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## Feed technological and nutritional properties of hydrolyzed wheat gluten when used as a main source of protein in extruded diets for rainbow trout (*Oncorhynchus mykiss*)



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## ABSTRACT

Four diets with hydrolyzed wheat gluten (HWG) replacing graded amounts of crude protein (CP) (0, 12.5, 25, 50%) from LT fishmeal (FM) were extruded. For the diets with 0–25% replacement of FM, durable and sinking pellets were produced by adding 19–20% water in the extruder, at a constant feeding rate. The die pressure, specific mechanical energy (SME) and torque decreased with increasing HWG incorporation during extrusion of the diets with 0-25% replacement. Diametric expansion increased with increasing HWG inclusion. In uncoated pellets, sinking rate in water as well as lipid loss increased with increasing HWG. Diametric expansion increased by lipid coating. In coated pellets, water stability decreased with increasing HWG. The visco-elastic properties of the HWG prevented transport through the extruder with 19–20% water added in the barrel at 50% replacement, and water addition was reduced to 11% in order to obtain stable material flow. This diet had notably reduced die pressure, and increased SME. Diametric expansion and oil retaining capacity were low, while durability was high in uncoated pellets. Diametric expansion and water stability were reduced compared to the pellets produced with 19-20% water, while the pellets sank faster. Each diet was fed to duplicate groups of 0.4 kg rainbow trout for 56 days. The fish nearly doubled their weight, and no significant diet effects were seen on feed intake or growth. All diets resulted in feed conversion ratios (FCR) near 0.8 g dry matter intake per g gain. The apparent digestibilities (AD, %) of nitrogen, total and individual amino acids were high for all diets. AD of protein, cyst(e)in and phenylalanine significantly improved with increasing inclusion of HWG, while AD of isoleucine decreased. In conclusion, HWG had a strong effect on extrusion parameters and physical quality, and had nutritional value similar to LT fish meal when supplemented with essential amino acids. Statement of relevance

The current research will help salmonid feed companies to optimally utilize a novel plant protein feed ingredient with high nutritional value and strong physio-chemical properties. This will both facilitate reduced use of fish meal, and increased use of other feed ingredients requiring increased binding. The current research also shows that HWG results in decreased SME, potentially decreasing production cost of extruded fish meal.

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#### 1. Introduction

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Wheat gluten has technological and nutritional qualities that make it a useful ingredient in diets for salmonids (Apper-Bossard et al., 2013). It has high concentration of protein, with lysine as the first limiting amino acid, followed by tryptophan and arginine. Due to the lenient technology employed in "Vital wheat gluten" production, the protein digestibility is high, and almost complete digestion of gluten protein was found in Atlantic salmon (*Salmo salar*) (Storebakken et al., 2000). Its carbohydrate fraction mainly consists of starch, rather than non-starch polysaccharides and indigestible sugars commonly found in legumes and oilseeds (Bach Knudsen, 1997), and wheat gluten has no profound effect on lipid digestibility in salmon (Storebakken et al., 2000). Wheat gluten has cohesive and visco-elastic properties (Day et al., 2006), and serves as an efficient, digestible binder when used in fish feeds. The feed technological features of wheat gluten are both attributed to monomeric, low weight proteins (gliadins) that contribute to viscosity of the extrudate, and glutenins with high molecular weight that contribute to elasticity and cohesiveness (Apper-Bossard et al., 2013).

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Hydrolyzing plant proteins with endoproteases may alter the physical characteristics of the protein, and add bioactive features such as antioxidant and anti-inflammatory capabilities (McCarthy et al., 2013). Thus, the first aim of this experiment was to find out how replacement of high-quality fish meal with a novel plant protein hydrolysate, hydrolyzed wheat gluten (HWG), affected the extrusion parameters and physical pellet quality of feed for rainbow trout (*Oncorhynchus mykiss*). The 2nd aim was to find out if replacing fish meal with HWG significantly affected feed intake, growth parameters, or utilization of dietary protein by rainbow trout.

#### 2. Materials and methods

An experiment was designed to compare feed technological aspects and the nutritional value of hydrolyzed wheat gluten (HWG) and LT fish meal (FM) as sources of protein in extruded diets for rainbow trout.

#### 2.1. Diets and feed processing

Four diets were produced by extrusion technology at Norwegian University of Life Sciences (NMBU) Centre for Feed Technology. Ingredients were milled in a Münch hammer mill (HM 21.115, Wuppertal, Germany) fitted with a 1-mm screen, and mixed (Dinnissen twin shaft mixer, Pegasus Menger 400 l, Sevenum, Holland). Then the mixture was fed into a double shaft conditioner (no water addition, 62 s retention time, BCTC 10, Bühler, Uzwil, Switzerland) before being extruded in a co-rotating five section twin-screw extruder with four dies of 4 mm in diameter (BCTG 62/20 D, Bühler, Uzwil, Switzerland). The extruded pellets were first pre-dried in a NMBU-FORBERG Fluid bed dryer (Forberg, Oslo, Norway), before finally being dried in small experimental batch dryers (10 kW heater, 2550 m<sup>3</sup> h<sup>-1</sup> fan capacity, product temperature not exceeding 55 °C) to final dry matter (DM) contents of 920–950 g kg<sup>-1</sup>.

The extruder screw configuration was built up by the following elements, from inlet to outlet: 80R80-80R80-60R60-60R60-60R60-60R60-60R60-80R80-100R100-P120-60L20-(90° twist off)-60L20-100R100-80R80-80R80-80R80-60R60-60R60-60R20-40R40. The screw elements are defined as follows: The first figure is the flight length, "R" denotes a forward and "L" denotes a backward conveying element. The last figure is its length (mm). "P120" is a polygon block (kneading element), 120 mm long. In general terms, this screw configuration is normally referred to as a "medium SME configuration". This configuration (Fig. 1) results in a small amount of energy being generated by friction, as are most forward conveying elements, except for the kneading section. The kneading section is followed by reversed elements, which facilitate increased duration of kneading.

The diets were formulated based on crude protein from HWG replacing 0, 12.5, 25 and 50% of crude protein from high-quality LT fish meal. Chemical composition of the HWG and fish meal is given in Table 1. Diet formulation and proximate composition are presented in Table 2. According to the specifications of the producer, the peptide size distribution in the HWG (% of SDS buffer soluble protein) was: F1 > 779.6 kDa, 0.8%; 779.6 kDa > F2 > 96.7 kDa, 3.8%; 96.7 kDa > F3 > 58.1 kDa, 2.2%; 58.1 kDa > F4 > 20.5 kDa, 19.7%; 20.5 kDa > F5 > 6.2 kDa, 62.3%; 6.2 kDa > F6 > 0.47 kDa, 11.2%.



Fig. 1. Configuration of the extruder screw elements.

#### Table 1

Composition of hydrolyzed wheat gluten and fishmeal.

Ingredient	Hydrolyzed wheat gluten (HWG) <sup>a</sup>	Fishmeal <sup>b</sup>
Composition, kg <sup>-1</sup>		
Dry matter (DM), g	944	911
Crude protein, g	893	749
Crude fat, g	45	99
Starch, g	74	-
Ash, g	9	157
Essential amino acids (EAA) <sup>c</sup> , g 16 g <sup>-1</sup>	N	
Arg	3.26	5.27
His	1.74	1.87
Ile	3.37	3.69
Leu	6.49	6.26
Lys	1.57	6.92
Met	1.92	2.42
Phe	5.69	3.37
Thr	2.54	3.65
Trp	0.76	0.73
Val	3.71	4.03
Total EAA	31.0	38.2
Total NEAA	66.4	40.2
Total AA	97.4	78.4
Minerals, g kg $^{-1}$		
Phosphorus, P	1.0	23
Calcium, Ca	1.0	26

<sup>a</sup> Solpro 508, hydrolyzed wheat gluten, Syral Belgium N.V., Aalst, Belgium.

<sup>b</sup> Norse LT-94®, low-temperature dried fish meal, Norsildmel, Bergen, Norway.

<sup>c</sup> Presented in dehydrated form.

The diets with HWG were supplemented with the first four limiting essential amino acids to the level found in fish meal, taurine (in excess of the optimum level found by Gaylord et al. (2007)), and mono calcium phosphate. The extrusion parameters were varied in order to produce diets that would sink in freshwater (minimum 460 g dm<sup>-3</sup> in uncoated pellets with approximately 300 g water dm<sup>-3</sup>). The highly visco-elastic properties of the HWG prevented transport through the extruder when 20% water was added in the extruder and the rate of CP replacement

#### Table 2

Feed formulation and analyzed proximate composition.

Crude protein from HWG, %	0	12.5	25	50
Ingredients (g DM) per kg diet DM				
Hydrolyzed wheat gluten <sup>a</sup>	0.0	67.2	134.6	269.4
Fish meal <sup>a</sup>	641.1	550.7	462.3	285.3
Wheat starch <sup>b</sup>	141.0	134.6	133.5	129.2
Fish oil <sup>c</sup>	201.0	206.0	212.0	222.0
Mono calcium phosphate	0.0	6.4	16.0	34.2
Lysine <sup>d</sup>	0.0	4.1	7.7	15.0
Methionine <sup>e</sup>	0.0	0.6	1.1	1.9
Threonine <sup>f</sup>	0.0	1.1	1.9	3.7
Arginine <sup>g</sup>	0.0	1.8	3.4	6.4
Taurine <sup>h</sup>	0.0	5.3	5.3	5.3
Limestone <sup>i</sup>	10.7	16.0	16.0	21.4
Constant ingredients <sup>j</sup>	6.2	6.2	6.2	6.2

<sup>a</sup> See Table 1.

<sup>b</sup> Meritena 200, wheat starch (food grade), Syral Belgium N. V., Aalst, Belgium.

<sup>c</sup> Silfas, Karmsund, Norway.

<sup>d</sup> L-Lysine-HCl, 99% feed grade, CJ Indonesia, Jakarta, Indonesia.

<sup>e</sup> Rhodimet® NP 99. DL methionine, 99% feed grade, Adisseo Brasil Nutricao Animal Ltda.. Sao Paulo. Brazil.

<sup>f</sup> L-Threonine, 98.5% feed grade, Ajinomoto Eurolysine S.A.S., Paris, France.

<sup>g</sup> SAFC L-arginine 98.5% pharma grade, Sigma-Aldrich, Steinheim, Germany.

<sup>h</sup> Taurine-JP8, Qianjiang Yongan Pharmaceutical Co., Ltd., Hubei, China.

<sup>i</sup> Ground limestone.

 $^{j}$  Vitamin and micromineral premix, 4.4 g (kg diet)<sup>-1</sup> (supplies per kg diet: vitamin A: 2000 IU, vitamin D<sub>3</sub>: 1200 IU; vitamin E: 160 mg; vitamin K<sub>3</sub>: 8 mg; vitamin B<sub>1</sub>: 12 mg; vitamin B<sub>2</sub>: 20 mg; vitamin B<sub>3</sub>: 60 mg; vitamin B<sub>5</sub>:24 mg; vitamin B<sub>6</sub>: 12 mg; vitamin B<sub>9</sub>: 4 mg; vitamin B<sub>1</sub>: 0.016 mg; vitamin C polyphosphate: 150 mg; biotin: 0.2 mg; Ca: 876 mg; Cu: 4 mg; Co: 0.8 mg; I: 2.4 mg; Mn:12 mg; Ca: 96 mg); 0.2 g inert marker (Y<sub>2</sub>O<sub>3</sub>), Metal Rare Earth, Ltd., Shenzen, China; 1.6 g choline chloride.

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