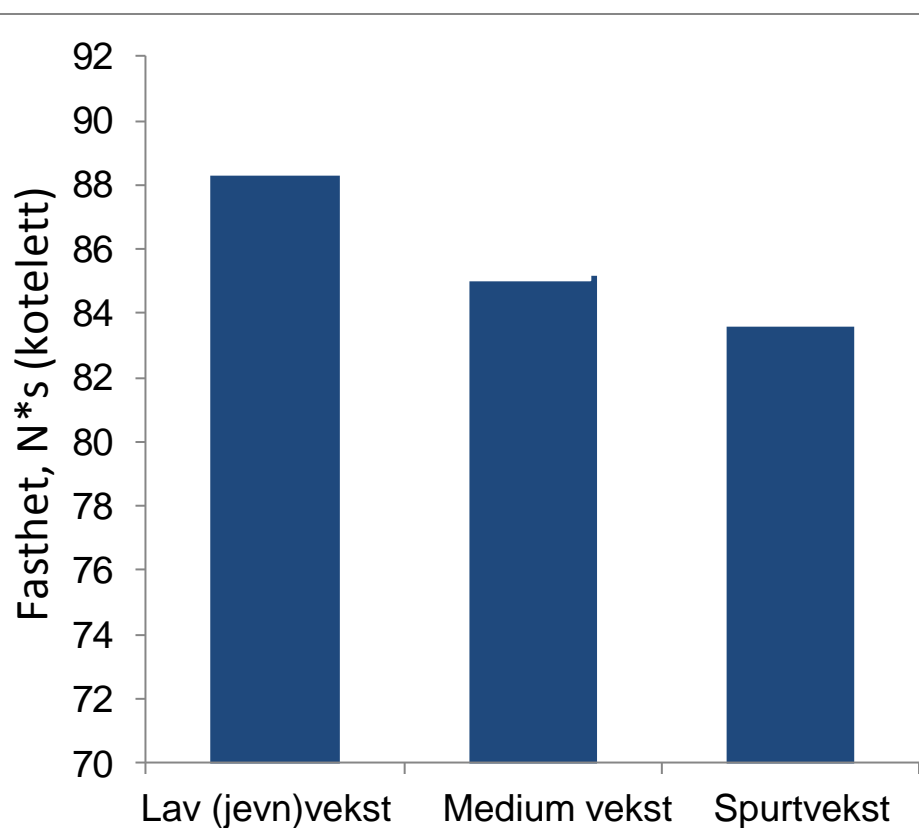
Resultater oktober

Filetspalting, vist som andel laks med problematisk gaping (% fisk). Resultatene viser at spurtveksten kombinert med høy (ektrem..) fettdeponering til laks med spurtvekst førte til betydelig gaping - nærmere 20% av fisken mens mindre enn 5% av laksen med jevn vekst hadde gaping. En liten og mest sannsynlig ubetydelig positiv effekt av ekstra tilsetning av protein i fôret



Resultater oktober

Fasthet i rå kotelett viste samme mønster som problematisk filetspalting, men her var det en mer fremtredende effekt av ekstra tilsetning av protein i fôret



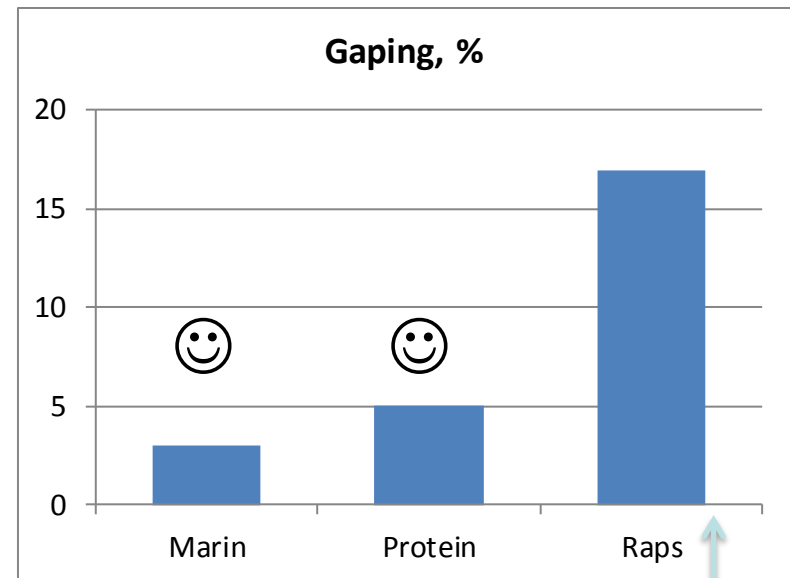
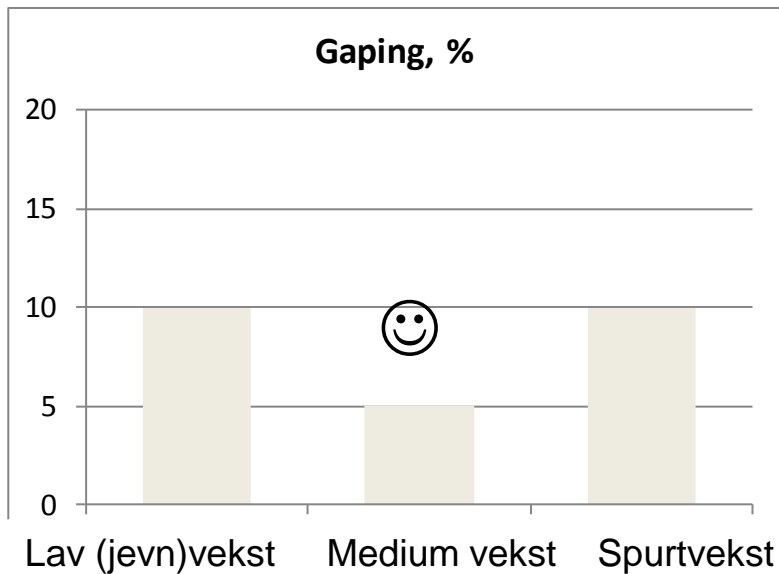
Væsketap viste samme mønster

Resultater fra møtet "Gullfanden - Kvalitetssjekk og fôrsystemer i henger". 30.09.2010. T. Mørkøre

Resultater desember

Gaping, % fisk

- Hoved-diet, aug-des



UPS – denne ser ikke bra ut....
For 3 av 4 nøter var denne forskjellen
ennå mer markant, mens for 4. rapsnota
var det mindre gaping

Gaping og tekstur i mars

Målingene ble utført 6 dager etter slakting, n = 90 per fôrgruppe. Snittvekten var 6,57 kg.

• Gaping score > 3 i hht Andersen et al. 1994

Marin olje **1,9%^{ab}**

Rapsolje **0%^b**

+ protein

Rapsolje **3,9%^a**

Tilleggs kommentarer:

Generelt lite gaping

Laksen som fikk det feite fôret fra mai-aug
(mest jevn vekst) hadde minst gaping

• Fasthet, rå kotelett (N*s)

Marin olje **92,3^a**

Rapsolje **95,6^a**

+ protein

Rapsolje **92,8^a**

Tilleggs kommentarer:

All fisken var fast i kjøttet – betydelig fastere
enn i oktober

Fast tekstur

- Styrt fôrsammensetning (tenke nytt...)
- Bedre forståelse av laksens energiomsetning – hva skjer egentlig inne i cellene og kan vi styre omsetningen i ønsket retning (stimulere til fettforbrenning)
- ”Oksygenmangel” ?
- Tilpasse produksjonen (fôr/fôring mm) til årstid / temperatur / vekstmønster ?
- Helsestatus ?
- Slaktefôr ?

Bløt filet ved vekstspurt

Bindevevsdannelsen henger etter

The rapid muscle growth, especially in turkey, is connected with atrophy of fibers and loss of connective integrity [Sosnicki & Wilson, 1991]. The connective tissue (endomysium) associated with individual muscle fibers cannot keep up with rapid muscle fiber growth and in result is less developed and immature [Swatland, 1990]. The fiber necrosis and focal myopathy (FM) incidence in poultry proves that muscle overgrows its own supporting system. It is not clear, in spite of similarities in the histopathological

Vekstspurt

- Fôrsammensetningen spesielt kritisk
- Høyt nivå rapsolje kombinert med lavt proteinnivå ser ut til å være ugunstig
- Økt behov for Vitamin E, C, Se, Zn ?
- Økt behov spesifikke aminosyrer !
- Næringsbehov

max tilvekst ≠ behovet for max kvalitet

Torunn Taksdal¹, Jannicke Wiik-Nielsen¹, Sveinung Birkeland², Paw Dalgaard³, Turid Mørkøre⁴

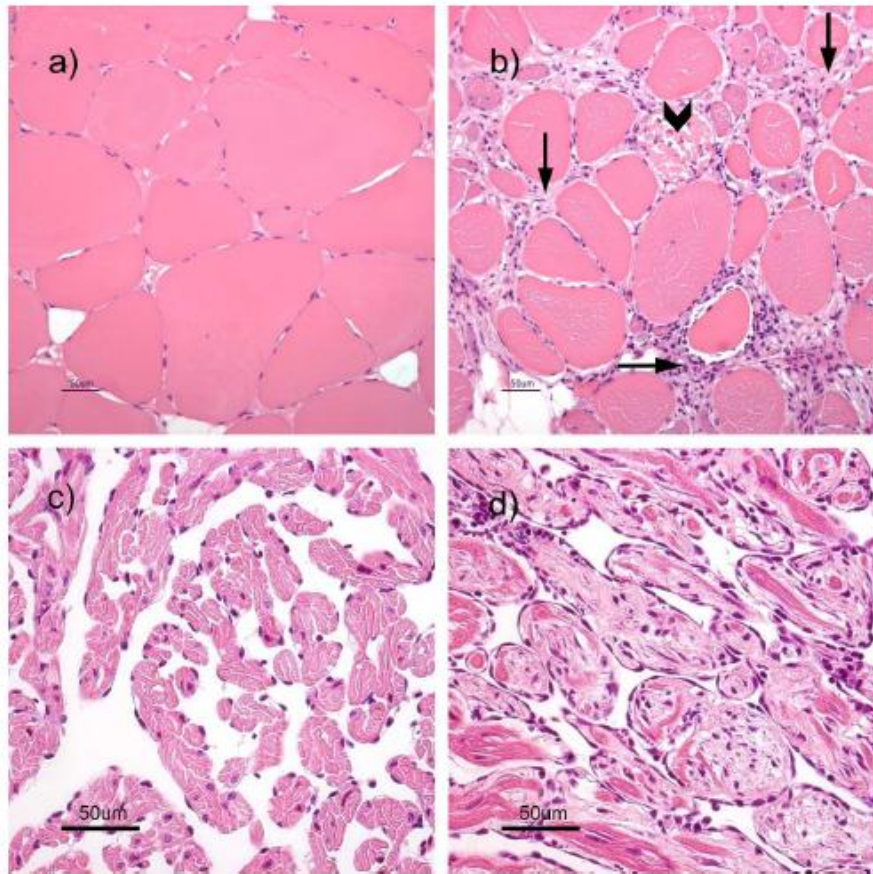


Figure 2. Light microscopy of clinically healthy, harvest-ready Atlantic salmon from a PD-affected fish farm. (a, b) White/anaerobic skeletal muscle with (a) no changes (score 0) and (b) severe changes (score 4) with necrosis of a single muscle fibre (arrowhead), inflammation (horizontal arrow) and fibrosis (vertical arrows). (c, d) Spongy myocardium with (c) no changes (d) subendocardial fibrosis, the muscle fibres appearing thickened due to the fibrosis and thickened endothelium (haematoxylin & eosin, bars = 50µm).

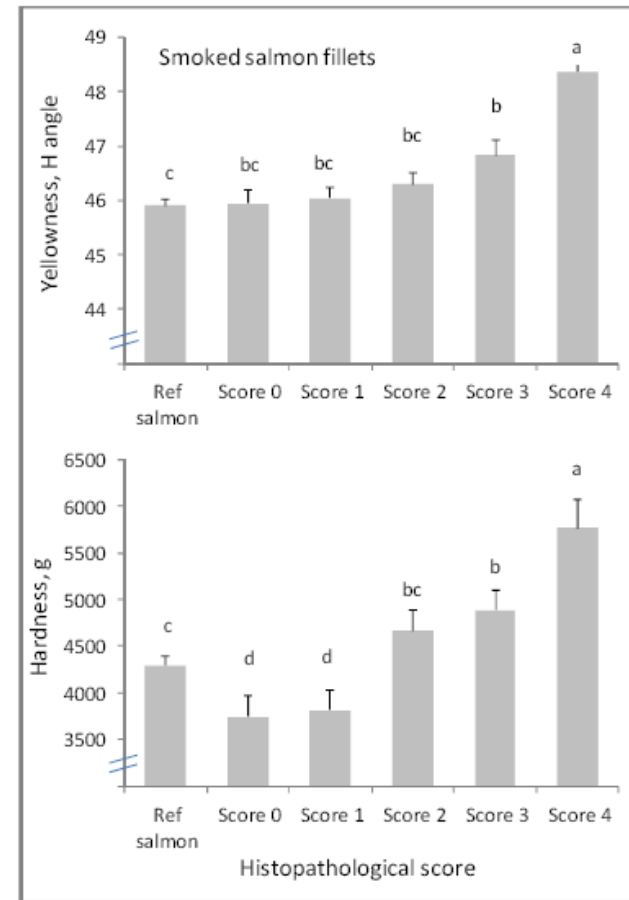
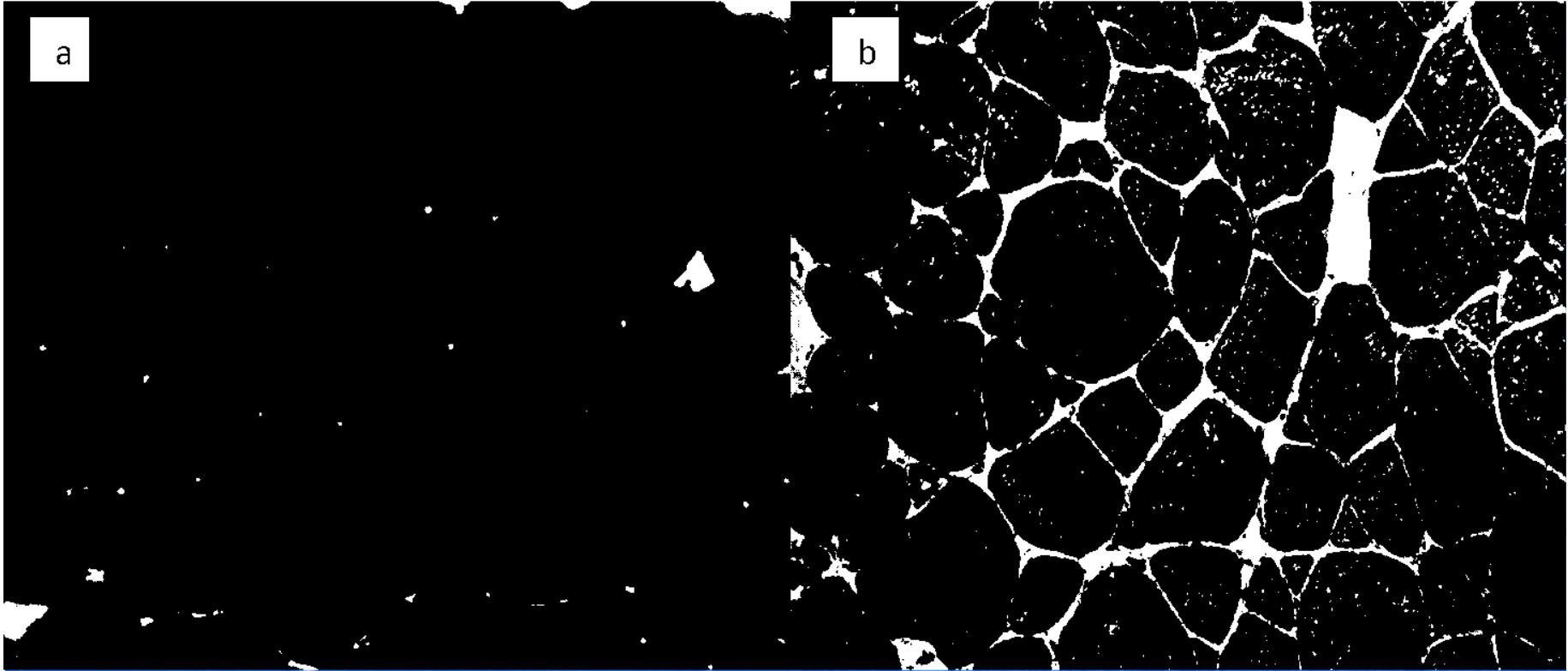


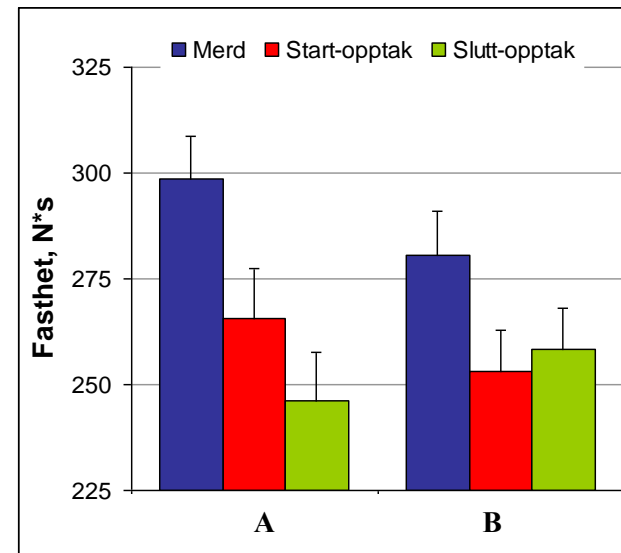
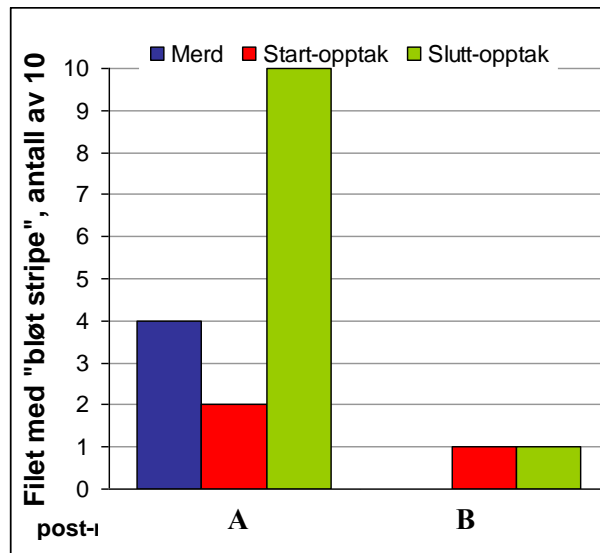
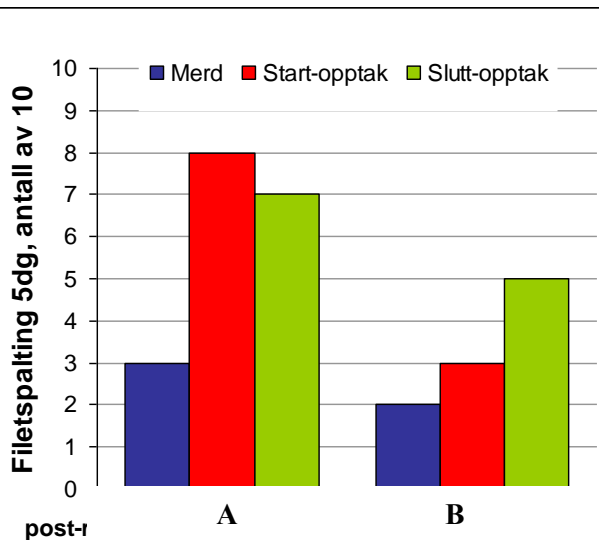
Figure 4. Yellowness and hardness of smoked fillets of 50 fish from a reference fish farm (Ref salmon) and 50 clinically healthy, harvest-ready Atlantic salmon from a PD-affected farm, the latter divided into bars according to the severity of muscle changes scored by light microscopy, from 0 = no changes to 4 = severe changes. Mean ± SE are shown. Different letters indicate significant differences between the yellowness and hardness for each score ($P < 0.05$).

Stress ved slakting kan føre til «krakelering» av muskelen



Laks med god vekst

Analysert etter 5 dagers lagring



Mye kunnskap fra tradisjonelt husdyrhold er overførbart til lakseoppdrett



Flyter i vannet - spretten
Lite bindevev
Tåler høye nivåer av melkesyre

- Myopathy in terrestrial species due to vitamin E and selenium deficiencies is well documented and often occurs in animals which undergo a sudden increase in muscular activity following a period of inactivity and poor nutrition (Anderson et al. 1977).

Pale, soft, exudative meat, PSE

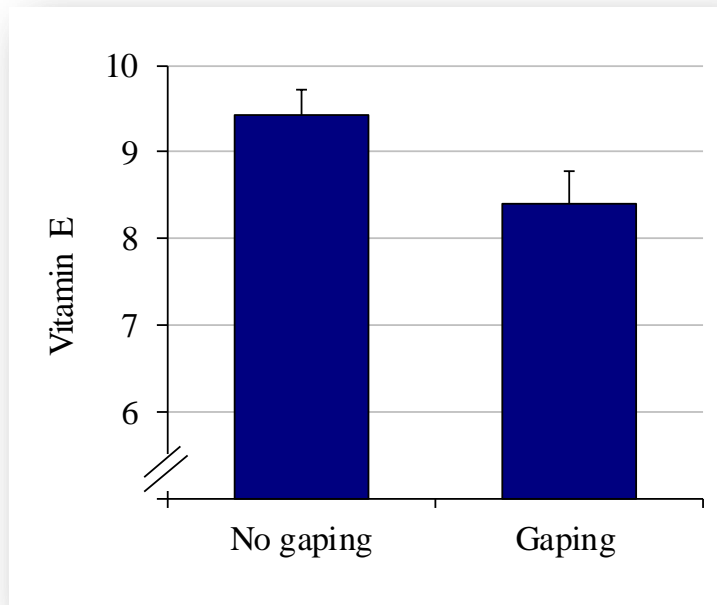
(PSS, porcine stress syndrome)

Art	Effekt	Observasjoner	Referanse
Gris	Redusert	1000mg Vit E i 46 dg reduserte utskillelse av Ca ²⁺ og PSE frekvens. Årsak, hypotese VitE stabiliserer membran	Buckley et al. 1995
Gris	Redusert	Ekstra tilsetning av Trp (0.5%) i slaktefôr 5 dg ⇒ reduserte PSE fra 9% til 6%	Anon 1991 (
Gris	Redusert	Ekstra tilsetning av Trp i slaktefôr ⇒ redusert stressrespons og redusert PSE	Adeola&Ball 1992
Kalkun	Økt	Rask vekst (muskelfiber atrofi, tap av bindevevsstyrke)	Sosnicki & Wilson, 1991
Gris	Økt	Tryptofan (Trp) mangel ⇒ redusert produksjon av serotonin i hundyr	Henry et al. 1992

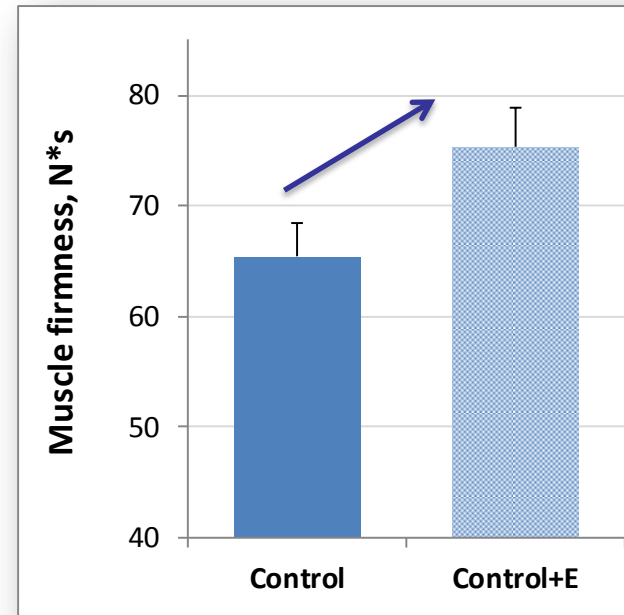
Gris	Økt	Stress ved slakting	Grandin 1989; Pedersen et al. 1993
Gris	Økt	Stress ved transport; bedre å gå fra mørke mot lys	Van putten et al. 1982
Gris	Økt	Blande dyregrupper ved transport/slakting (sosial uro)	Karlsson& Lundstrøm 1992

Vitamin E

Vitamin E i muskel lavt i laks med spalting



Økt vitamin E i fôret før slakting (2,5 mån) ga fastere filet

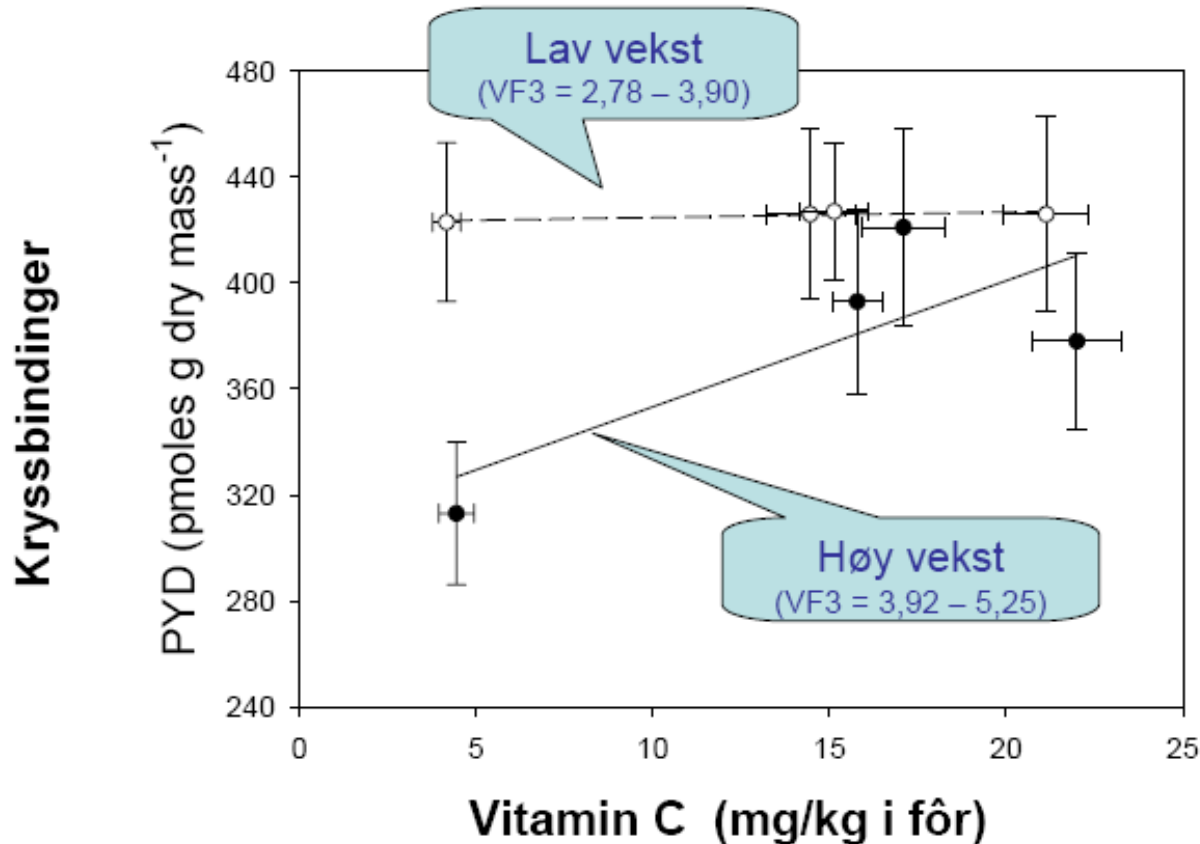


Takk for meg !

Fôringsforsøk med med smålaks (160 g – 675 g)



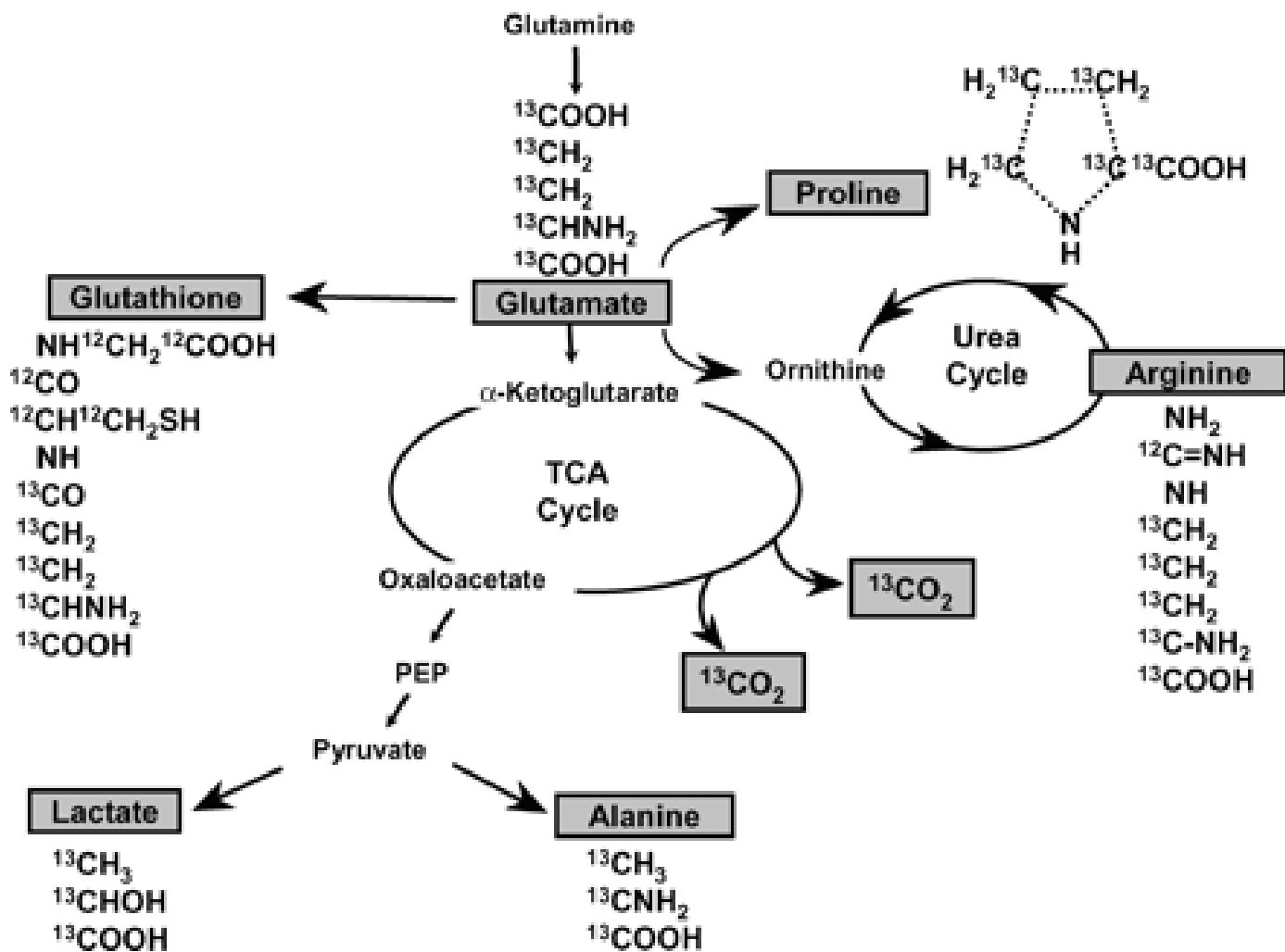
Vitamin C innhold og kryssbindinger i kollagen



- Kollagenmengden påvirker fastheten
- Flere kryssbindinger i kollagen gir økt fasthet
- Veksthastighet påvirker fasthet på filét
- Det er et samspill mellom innhold av vitamin C og veksthastighet på fasthet i filét

Kilde: Sigholt & Bickerdike 2008





B)

