EVALUATION OF DIETARY WHEAT GLUTEN PRODUCTS AND scFOS ON GUT HEALTH AND GROWTH PERFORMANCE OF RAINBOW TROUT *Oncorhynchus mykiss*

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Image: treehugger.com

Aquaculture and sustainability

- Fish account for 16.9% of the worlds animal protein intake
  - 6.5% of all protein consumed.
- 49.9 million tonnes of fin fish produced in 2014 (>$101 billion)
- 56.4% of total fin fish production is accounted for by fresh water species
  - Rainbow trout production 2014 = 812,940 metric tonnes (FAO).

- Concerns over the stainability of the industry in regards to:
  - Disease
  - Parasites
  - Impact on the environment
  - Fishmeal inclusion in aquafeeds

Plant protein sources

- Enables expansion of the industry despite stagnated levels of fish meal production
  - 5 million tonnes produced globally annually
- Salmonids require a high protein diet,~45% (~20% lipid)

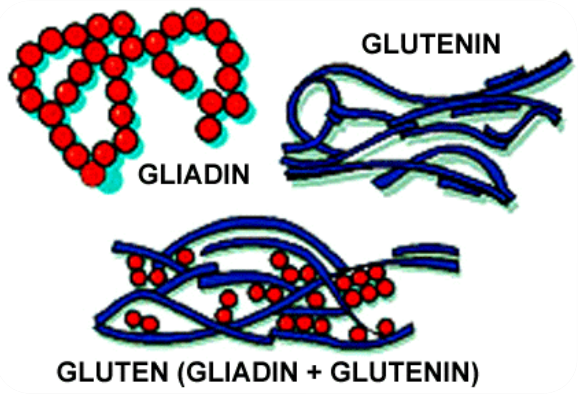
- Alternative plant protein sources accepted in aquafeed industry
  - Blended formulations reduce reliance on one PP source, and their individual limitations.
    - Legumes (especially soybean)
    - Oilseeds
    - Cereal grains

- Antinutritional factors (ANFs) major consideration in the inclusion of PP's in aquafeeds
  - Saponins
  - Tannins
  - Pectins
  - Protease inhibitors
  - Glucosinolates
  - Non-starch polysaccharides
  - Oligosaccharides
  - Alkaloids
  - Antigenic compounds
  - Cyanogens
  - Antivitamins
  - **Phytates**

Wheat gluten

- High protein co-products from the removal of starch from cereal grains
  - 75-85% protein
  - 5-10% lipid
- Comprised of hundreds of polypeptides, which give wheat gluten its characteristics:
  - 50% Monomeric gliadins - viscosity and extensibility
  - 50% Polymeric glutenin - elasticity and resistance to deformation

- Drying into its vital state increases ease of transportation and storage
- Rehydration restores functional properties



- Low carbohydrate and antinutritional factors, especially phytate.

Amino acid composition and functional amino acids

- Wheat gluten typically consists of~80% crude protein.

- Wheat gluten is highly digestible for salmonids
  - Protein - 97%
  - Energy - 84% (Davies et al. 1997)

- Low lysine content (1.5-1.7g/100g of wheat gluten)
  - Arginine and threonine

(Li et al. 2009)

- Glutamine – comprises 35-40% of wheat gluten
  Utilised throughout the fish
  - –Synthesis of purines and pyrimidines (RNA/DNA)
  - –Energy source for all rapidly proliferating cells (vital for enterocytes in the GI tract)

- Evidence of glutamine acting in other areas as well
  - Substrate for immune cells (leukocytes)
  - Free radical and reactive oxygen species protection
  - Precursor to the synthesis of glutathione (antioxidant)

Wheat gluten products investigations

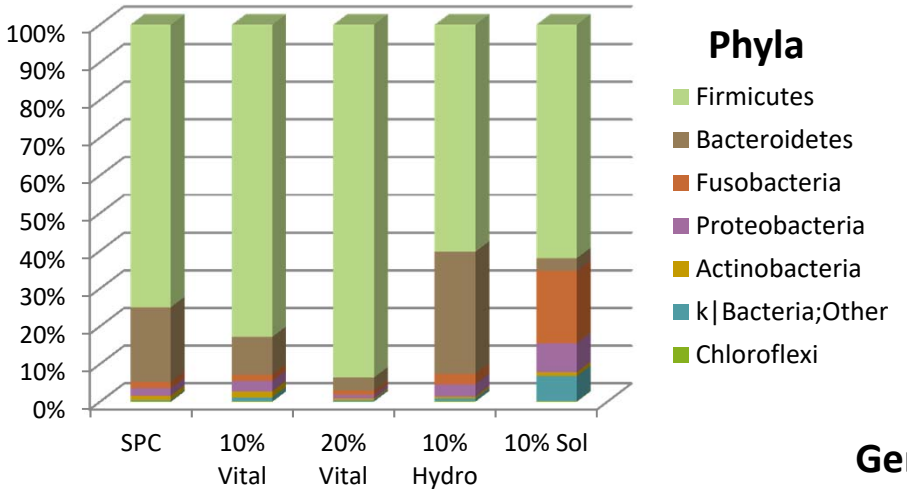
- Series of growth trials.
- Conducted with the Aquatic Animal Nutrition and Health Research Group at the University of Plymouth
- Wheat gluten inclusions at the expense of soy protein concentrate

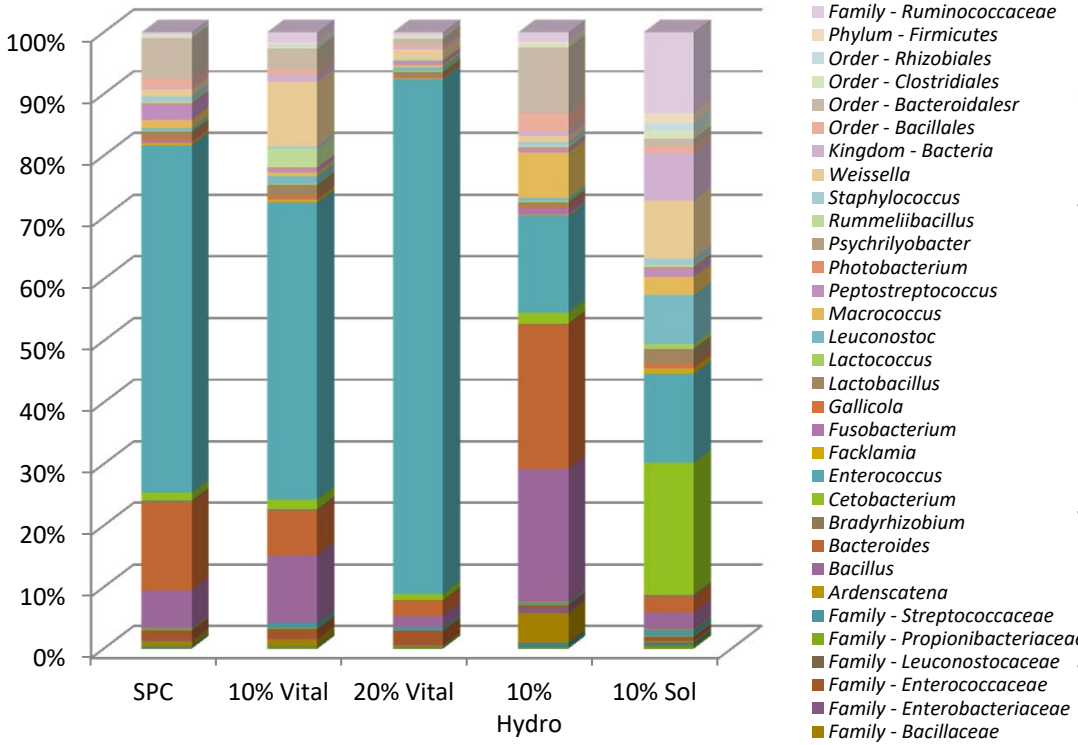
Parameter	Investigation 1	Investigation 2
Trial length	66 days	56 days
N° of tanks	15	18
Fish per tank + mean fish weight	31 (24.80 ± 0.31g)	30 (26.70 ± 0.12g)
Wheat gluten types	Vital, Hydrolysed, Soluble.	Vital, Soluble, Blend of the two.
Dietary inclusions (% of diet)	10% Vital 20% Vital 10% Hydrolysed 10% Soluble	20% Vital 20% Soluble 20% Blend 25% Blend 30% Blend

	SPC (Basal)
Ingredient (%)	
Soya protein concentrate	52
Fish Oil	16.49
Hydrolysed wheat gluten	-
Vital wheat gluten	-
Soluble wheat gluten	-
Corn starch	7.63
Herring meal	10
Soyabean meal	10
L-Lysine HCl	1.85
Calcium carbonate	1
Vitamin mineral premix	0.5
CMC-Binder	0.5
Antioxidant mix	0.03
Proximate composition (%)	
Moisture	4.36
Protein	48.04
Lipid	19.56
Ash	6.11
Energy (MJ/Kg)	22.55

Next generation sequencing of gut microbiota

- >11,200 reads per sample.
- Ca 2,500 reads = >0.99 coverage
- Phred score = 20.
- 16S v1 – v2 region





Phylogenetic composition of digesta samples per treatment.

- No dietary effect of wheat gluten types on Phyla composition

Genera of gut microbiota

- *Enterococcus* spp. (*E. faecium*)

- 20% vital > 10% Sol, 10% Vital, 10% hydro

- *Bacillus* spp. – *B. coagulans*

- 10 Hydro > all other treatments

- *Weissella* spp. – *W. confusa*

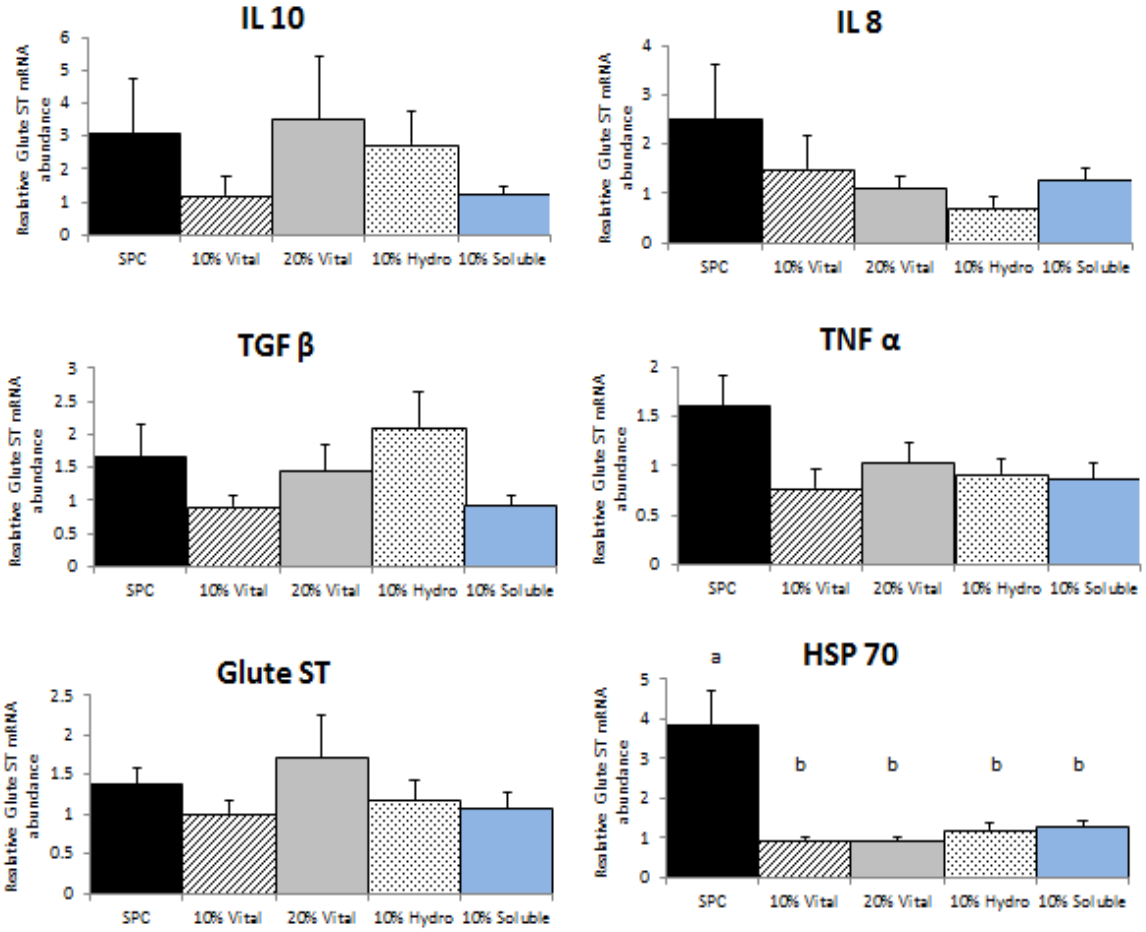
- 10 vital> SPC, 10% hydro

- *Leuconostoc* spp.

- 10% Sol > all other treatments

- Ecological diversity parameters unaffected

Intestinal response



Microscopy

- Increased IELs in 10% vital and 10% soluble treatments
- Increased lamina propria width in 10% vital treatments
- Microvilli density unaffected
  – Scanning electron microscopy

- Pro-inflammatory cytokines- TNF-α, IL-8
- Anti-inflammatory cytokines – IL 10, TGF-β
- Intestinal stress – HSP 70
- Oxidative stress – Glutathione S-transferase (Glute ST)

Growth performance

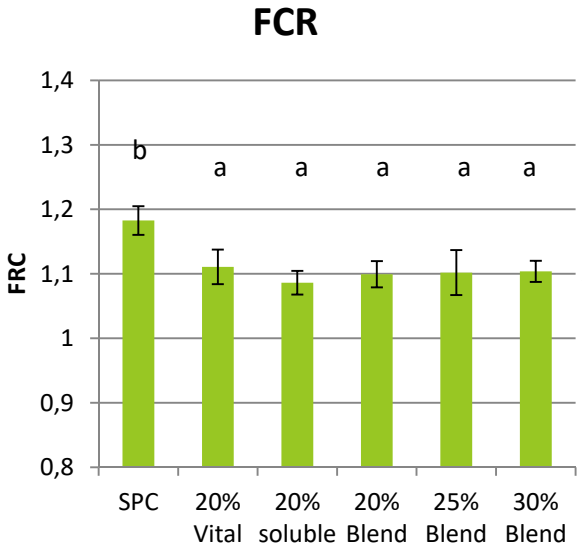
Investigation 1

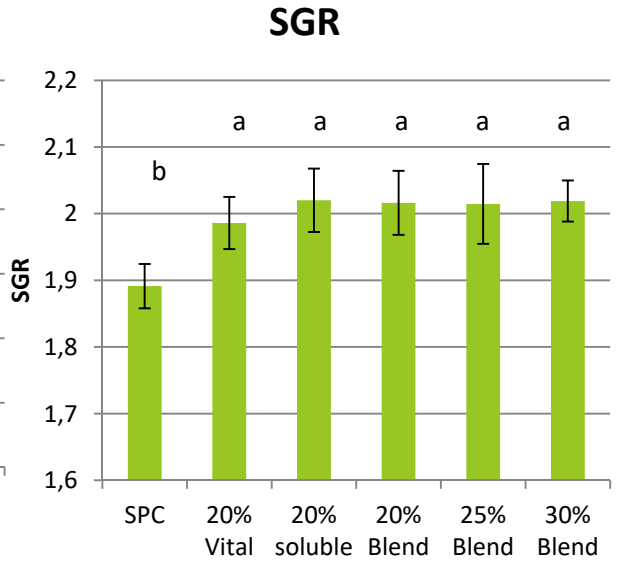
- no significant difference compared to the SPC,
- All treatments preforming very well.
- FCRs ranging from

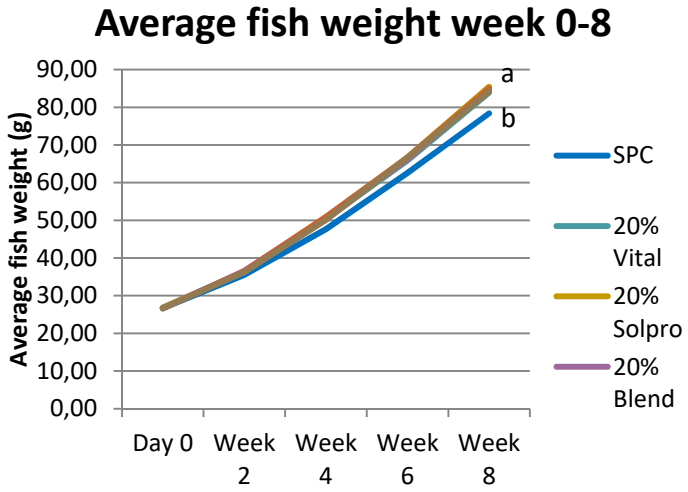
  0.92±0.02 to 1.10±0.08.

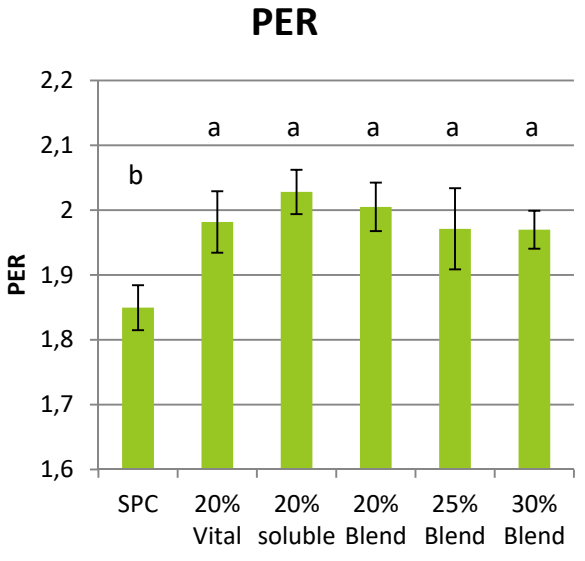
  SPC = 0.99±0.03

Investigation 2









scFOS in aquafeeds for juvenile rainbow trout.

- Fructooligosaccharides naturally occur in a number of edible plants
  - e.g. onions, wheat and asparagus.
- Produced commercially via:
  - 1) from sucrose, utilising fungal fructosyltransferase
  - 2) the partial hydrolysis of inulin

- Potential to promote “good” bacteria in the intestine.
  - Aid digestion and nutrient absorption
  - Aid defence against pathogenic attack

- Production of SCFAs in the intestine
  - Energy supply for rapidly proliferating cells of the intestine.

Wheat gluten and scFOS investigations

- Conducted at Exmoor fisheries, Somerset, UK.
- Wheat gluten included at the expense of soy protein concentrate

Parameter	Investigation 3
Trial length	10 week
N^{o} of tanks	12
Fish per tank + mean fish weight	72 (14.65 ± 0.07g)
Wheat gluten types	Blend of Vital and Soluble
Dietary inclusions	Wheat gluten – 20% scFOS – 0.3%

	Diet	
	SPC (basal)	WG (basal)
Ingredient (%)		
Soya protein concentrate	52	27.21
Fish Oil	16.49	15.58
Vital wheat gluten	-	18
Soluble wheat gluten	-	2
Corn starch	7.36	13.31
Herring meal	10	10
Soyabean meal	10	10
L-Lysine HCl	1.85	1.6
Calcium carbonate	1	1
Vitamin mineral premix	0.5	0.5
CMC-Binder	0.5	0.5
scFOS	-	-
Antioxidant mix	0.03	0.03
Proximate composition (%)		
Moisture	5.08	5.35
Protein	46.94	47.39
Lipid	18.09	17.35
Ash	6.57	5.13

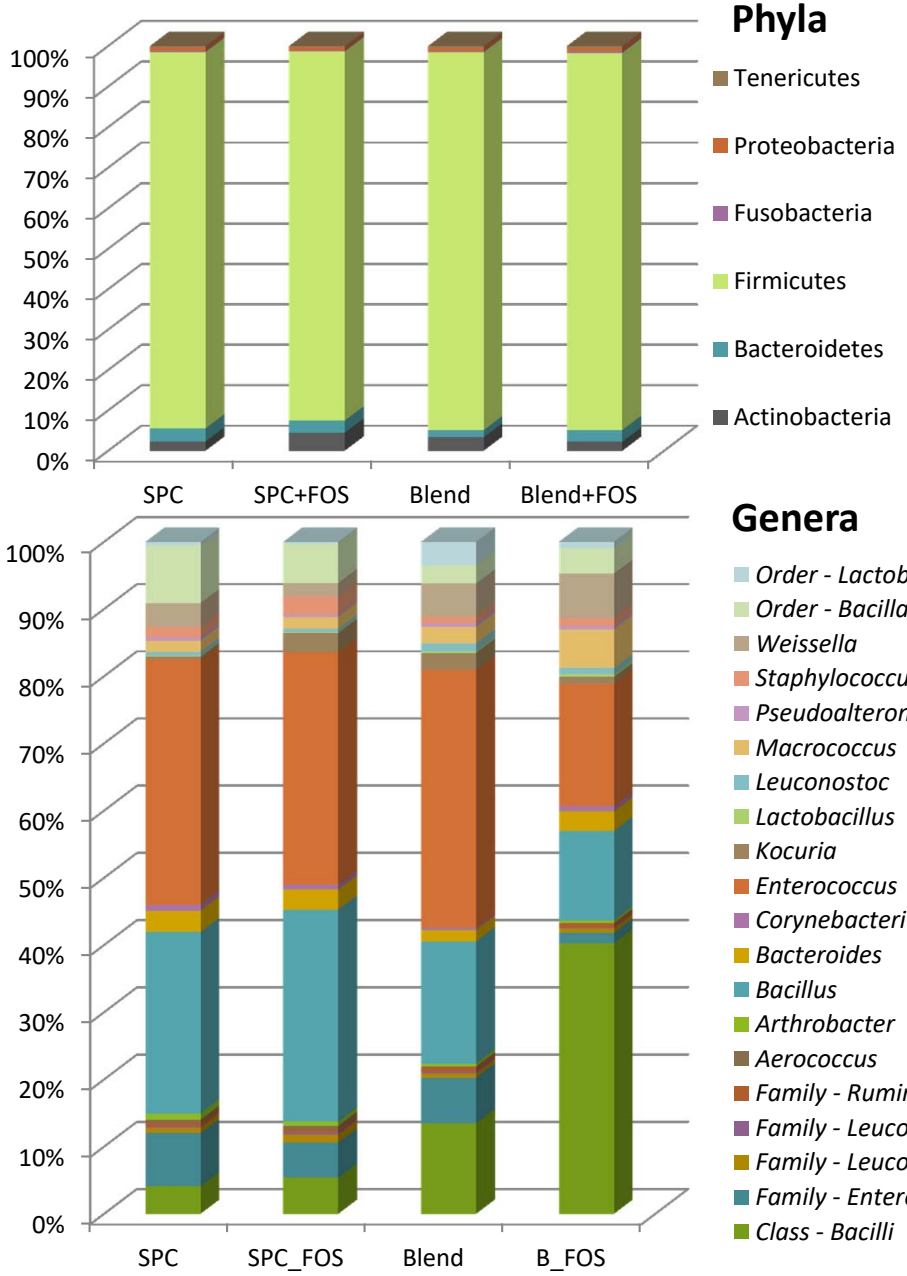
Growth performance

	SPC	SPC+ FOS	20% Blend	20% Blend + FOS
FCR	0.94±0.01	0.95±0.05	0.95±0.03	0.94±0.03
SGR	2.05±0.01	2.05±0.05	2.07±0.03	2.07±0.05
Mean weight end point (g)	60.15±0.49	61.23±1.02	61.66±1.00	60.934±1.85
Protein efficiency ratio	2.26±0.028	2.27±0.11	2.26±0.07	2.24±0.08
K-Factor	1.54±0.10	1.47±0.07	1.54±0.06	1.54±0.10
Survivability (%)	100±0.00	99.08±1.59	99.54±0.78	100±0.00

- All treatments performed extremely well
- No effect observed between dietary treatments
- No effect on carcass composition
- Previously observed reduced FCR under sub-optimal conditions

Next generation sequencing of gut microbiota

- >41,700 reads per sample.
- Ca 3,500 reads = >0.99 coverage
- Phred score = 20.
- 16s v1 – v2 region



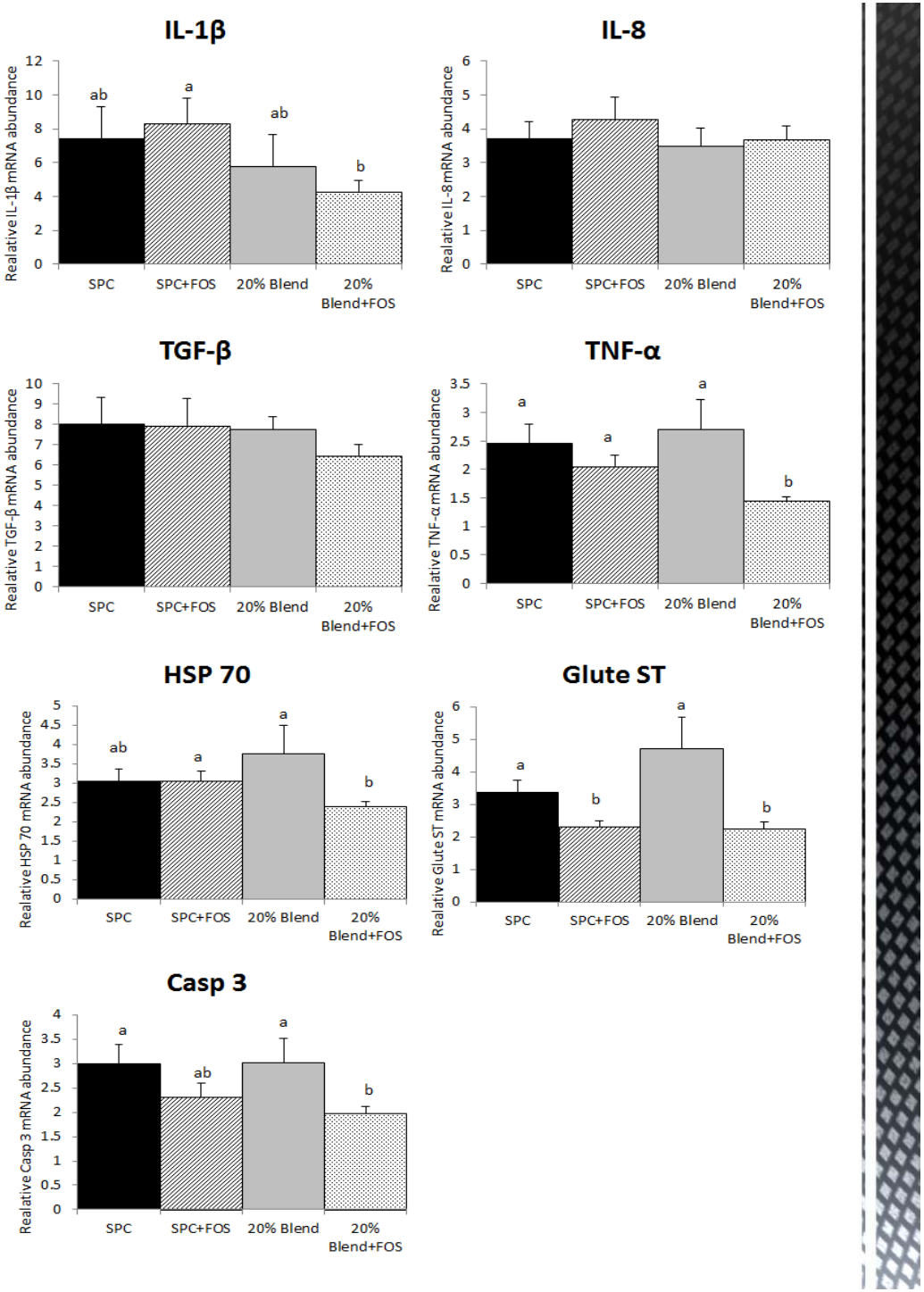
Phylogenetic composition of digesta samples per treatment.

- No dietary effect of wheat gluten or scFOS on Phyla composition
  - *Enterococcus*
    - 20% blend + FOS > 20% blend
  - Family Enterococcaceae
    - 20% blend > 20% blend + FOS
  - Class Bacilli
    - 20% blend > SPC treatments
    - 20% blend + FOS > 20% blend
  - *Kocuria*
    - SPC + FOS > SPC

Ecological diversity parameters unaffected.

Intestinal response

- Pro-inflammatory cytokines
  - TNF-α, IL-8, IL 1β
- Anti-inflammatory cytokines
  –TGF-β
- Intestinal stress and apoptosis
  – HSP 70, Caspase 3
- Oxidative stress
  – Glutathione S-transferase (Glute ST)



Summary – wheat gluten

- Wheat gluten has promising characteristics as an alternative plant protein source:
  - Growth performance with ≥20% inclusions
  - Intestinal microflora
    - ***Bacillus*** spp. promoted in hydrolysed wheat gluten treatment
    - ***Weissella*** spp. Promoted in 10% vital wheat gluten treatment
    - ***Enterococcus*** spp. dominated 20% vital treatment

- Inferences from gene expression
  - Absence of inflammation
  - Reduced intestinal stress with wheat gluten inclusions
- Possible elevated immunological capacity (light microscopy)

Summary – scFOS

- Promising applications for juvenile rainbow trout:
  - Growth performance under sub optimal conditions
  - Intestinal microbiota
    - ***Enterococcus*** enhanced with supplementation of scFOS in wheat gluten diets
    - **Class Bacilli** enhanced with supplementation of scFOS in wheat gluten diets
    - ***Kocuria*** enhanced with supplementation of scFOS in soy protein based diets

- Positive observations with gene expression analysis of posterior intestine:
  - **Down** regulation of **pro-inflammatory cytokines** in 20% blended wheat gluten + FOS treatment
  - **Down** regulation of **intestinal stress** and **apoptosis biomarkers** in 20% blended wheat gluten + FOS
  - **Down** regulation of **antioxidant biomarker** with the addition of scFOS in both wheat gluten and soy protein diets.

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Thank you for your time,

Any questions?

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