Supplementary Material

# Supplementary Methods

## Zootechnical parameters

Zootechnical performance parameters were calculated as follows: weight gain (WG) as FBW – IBW, g, daily weight gain (DWG) as WG × day-1, g; specific growth rate (SGR) as {100 × (ln (FBW/IBW)]} × d-1 (% BW d-1), and feed conversion ratio (FCR) as feed intake × biomass gain-1 as fed basis.

FBW = final body weight;

IBW = initial body weight

## Sampling

### Whole fish samples

At the beginning and at the end of the trial, whole fish were sampled and frozen at -80ºC to analyze whole body retention. One pool of 10 fish was sampled for initial analyses, whereas at the end of the trial 3 pools (replicates) of 5 fish per tank were collected.

### Plasma samples

Plasma samples for enzymatic and oxidative stress analysis (super oxide dismutase (SOD), catalase (CAT), total antioxidant power (PAOT) and isoprostanes) was taken at the end of the trial from 5 fish per tank (15 fish per treatment).

Prior to the sampling fish were individually anaesthetized using 80 mg MS222·L-1. Four mL of blood were sampled using a lithium-heparin 4.5 mL syringe and kept in ice until further processing. Blood samples were centrifuged (10 min, 2000g, 4ºC) and 1.5 mL plasma aliquots were frozen (-20ºC) until analysis. Samples were analyzed individually and tank was considered as a replicate for statistical purposes (n=3).

### Samples for Apparent digestibility coefficient (ADC)

Three different faeces collection were done on the 2nd, 9th, and 16th of November 2021 to determine macro ingredient, mineral and amino acid digestibility. Animals were slightly anaesthetized (80 mg MS222·L-1) and fecal material from all fish in each tank was collected by manually stripping faeces from the distal portion of the intestine by applying pressure to the abdominal cavity using three passes for each fish. Samples from the three collection days were pooled and one sample per tank was lyophilized prior to storage and analysis.

### Tissue samples

Liver, intestine, and white muscle samples were taken after the blood sampling (5 fish per tank; 15 replicates per treatment). Fish were eviscerated for the collection of the liver, posterior intestine tissue and posterior intestine content. The head kidney was sampled and directly analysed in the lab for oxidative burst and phagocytosis on one fish per tank, on two consecutive days.

### Analysis and calculations

*Proximate and amino acid analysis.*

The analyses of the nutrient content in the feed, digesta and excreta samples were performed according to standard methods (VDLUFA 1976; AOAC, 2006). The crude protein was determined by a nitrogen analyser (FP 528, LECO, St. Joseph, USA) using the Dumas method (CP=N\*6.25). Gross energy measurements were performed using an adiabatic bomb calorimeter (C 2000 basic, IKA, Staufen, Germany). The content of amino acids was determined according to the method 982.30 E (a, b) (AOAC, 2006). The lipid analyses of feed were performed at the external company Larebron by hot acidic etching with hydrochloric acid, followed by continuous extraction with petroleum ether and determination with a gravimetric method.

*Mineral analysis*

Yttrium oxide, Calcium, Phosphorus and Zinc concentrations in feed, excreta, whole fish, and water samples were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, 5100 Dual View, Agilent) with an internal method based on DIN EN ISO 11885:2007 (AOAC, 2006) after sulfuric acid mineralization.

*Calculations*

The concentration of the marker in feed and excreta together with the content of the nutrients in the feed and excreta were used to calculate the Apparent Digestibility Coefficient (ADC) of nutrient according to the following equation:

ADC (%) = 100 - [(CMf/CMe) x (CNe/CNf)] x 100

CMf =concentration of marker in feed; CMe =concentration of marker in faeces;

CNf = concentration of nutrient in feed; CNe =concentration of nutrient in faeces

The following equation (Chen et al., 2018), was used to calculate the retention:

Nutrient Retention (%) = 100 x [(FBW x Nutrientf) - (IBW x Nutrienti)] / (FI x Nutrientd)

FBW = final body weight; Nutrientf = nutrient in the final biomass

IBW = initial body weight; Nutrienti = nutrient in the initial biomass

FI = feed intake; Nutrientd = nutrient in the diet

*Tissue preparation*

Head kidney suspensions were obtained by gently forcing the tissue through cell strainer (40 μm). The cell suspensions were placed on discontinuous Percoll gradient (34%-50%). After centrifugation, leucocytes were recovered on the two interphases. After washes, the cells were counted using flow cytometer and adjusted to 1.106 leukocytes/ml for oxidative burst analysis and 1.107 leukocytes/ml for phagocytosis analysis in L-15 complete medium (15% Fetal Calf Serum, 1% penicillin/streptomycin and 1% L-Glutamine).

*Oxidative burst measurement*

Leukocytes were seeded in white 96-well plates (1.105 leukocytes/well) at 22°C in complete L-15 medium. The next day, floating cells were removed, and adherent cells (only phagocytes) were treated. Oxidative burst response was induced by the addition of PMA (phorbol myristate acetate) at 2 μg/ml. Luminol is used as substrate to determine the total production of ROS. This reaction results in a light emission which is quantified with luminometer. Total oxidative burst response was analyzed over time (45 min) as area under curve of luminescence. Then, the area under the curve was normalized with the percentage of phagocytes in blood.

*Statistical analysis*

Each tank was treated as one experimental unit, n= 3. All percentage data will were arcsine transformed. Performance, retention and digestibility data were analyzed by two way ANOVA (Factors: protein source and inclusion followed by posthoc multiple comparison tests (Tukey HDS). The accepted significance level was p <0.05. Plasma parameters, were analyzed by one-way ANOVA followed by the Newman-Keuls multiple comparison test. The effects of treatment were considered significant at p <0.05, 1 tank = n of 1. Statistical analysis were performed using JMP 15.1.0 software.

*Environmental conditions during the trial*

# Levels of waterborne ammonia (total ammonia nitrogen-TAN), pH, nitrites and nitrates were maintained within the recommended limits for rainbow trout (TAN:<1.0 mg/L; pH: 6.5-8.5; nitrites:<0.1 mg/L; nitrates:<1000 mg/L). During the trial period, pH and oxygen were maintained within 8.05±0.14 and 9.22±0.32 mg/L, respectively.”Supplementary Tables

## Supplementary Tables

Supplementary Table 1. Feed diets composition

| **Ingredient** | **Control** | **SCP1 - 5** | **SCP1 - 10** | **SCP1 - 20** | **SCP2 - 5** | **SCP2 - 10** | **SCP2 - 20** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Fish meal1 | 10 | 5 | 0 | 0 | 5 | 0 | 0 |
| Soybean meal | 5 | 5 | 6 | 3 | 5 | 6 | 3 |
| Soy protein concentrate2 | 19 | 20.5 | 21 | 12.5 | 20.5 | 21 | 12.5 |
| Rapeseed meal | 9.0 | 9.5 | 5.9 | 7.6 | 9.5 | 5.9 | 7.6 |
| Wheat | 13.13 | 10.23 | 9.98 | 9.18 | 10.23 | 10.08 | 9.18 |
| Wheat gluten | 13.0 | 13.4 | 14.6 | 16.5 | 13.4 | 14.6 | 16.5 |
| Corn gluten | 7.0 | 6.5 | 7.0 | 7.0 | 6.5 | 7.0 | 7.0 |
| SCP1 | - | 5 | 10 | 19 | - | - | - |
| SCP2 | - | - | - | - | 5 | 10 | 19 |
| MCP3 | 0.65 | 1.15 | 1.7 | 1.9 | 1.15 | 1.7 | 1.9 |
| Soy lecithin | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Choline chloride 70% | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Fish oil | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 |
| Rapeseed oil | 12.8 | 13.0 | 13.0 | 12.7 | 13.1 | 13.0 | 12.7 |
| Vitamin Premix | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| L-Lysine | 0.6 | 0.8 | 0.9 | 1.0 | 0.8 | 0.9 | 1.0 |
| L-Methionine | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Yttrium | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
|  |  |  |  |  |  |  |  |
|  | **Calculated energy and nutrient contents** | | | | | | |
| Crude protein | 39.9 | 39.8 | 39.8 | 39.7 | 39.8 | 39.9 | 39.9 |
| Crude lipid | 24.5 | 24.7 | 24.7 | 24.6 | 24.8 | 24.7 | 24.8 |

1 Norvik LT, 66.9% protein

2 X-SOY600; 60.5% protein

3 Mono Calcium Phopshate

**Supplementary Table 2.** Effect of experimental diet on oxidative burst of the head kidney of rainbow trout. Values show average ± standard deviation.

| **Treatments** | **Oxidative burst**  **(%)** |
| --- | --- |
|
| Control | 0.012 ± 0.012 |
| SCP1-5 | 0.011 ± 0.007 |
| SCP1-10 | 0.020 ± 0.008 |
| SCP1-20 | 0.022 ± 0.013 |
| SCP2-5 | 0.029 ± 0.006 |
| SCP2-10 | 0.020 ± 0.012 |
| SCP2-20 | 0.028 ± 0.013 |
|  |  |
| ANOVA |  |
| *p* value | 0.33 |

Means of two individuals (from the same tank) were determined and significant differences among groups were determined using one-way ANOVA. In this case, each tank was considered a replicate. Statistical analyses were conducted in JMP software (<https://www.jmp.com>).