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Evaluation of apparent nutrient digestibility in Atlantic salmon fed diets with differently processed faba bean

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Abstract

This study was conducted to evaluate the apparent digestibility coefficients (ADC) of three different diets with faba bean in Atlantic salmon (*Salmo salar*). A basal diet was prepared with fish meal, soy protein concentrate and vital wheat gluten as the primary source of proteins. Yttrium Oxide (Y_2O_3) was included in basal diet at 0.01% dry matter basis as inert marker for nutrient digestibility assessments. A control diet was prepared by adding 25% whole untreated, milled faba bean to basal diet. In diet 2 the same amount of faba bean has been pre-incubated with 0.01 % NSPase (Econase xt 25 L) and 0.03% phytase (Quatntum blue 5 L) for 30 minutes at 37°C and added to dry basal diet mix before extrusion, without prior drying. In diet 3 the same amount of faba bean was sprouted for 5 days and dried over night at 60°C, milled and added to dry basal diet before extrusion.

Each diet was provided to salmon in three tanks (3 diets × 3 tanks, 9 tanks in total), each tank containing 10 fish with initial weight of 374.1± 2.8 g. Water temperature and dissolved oxygen concentration of the outlet water was maintained at 14.3 ± 0.4 ºC and 83.5 % of saturation, respectively.

Apparent digestibility coefficient of Kjeldahl and Dumas nitrogen, starch, lipid, carbon, hydrogen, energy, sulphur, magnesium, phosphorous and zinc was evaluated in three diets in the faeces samples collected from a wedge wire screen. In addition, digestibility of Dumas N, carbon, sulphur, magnesium, and zinc in faeces samples collected by stripping methods were also estimated and compared with those of collected from wedge wire screen. ADCs of N from both Kjeldahl and Dumas in faeces samples collected from wedge wire screen were high in all three diets. Enzyme treated and sprouted faba bean diet showed significantly higher N digestibility compared to the untreated faba bean probably due to the effects of enzyme treatment and germination on reducing the amount of antinutrients which bond with proteins or proteases and restrict their utilization and hydrolyzation in fish. N digestibility results from Dumas and Kjeldahl methods in faeces from wedge wire screen was very similar to each other. It could be concluded that both methods are precise for N digestibility evaluation.

ADCs of lipid, carbon, phosphorous and energy in faeces samples collected from wedge wire screen significantly improved in germinated and enzyme treated faba bean diet compared to untreated faba bean diet probably due to the reduction of antinutrients as well as structural polysaccharides presented in faba bean.

ADC of Zn, Mg, S, and H did not show significant improvement in treated diets compared to untreated diet in faeces samples collected from wedge wire screen. Nevertheless, as they showed a high and variable standard error mean, it could be concluded that these insignificancies between diets could be preferably related to the error in sampling and analytical errors and not lacking of response to the treatment. Therefore, it could be concluded that enzyme treatment and germination were effective method to improve nutrient digestibility in diets containing 25% faba bean.

ADCs of Dumas nitrogen, carbon, sulphur, magnesium and zinc did not show significant difference between three diets in stripped faeces samples.

ADSs of Dumas N, carbon, sulphur, magnesium, and zinc from wedge wire screen was significantly higher compared to the samples collected from stripping method. It could probably be related to the digestibility overestimation by wedge wire screen method due to the leaching of nutrient to the water or underestimation of stripping method due to the contamination of faeces with digesta.

Keywords: Atlantic Salmon *(Salmo Salar)*, Faba bean, Enzyme treatment, Germination Digestibility, Apparent digestibility coefficient, Faecal collection, Stripping, Wedge wire screen

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Abbreviations

Apparent digestibility coefficient (ADC)

Antinutritional factor (ANF)

Calcium (Ca)

Dry matter (DM)

Standard error mean (SEM)

Magnesium (Mg)

Nitrogen (N)

Sulphur (S)

Yttrium Oxide (Y_2O_3)

Hydrogen (H)

Zinc (Zn)

phosphorous (P)

Non-starch polysaccharide (NSP)

Iron (Fe)

Copper (Cu)

one-way analysis of variance (ANOVA)

1. Introduction

1.1 Faba bean (Vicia faba L.)

Faba bean, (*Vicia faba L.*) also known as the broad bean, horse bean or field bean, belongs to the family Leguminosae (Fabaceae) (Adsule et al., 1996). Faba bean have been cultivated and consumed as human food in North Africa and Near East for more than 10,000 years (JA, 1981). It is also widely used as animal feed for piglet, ruminants, and poultry worldwide (Crépon et al., 2010). Faba bean is a legume with high amount of protein, carbohydrate, B-vitamins and minerals. However, there is a large genetic variability for protein, starch and fibers content of faba bean (Duc et al., 2011).

Faba bean protein content ranges from 20% to 41%, depending on the variety (Kadam et al., 1989). Albumins (20%) and globulins (80%) are two main protein fractions (Revilla, 2015). Globulins consist of legumin and vicilin which are high molecular weight proteins. Legumin and vicilin have similar amino acid composition. They are rich in glutamic and aspartic acids whereas the concentrations of sulfur containing amino acids cysteine and methionine and also tryptophan residues are low (Multari et al., 2015).

Faba bean seeds contain 51% to 68% of carbohydrate in total. The major proportion of carbohydrates is composed of starch (41–53%), soluble sugar, and dietary fiber (Revilla, 2015).

The main soluble sugars in faba bean are raffinose family oligosaccharides raffinose, stachyose, and verbascose. Sucrose is the predominant constitute of low molecular weight carbohydrates in mature faba bean while the monosaccharides fructose and glucose are generally negligible in mature faba bean seed (Landry et al., 2016). The content of dietary fiber shows diversity in faba bean which seems to depend on the seed variety and seed size (Giczewska et al., 2003; Vidal-Valverde et al., 1998). Different authors reported a crude fiber content from 5% to 30%, with hemicellulose as the major fiber component (Vidal-Valverde et al., 1998).

Faba bean has low fat content (Crépon et al., 2010). Saturated fatty acids compromise, on average 21.6% to 23.4% of total fatty acids in faba bean. Unsaturated fatty acids, especially linoleic acid, constitute a relatively high proportion of fatty acids in faba bean (18–18.4% of monounsaturated

fatty acids and 31.9–35.1% of polyunsaturated fatty acids) (Grela et al., 1995). Thiamine, riboflavin, and niacin are the most significant water-soluble vitamins in faba bean and their contents range from 0.253–0.640, 0.123–0.190, and 1.52– 2.00 mg/g DM, respectively (Prodanov et al., 2004; Revilla, 2015). Regarding liposoluble vitamins, faba bean contains intermediate amount of α-tocopherol (15–17 mg/kg) (Grela et al., 1995). Faba bean is a good source of dietary minerals. It is relatively high in iron, copper, zinc, phosphorus, magnesium, potassium, and sodium and relatively low in manganese and calcium (Revilla, 2015). However, more than 40-60% of phosphorus is unavailable as phytates (Vidal-Valverde et al., 1998). Table 1.1 shows the composition of raw faba bean.

*Table 1. 1*Chemical composition of faba bean in dry matter basis

Compound	(Duc et al., 1999)		(Khalil et al., 1995)
	T^+	T^-	
Crude protein (g/kg)	310	319	292
Starch (g/kg)	412	427	441
Crude fiber (g/kg)	99	88	50
Reducing sugar (g/kg)	38	44	72
Fat (g/kg)	19	20	11
Ash (g/kg)			42
Na (mg/100g)			297
Ca (mg/100g)			220
K (mg/100g)			748
Cu (mg/100g)			2.5
Zn (mg/100g)			11.7
Fe (mg/100g)			6.6
Mg (mg/100g)			281
Mn (mg/100g)			2.3
Tannins(gr/kg)	6.6	0.1	14.5
Vicine+Convicine (g/kg)	8.3	7.6	8.5
Phytic acid (g/kg)	2.9	2.9	3.90
Raffinose+Stachios(gr/kg)			18.1
Trypsin inhibitor activity (TIU/mg)			2.8

Table is adapted from (Duc et al., 1999), means of four high tannin lines(T^+) and four low tannin lines (T^-), and (Khalil et al., 1995) , means of triplicate measurement of one variety

1.2 Antinutritional factors in faba bean

Antinutritional factors (ANFs) are biological substances present in plant generated by the normal metabolism of species and by different metabolic pathways. Antinutritional factors play a role in protection of plant against attack by herbivores, insects and pathogens or as a means to survive in unfavorable growing condition (Khokhar et al., 2003). They reduce the bioavailability of nutrients or food intake or have adverse effects on animal health and productivity.

Protease inhibitors, lectins, condensed tannin, phytic acid, vicine and convicine, are the most important ANFs in faba bean (J. Helsper et al., 1991).

The seed albumin fraction includes two antinutritional factors, trypsin inhibitors and lectins.

Trypsin inhibitors binds to the digestive enzyme, trypsin, making it partially or fully inactive (Hajra et al., 2013). Kunitz and Bowman-Brike are the two most important trypsin inhibitors. Some authors reported chymotrypsin and α-amylase activity in faba bean. Shi et al. reported a low trypsin inhibitor content (5.96–6.10 TIU/mg) in faba bean seeds (Shi et al., 2017). They also reported the low chymotrypsin activity in faba bean (1.12–1.67 CIU/mg). Alonso et al. founded the trypsin, chymotrypsin and α-amylase content of Spanish *Vicia faba* L. (var. Equina) 4.47±0.21, 3.56±0.16 and 18.9±1.84 IU/mg DM, respectively (Alonso et al., 2000).

Lectins or Haemaglutinins are proteins which bind reversibly to membrane carbohydrate moieties of complex glyco-conjugates of the cells (Francis et al., 2001). They are resistant to proteolysis and could bind to the glycosyl groups of the membrane cells of digestive tract. As the result of this interaction they can disrupt gut epithelial cells and affect small intestinal metabolism, damage to the villi, and interfere with nutrient digestion and absorption (Vasconcelos et al., 2004).

Makkar et al. found very low lectin activity in twelve faba bean cultivars *(Vicia faba L.)* and suggested that the lectin activity of these cultivars would not be of any physiological significant (Makkar et al., 1997).

Faba bean is a source of condensed tannin which are antinutritional component considered to reduce faba bean protein digestibility (Adsule et al., 1996). White flowered faba bean has lower condensed tannin compared to colored flower faba beans (brown and yellow) (Martin et al., 1991).

Hence, tannin free faba beans can be produce by selection using the recessive white flower genes (J. P. Helsper et al., 1993). Duce et al., reported 6.6 and 0.1 g/kg tannins in dry matter in high tannin and low tannin faba bean seed, respectively (Duc et al., 1999). Tannins are water-soluble phenolic biomolecules with molecular weights between 500 and 3,000. They are widely distributed in plants and are generally divided in tow classes, the hydrolysable and the non-hydrolysable or condensed tannins (Bate-Smith, 1954). The hydrolysable tannins do not have any antinutritional effect in animal feed as they easily eliminate by gastric juice (Martin et al., 1991). Condensed tannin could interfere with the digestive processes and decrease digestibility either by binding to the enzymes or by binding to nutrients such as proteins or minerals (Liener, 1989). They also may reduce the absorption of vitamin B_{12} and reduce feed palatability due to their astringent, bitter flavor (Hemre et al., 2018). Tannins also could interact with other antinutrients and feed components (Francis et al., 2001). For instance, tannins and lectins interaction could eliminate the inhibitory action of tannins on amylase (Fish et al., 1991), and tannins and cyanogenic glycosides interaction will reduced the detrimental effects of the cyanogenic glycosides (Goldstein et al., 1985). Nevertheless, tannin compound also have some health beneficial effects such as antioxidant activity, immune system stimulation (Chung et al., 1998) and antimicrobial activity on fish pathogens (Schrader, 2008).

There is little investigation on biological effects of tannin on fish, but these investigations suggest that fish are sensitive to tannins (Hemre et al., 2018). A study showed that common carp could tolerate 20 g/kg condensed tannin powder without any effect on feed intake and growth (Becker et al., 1999). Faba bean meal with high level of condensed tannin had lower protein digestibility than soybean meal in carp and tilapia under simulated condition and the reduced protein digestibility was more pronounced in carp than the rainbow trout (Grabner et al., 1985). These differences probably show the differences in tolerances of different fish species towards tannin in the diet (Francis et al., 2001). However, since faba bean is dehulled in feed processing, we may assume that condensed tannins are not problematic in fish feed.

Vicine and convicine are glucopyranosides accumulated in the cotyledons of faba bean seeds (Griffiths et al., 1996). They are antinutritional compound which are likely to contribute in plant defense mechanisms against pathogens. Wang et al, reported 6.68 ± 1.12 mg vicine and 2.33±0.59 mg convicine per gram dry bean in twenty one varieties of faba beans in Germany (Wang et al.,

1990). Faba bean lines with reduced amount of vicine and convicine can be produced by selection methods (Duc et al., 1989). Several studies have been demonstrated the detrimental effects of vicine and convicine in monogastric animals (Crépon et al., 2010). They have been shown to decrease feed consumption and weight gain in chickens (Khamassi et al., 2013). However, There is no report on negative effects of vicine and convicine on fish (Christian De Santis et al., 2016).

More than 40–60% of total phosphorus content in faba bean is presented as phytic acid or its salt form phytate (Vidal-Valverde et al., 1998). Phytic acid content of faba bean significantly vary according to genotype, location of the plant and selection methods (Griffiths et al., 1981). Oomah et al, reported phytic acid content of thirteen low-tannin Canadian faba bean genotypes ranged from 5.9 to 15.1 g/kg, varied significantly among genotypes and between locations (Oomah et al., 2011).

Phytic acid and phytate have been considered as antinutrients due to their ability to complex with minerals such as Zn, Fe, Ca, and Mg and reducing the amount of phosphorus and associated metal ion available for absorption in the intestinal tract of animal. Moreover, they possibly form phytate-protein and phytate-mineral-protein complexes, and hence reduce the availability of proteins. phytic acid has been shown to reduce protein digestibility, growth and mineral retention in Atlantic salmon (Hemre et al., 2018). Decreased growth rates in rainbow trout fed a diet containing 5 g/ kg synthetic phytic acid was reported by (Spinelli et al., 1983). However, there is controversial results about the effects of phytic acid on protein. In a dose response study, no clear relationship was found between different doses of phytic acid and the digestibility of main nutrients (Denstadli et al., 2006).

In addition, in aquaculture, the excretion of the phytate phosphorus in freshwater system is important, since the excess phosphorus can increase the growth of phytoplankton and algae (Hajra et al., 2013). It is reported that salmonid could tolerate 5 to 6 g/kg of dietary phytate in the feed (Francis et al., 2001).

Faba bean soluble sugars, including raffinose, stachyose and verbascose consider as antinutritional components in fish feed. Faba bean also contains non-starch polysaccharides (NSPs) such as cellulose, pectin, rhamnose, arabinose, xylose, mannose, galactose, and uronic acids. NSPs are soluble and insoluble polysaccharides composed predominantly of linked monomers of hexoses and pentoses, present in primary or secondary plant cell wall (Selvendran,

1984). NSPs cover a large variety of polysaccharides. cellulose, non-cellulosic polymers and pectic polysaccharides are three main group of NSPs. Non-cellulosic polymers compromise Arabinoxylans, mixed-linked β-glucans, mannans, and xyloglucan, while pectic polysaccharides compromise polygalacturonic acids substituted with arabinan, galactan and arabinogalactan (Sinha et al., 2011). 19.0 % of dry matter (DM) total NSP, containing 8.1% of DM cellulose, 3.2% of DM Uronic acids, 2.4% of DM Arabinose, 1.2 % of DM Xylose, 0.2 % of DM Mannose, and 0.6% Galactose is reported in faba bean (Knudsen, 2014). Soluble NSPs constitute 24% of total NSPs. Vidal et al, found 0.28 %, 1.10 % and 2.29 % of DM, Raffinose, stachyose and Verbascose, respectively, in faba bean (Vidal-Valverde et al., 1998). The antinutritional effects of NSPs have been investigated in different animal species, however there is few studies on fish. NSPase enzymes such as β- glucanase and β- xylanases absent in different fish species or have very low activity (Kuz'Mina, 1996). It could negatively affect animal performance, as the NSPs remain intact and undigested (Sinha et al., 2011). However, diets containing NSPs was digested by Nile tilapia (Haidar et al., 2016).

Leenhouwers et al, found that soluble NSPs reduced the digesta viscosity in African catfish gut (J. I. Leenhouwers et al., 2007), while Kraugerud et al, did not find any effect of diet containing NSPs on gut viscosity in Atlantic salmon (Kraugerud et al., 2007).

Reduction in digesta viscosity could negatively affect the metabolism of glucose, lipid, amino acids and mineral, due to the partial distribution of digestive enzymes in a viscous solution (J. Leenhouwers et al., 2006). Moreover, reduction of growth performance, reduced nutrient digestibility, and reduced body weight is reported in different fish species including Atlantic salmon, fed by feeds containing various level of NSPs (Sinha et al., 2011).

The insoluble NSPs such as cellulose and hemicellulose provide physical bulk in feed and have been used as binders in fish feed. They are indigestible by fish, as the enzymes for digestion of insoluble NSPs are very low or scarce in fish. They also increase the passage rate and there is some argues that this may decrees nutrient digestibility (Choct, 1997).

1.3 Effect of different processing method on nutritional and antinutritional factor of faba bean (Vicia faba L.)

Different processing methods are used to improve the nutritional quality and effective utilization of legume in animal feed. These methods could improve the protein and starch digestibility in legume and hence, improve the utilization of protein and starch by animal (Alonso et al., 2000). In addition, processing could reduce the antinutritional factors in seeds and beans (Khokhar et al., 2003).Wide range of processing techniques such as dehulling, soaking, cooking, extrusion cooking, autoclaving, enzymatic treatment, fermentation, and germination are used to improve the biological value of legume and in particular faba beans. As enzymatic treatment, and germination are used in this project, they are discussed following.

1.3.1 Using of enzymes in aquafeed

Enzymes are used in livestock feed for decades to inactive or reduce the antinutrients and improve the digestion of nutrients and animal performance. Use of enzymes in aquaculture has been relatively low in the past perhaps due to the using of fish meal as the major source of protein which is highly digestible and lacks the antinutritional factors (Rana et al., 2009). However, increasing the utilization of plant products in fish feed as partial or complete replacement of fish meal, increases the need for application of enzymes in aquafeed. As discussed in previous chapter, faba bean anti-nutritional factors may limit its nutrient availability and have impaired effects on salmon. In Salmonid diet a great concern is non-starch polysaccharides and phytic acid. Since phytase and non-starch polysaccharide hydrolyzing enzymes (NSPas) are used in this study to inactive or reduce the effects of phytate and NSPs, these two enzymes and their effects are investigated here.

- **Phytase**

As has been mentioned earlier, phytic acid is the primary phosphorous (P) storage in plant seeds. Monogastric animals, including carnivorous fish lack the enzyme phytase for releasing P from phytic acid. It reduces the availability of P for animal. Moreover, phytic acid form chelate with other minerals such as Zinc (Zn), magnesium (Mg), and calcium (Ca) and complex with protein and amino acids, thus reduces the availability of these minerals and digestibility of proteins (Encarnação, 2016). In addition, undigested P will be loaded to the water and result in phosphorous pollution. Phytic acid is a heat stable antinutrient and phytase is added to feed to hydrolyze phytate. Phytase chemically known as myoinositol (1,2,3,4,5,6)- hexaphosphate phosphohydrolase, is increasingly used as supplement in fish feed during the last decades to enhance the bioavailability of dietary P and other macro and micro minerals, nutrient utilization and digestion, growth performance and reduce the P pollution in aquatic environment (V Kumar et al., 2012).

Some researchers investigated the impact of phytase application in fish feed. The positive effects of phytase on total P availability and digestibility for fish is investigated in many studies (Cao et al., 2007). P is an essential element in growth, reproduction and skeletal development of fish (Hardy et al., 1985; Åsgård et al., 1997). Phosphate uptake from water is negligible in fish, so fish must obtain P from the feed. Meanwhile, P exertion to the water is a critical source of pollutant in aquatic environment. The inclusion of phytase in fish diet reduce the phosphorous concentration in effluent by making it available for fish and hence reducing the amount of faecal P. Rainbow trout fed phytase treated soybean meal diet released lower P to the water compared to the fish fed untreated soybean meal (Cain et al., 1995).

Some authors also reported increasing the bioavailability of other minerals by phytase supplementation in fish feed. Supplementation of the soybean meal diet with phytase significantly increased the apparent absorption of Ca, Mg, Cu, Fe, Sr and Zn (Sugiura et al., 2001).

There are inconsistent results regarding the impact of phytase on fish growth. Some authors reported the positive impact of phytase on fish growth in Rainbow trout (Vielma et al., 2002), African catfish (Weerd, 1999), and Striped bass (Papatryphon et al., 1999). In contrast, some

studies observed no improvement in feed intake and fish growth by phytase supplementation in fish feed. Atlantic salmon fed canola meal based diet with or without phytase have not showed any significant difference in weight gain (Sajjadi et al., 2004).

The impact of phytase on protein availability and digestibility is a matter of controversy. Theoretically, phytase could improve protein digestibility by hydrolyzing the phytate protein complexes. Improved protein digestibility was reported in Atlantic salmon fed with phytase treated soy concentrate diet (Storebakken et al., 1998). Phytase supplementation in soybean meal resulted to increased apparent digestibility of protein in rainbow trout (Sugiura et al., 2001). In contrast no significant difference in apparent protein digestibility was found in tilapia fed soybean meal diets with or without phytase treatment (Riche et al., 2001).

Phytase activity was detected in rice decades ago (Suzuki et al., 1907). Natuphos, the first commercial phytase was made from *Aspergillus Niger* in 1991 (V Kumar et al., 2012).

Phytase could be isolated from microorganisms, plants and animal tissues. Phytase can be categorized into acid and alkaline phytase, depending on their optimal pH. Acid phytase have the optimal activity at pH about 5.0, while the alkaline phytase optimal activity is at pH near to 8.0 (Baruah et al., 2007). Feed phytase could also be divided into 3-phytase, 5-phytase and 6-phytase according to the site where the hydrolysis of phytate molecule is initiated (Rostami et al., 2013). Animal phytase activity is very low compared to plant and microorganisms phytases. Plant phytases are sensible to heat and have narrower pH spectrum activity compare to the microbial phytase(Cao et al., 2007). Microbial phytase is favorable as feed supplement, due to the high temperature tolerance which allow it to survive the feed pelleting process, broad optimal pH, and higher specific activity (Cao et al., 2007). Microbial phytase could be isolated from fungi, yeast, bacteria, and protozoa (Lei et al., 2007). A wide spectrum of microbial phytase have been characterized and commercialized as fish feed additives. The most commonly used are produced from fungi (*A. niger*) and bacteria *(E. coli*) (V Kumar et al., 2012).

Numerous factors impact microbial phytase activity. Regarding pH, phytase usually shows the high activity in two wave crest of pH: the highest activity around pH 5.0 to 5.5, and the second highest around 2.5 (Cao et al., 2007). Phytase activity is also various in different fish spices. It shows poor efficiency in agastric fish because of high digestive system pH, while in gastric fish

with lower pH in digestive system phytase shows much better activity (Cao et al., 2007). Using neutral phytase or pre-treatment of feed with phytase could solve this problem in agastric fishes.

Phytase is heat and pressure sensible and temperatures above 70°C could inactive phytase partially or totally. As the fish feed processing usually needs the temperature higher than 85°C, pre-treatment of feed with phytase and liquid phytase spraying onto the feed pellet after feed processing could be good choices to avoid phytase denaturation by heat. Using thermostable phytase could be another alternative choice to combat heat deactivation of enzyme.

Different feed additives such as citric acid and vitamin D are shown to complement the efficacy of phytase in fish feed specially for agastric fish (V Kumar et al., 2012).

Angel et al. reported that high concentration of calcium or high Ca:P ratio in poultry feed reduce the effectiveness of phytase activity(Angel et al., 2002).

Phytase may be added to the fish feed as powder, granulate or liquid (Cao et al., 2007). Raw materials could be pretreated by enzyme or enzyme could be added post pelleting by liquid spray coating to prevent enzyme inactivation at high extrusion temperature. Using thermostable phytase could be of benefit to prevent degradation of enzyme during extrusion process. Phytases of Schwanniomyces castellii, Arxula adeninivorans and various Pichia spp. reported thermostable in the range of 75–80 °C.

The inclusion phytase rate in fish feed depends on fish species, source of phytase, diet formulation and process. Phytase doses of 250 to 1500 FTU per kilogram feed is usually considered as optimum in many fish species.

Apart from using phytase, some other techniques also have been investigated to reduce the phytate content. Since the phytate is water soluble, soaking the seed and discarding the water could reduce the phytate content of the seed by endogenous phytase activity (Rostami et al., 2013). However, it also results in loss of minerals, water extractable proteins and vitamins. Fermentation are also beneficial in elimination of phytate. In addition, Low phytate crops and seeds could be developed by genetic alternation of plants. Finally, germination could be applied as a strategy for reducing phytate. Germination will be discussed in detailed further.

- **NSPas**

The concentration of digestive enzymes that specifically hydrolyzed β-glycosidic bond of NSPs seems very low or scarce in fish (Castillo et al., 2015). NSPas supplementation to plant-based fish diet have been shown to increase nutrient digestibility and reduce nutrient exertion. NSP enzymes or carbohydrases are enzymes which hydrolyze carbohydrates to low molecular weight oligo or polysaccharides. Xylanase and glucanase accounts for more than 80% of global carbohydrase market (Castillo et al., 2015). α-amylase, β-mannanase, α-galactosidase and pectinase are other commercial carbohydrase in the market. NSPase commonly are produced by microbial or fungal fermentation. They could be used either as a single component products such as xylanase or β-glucanase or as a blended enzyme usually contains two or three enzymes with different distinct target (Masey et al., 2014). They also could be produced in one single fermentation as cocktail enzymes which have multitude of activities. It is believed that NSP hydrolyzing enzymes act by influencing on digesta viscosity, cage effect and intestinal microflora (Simon, 2000) by hydrolyzing both soluble and insoluble NSPs. Water soluble NSPs absorb water and form viscus gel in intestine and hence, prevent enzymes from reaching nutrients and impeding digested nutrient from migrating to gut wall for absorption. It negatively affects the nutrient digestibility. NSPase has been reported to reduce the viscosity of NSP gels, and therefore improving nutrient digestions. Supplementation of β- glucanase and protease in juvenile rainbow trout fed solvent extracted soybean meal resulted in the breakdown of NSP structure and increase the apparent digestibility of some nutrients (Dalsgaard et al., 2016). In an another study, supplementing β-glucanase or protease to rainbow trout diet containing 344 g/kg soybean meal significantly improved the apparent digestibility of protein, fat, and phosphorous (Dalsgaard et al., 2012). It is suggested that carbohydrase may increase the mineral availability by hydrolyzing the targeted substrate and exposure the minerals to digestive enzymes (Castillo et al., 2015). In a study, supplementation of enzyme mix (trypsin, alkaline protease,acid protease, amyloglucosidase, amylase and cellulase) in Atlantic salmon fed by soya meal based diet led to significant improvement in growth rate and feed efficiency (Carter et al., 1994). In contrast, in another study, no significant effect was found on protein digestion, dry matter and energy by supplementation of an enzyme blend (containing

β-glucanase and β-xylanase) in silver perch fed diets containing 30% wheat or dehulled lupins (Stone et al., 2003). Insoluble NSPs can impair nutrient availability by preventing the access of digestive enzymes to the nutrient fence in the cell rumen, the so called "cage effect" (Smeets et al., 2018). It suggested that the partial digestion of NSPs by NSP hydrolysis enzymes may decrease the cage effect and make the nutrient more accessible for digestive enzymes (Simon, 2000).

Finally, carbohydrates may improve the gut health in animal by modification of the intestinal microbial communities. Reducing gut viscosity and mucin formation, and following reducing the microbial adhesion, partial degradation of NSPs and improve the availability of some oligomers for useful bacteria, and reducing ammonia production and increasing volatile fatty acid concentration could be some of hypothetical mechanism of how NSPase might modify the intestinal flora (Castillo et al., 2015; Simon, 2000).

1.3.2 Germination

The germination process has been used for centuries in human nutrition for improving the nutritional value, reducing antinutrient and increasing the bioavailability of micro and macro nutrients (Frias et al., 2005). Germination is the first stage of plant growing during which the primary root breaks the seed coat and sprouts (Savelkoul et al., 1992). Following the uptake of water by the seed the metabolic activity of resting seed increases and complex biochemical changes occur during hydration and sprouting. These changes are associated with the activation of endogenous enzymes which could breakdown the complex components to a simple form and transform them into essential constituents. Germination has been also reported as an effective means to reduce antinutritional factors in seeds (Savelkoul et al., 1992). The effect of germination on carbohydrate, protein, and antinutrient are discussed in this section.

- **Carbohydrates**

Germination activate the endogenous enzymes such as α- amylase and hence, facilitate the breakdown of carbohydrates into simple sugars in a time-dependent manner (Oghbaei et al., 2016). It could be of benefit in salmonid by improving the digestibility of starch. Germination of faba bean for four days have been resulted in starch content decline followed by an increase in both reducing and non-reducing sugars (Hegazi, 1974). The duration of germination is a significant

factor in starch content decline (Nkhata et al., 2018). Starch content declined significantly from 60% to nearly 20% during germination of oat seeds (Tian et al., 2010).

- **Proteins**

There are controversial results about the effect of germination on protein and essential amino acids contents. Slight increase in protein content was found in sorghum sprouted between 24h and 168h (Otutu et al., 2014). Protein content of foxtail millet, wheat and chickpea increased after 48h controlled germination (Laxmi et al., 2015). However, a slight reduction in proteins after four days germination in faba bean was reported (Hegazi, 1974).

Increasing in protein content could be attributed to the loss of dry weight during germination due to the utilization of some carbohydrates and fat or synthesis of some amino acids (Nkhata et al., 2018). Protein losses could be attributed to the degradation of proteins during germination. Consequently, the net effect of synthesis and breakdown of proteins will dictate the actual protein content of seed after germination.

It have been claimed that germination could improve biological value of proteins (Nkhata et al., 2018). In vitro protein digestibility of some legume seeds increased upon 24h of germination (Ghavidel et al., 2007). In vitro protein digestibility of faba bean increased after 24, 48 and 72h of germination. The most profound increase was found in 72h germinated seed (Alonso et al., 2000). However, germinated lupin seeds had lower ileal digestibility of methionine and lysine compared to raw seeds in pig (Chilomer et al., 2013).

- **Fat**

Lipid content of cereal and flax decreased during germination, which could be attributed to the utilization of lipids as an energy source during germination process (Traoré et al., 2004; Wanasundara et al., 1999). However an increase in crude lipid of germinated rice have been reported (Kim et al., 2012).

- **Antinutrients**

It is believed that germination reduce the content of proteinaceous antinutrients by the act of protease (Savelkoul et al., 1992). Water soaking prior to germination also could reduce the antinutrient by leaching. In a review, it is claimed that soaking reduces the amount of tannins, trypsin inhibitors, phytic acid, saponins and raffinose family in faba bean (Revilla, 2015). Tannin content reduced by 50% after soaking. The reduced amount of phytic acid could be due to the activation of endogenous phytase during soaking. Observed decrease in saponin and trypsin inhibitor could be due to the water soluble nature of those antinutrient that permit the migration from seeds into the soaking water. The author reported that lectin leaching out in the water is not sufficient to reduce its antinutritional effects.

Germination processes up to 72h in buckwheat significantly reduced the content of phytic acid while the content of total tannin significantly increased (Benincasa et al., 2019). Trypsin inhibitor gradually reduced during germination in the same study. The reduction in phytic acid could be attributed to the activation of phytase enzyme in germinated seed.

Bohn et al, claimed that the endogenous enzymatic activity of phytase during germination is species dependent (Bohn et al., 2008). They also claimed that germination is not a suitable method to reduce phytic acid in seeds and using of phytic acid hydrolyzing enzymes would be a better approach to reduce the phytic acid.

Germination have been resulted in significant reduction in stachyose, tannins, phytic acid, vicine, trypsin inhibitor and haemagglutinin activity in faba bean (Khalil et al., 1995)

Condensed tannins were significantly reduced after 24 and 72h germination in faba bean (Alonso et al., 2000). In another study, the content of raffinose family oligosaccharides (RFO) in faba bean were markedly decreased after second day of germination (Goyoaga et al., 2011). Continues flow soaking of faba bean in tap water for 72 h at 50 °C, 60 h at 55 °C, or 48 h at 60 °C with a flow rate of 0.5 mL/min resulted in removing the whole vicine and convicine from faba bean seeds in all case (Jamalian et al., 2005). As soaking is applied prior to germination, it could be beneficial to remove these antinutrients.

The concentration of vicin and convicin reported very low, around 0.02 and 0.005 μmol, respectively, during the first 2 weeks of sprouting (Brown et al., 1972).

It is also claimed that germination could increase the mineral availability by decreasing the amount of mineral binding antinutritional factors such as phytate (Nkhata et al., 2018).

1.4 plant-based ingredients in carnivores fish feed

Aquaculture is one of the fastest food production sectors and continue to grow to reach 93.6 million ton by 2030, a growth rate of 47 percent over 2011 (Msangi et al., 2013). Atlantic salmon (*Salmo salar*) constituted 4% of world finfish production in 2016 (FAO, 2018). Norway and Chile are the largest salmon producer in the world. Atlantic salmon is the most important species in Norwegian aquaculture which accounts for about 90% of its total aquaculture production. Norway supplied 50% of the global salmon production and is expected to maintain this leading position in the coming years (EY, 2017).

Supplying feed ingredients is one of the major challenges in aquaculture specially for carnivorous fish. In 2016 more than 1 billion feed produced by feed manufacturing industry, which about 4% of produced feed was used in aquatic farming (EY, 2017). About 11% of aquatic feed was used in salmonid farming. Feed accounts for about 50% of production cost in salmon industry. Salmon feed are largely based on the fish meal and fish oil. Global fish meal and fish oil production reaches 6.5 and 1.3 million metric tons, respectively, during the past 20 years (Hardy, 2010). FAO reported that 70% of fish meal and oil applied in aquafeed was used in salmon, trout and shrimp industry (Hardy, 2000). This is because these resources meet the nutritional requirement of carnivorous fish which need high level of protein and lipid (Gillund et al., 2010). Figure 1 shows the composition of ingredients typically used as feed for Atlantic salmon. This figure shows that salmon diets still relies on 61% marine ingredients.

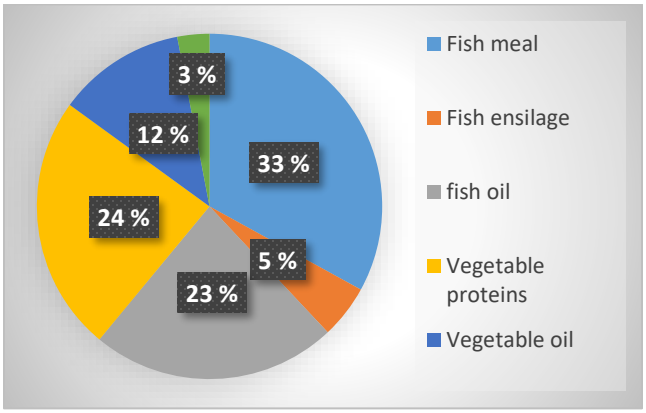

Figure1. 1 Composition of ingredients (percentage) used in Atlantic salmon feed (Ellingsen et al., 2009)

The shortage of marine resource, including fish meal and fish oil, and the arguments over the sustainability of using marine resource as fish feed, has led to exploration of economically viable and environmentally friendly alternative and new ingredients in aquaculture industry. Various alternative feed ingredients are currently being explored or are already practiced. Various species of lower trophic level such as krill, and zooplankton, by products of fishery and animal industry, insects, and Plants are among the ingredients which are used already in aqua feed or explored recently. Plants traditionally have been used as protein or energy source in feed. A range of plant ingredients are utilized in aquafeed such as grain (wheat, corn, barley, …), oilseeds (soybean, sunflower, rapeseed, cotton seed, …), and pulses (bean, lupin and peas, …). Soybean, wheat, rapeseed and corn are extensively used in animal diet. Soy protein products are among the best accepted protein source due to the high crud protein content, competitive price and reasonably balanced amino acids. However, it contains high level of antinutritional factor and low level of some essential amino acid for fish. Wheat and corn gluten are the major protein cereals which are used in salmonid feed. They both showed a high digestibility in salmon feed (Sørensen et al., 2011). Nevertheless, corn gluten have been reported to have negative impact on pigmentation in salmonids (Sørensen et al., 2011), and wheat gluten have undesirable effect on pellet quality because of its protein binding properties (Oliva-Teles et al., 2015)

The demands for soybean, wheat and corn is expected to rise worldwide as they are used increasingly in human nutrition, animal feed and industrial products (Lech et al., 2012). Relying on specific feed ingredients will result in increasing the price. Besides, using a combination of plants ingredients may increase the number of antinutritional factors but in the same time it may reduce the concentration of each individual antinutritional factor which may be beneficial for fish (C De Santis et al., 2015) .

Pulses or legums have also been used as feed for ruminant and nonruminant animals. They provide a low-cost binding agent for pelleted feed. Faba bean is a widespread and relatively unexploited crop in Europe (Christian De Santis et al., 2016). It contains less antinutritional factor compared to soybean, with most of the antinutrients concentrated in the seed coat which can be removed by de-hulling (El-Shemy et al., 2000). Additionally, it has high lysin content, comparable to fish meal (Oliva-Teles et al., 2015).

Whole and dehulled faba bean have been used in omnivorous and carnivorous fish feed with promising results (Christian De Santis et al., 2016).

Gaber reported that replacing 50% of fish meal with broad bean meal in Nile tilapia, resulted in the growth rate and protein digestibility comparable to the growth rate and protein digestibility of the 100% fish meal control diet (Gaber, 2006).

Booth et al, reported that whole and dehalled faba bean meal fed Australian Silver perch had high protein, energy and dry matter digestibility (Booth et al., 2001).

Ouraji et al, demonstrated that Nile tilapia could tolerate up to 30% faba bean meal in its diet (Ouraji et al., 2013).

Faba bean and field pea are two pulse which are used in salmon feed (Sørensen et al., 2011). Aslaksen et al, demonstrated that seawater salmon fed faba bean diet had comparable protein digestibility to fish meal based control diet, and concluded that faba bean could use as a potential plant ingredient for Atlantic salmon (Aslaksen et al., 2007).

Chikwati et al, also found a high digestibility of some amino acids in horse bean meal feeds for Atlantic salmon and resulted that horse bean meal could use as a valuable protein source for salmonids (Chikwati et al., 2012).

A moderate inclusion level of around 21% of air classified faba bean was a viable source of protein for both parr and post smolt salmon with most nutrient being highly digestible to fish and without any adverse effect on fish performance and health (Christian De Santis et al., 2015; C De Santis et al., 2016).

However, the challenges with faba bean, like most plant products, as a protein source in feed for carnivorous fish are: low protein content, high level of carbohydrates, imbalance amino acid profile and deficient in Sulphur amino acids such as methionine and cysteine, and presence of antinutritional factors (Sørensen et al., 2011). So, because of relatively low protein content it cannot be consider main protein source, and should mainly be used as complementary protein (Oliva-Teles et al., 2015). Poor amino acid composition and digestibility can be balanced by using a mixture of alternative protein source that may be complementary in terms of amino acid composition and use of additives such as amino acids, vitamins and minerals. Various strategies could applied to reduce or inactive antinutritional factor in plant ingredient. Technical treatments such as heat processing, solvent extraction, dehulling, using exogenous enzymes and breeding new plant species with improved amino acid profiles or low content of antinutrients could be used to

battle the problem of antinutritional factors in plant ingredients (Oliva-Teles et al., 2015). As stated before germination could also be used as a strategy for improving plant utilization in aquafeed. Heat processing methods such as extrusion cooking not only inactivates or reduces the heat liable antinutrients but also improves the digestibility of starch and carbohydrates in salmon (Qamar, 2018). Exogenous enzymes have been used in aquafeed with promising results on improve the digestibility of nutrients particularly of non-starch polysaccharides and phosphorous (Adeola et al., 2011; Ai et al., 2007; Dalsgaard et al., 2012). The effect of endogenous enzyme and germination on faba bean were discussed in detailed before.

A study by Schwediauer et al, investigated the effect of inclusion the germinated faba bean in piglet feed and concluded that germinated faba bean could be used in piglet feed up to 240 g/kg without reduction in feed intake (Schwediauer et al., 2018). However, they did not recommend germination as a method to improve nutrition value or acceptance of faba bean in weaner pigs. El-Hack et al, demonstrated that enzymatic treatment of faba bean with an enzyme mixture (beta-glucanase, xylanase , cellulase, alpha amylase and protease) significantly increase the body weight and feed conversion in laying hens (El-Hack et al., 2017).

To our knowledge no studies have been published on the potential of sprouted or enzyme treated faba bean as a feedstuff for Atlantic salmon.

1.5 Digestibility

Digestibility, by definition, is the amount of nutrient absorbed by the gastrointestinal tract (Lawrence et al., 2013) and represents the difference between the amount of feed ingested and the amount of nutrient retained in the faeces. Digestibility studies of a given diet or feed is important for evaluation of the diet as potential feed candidate, providing a low-cost diet with defined nutritional requirement and reducing the environmental impact of undigested feed (Vandenberg et al., 2001). Direct and indirect methods are two approach to asses diet digestibility (Maynard et al., 1969). Direct method relies on quantitative measurements of both feed input and faecal output. Direct method is an imprecise method in fish digestibility measurement due to the difficulties and errors in accurate data collection of feed intake and faeces (Glencross et al., 2007). Indirect method or indicator method could be an alternative in fish digestibility measurements. A marker is used in indirect method which become concentrated in faeces during transit through the gut (Jones et al.,

1998). This marker could be either internal markers which naturally presented in feed such as fibers or external inert markers. The external indicators should be indigestible and added to the feed in small quantity (1-2%). Different types of marker have been used in aquaculture digestibility studies such as metal oxides like Chromic oxide, radioactive isotopes, mineral salts, natural and artificial dye and rare earth metal oxides such as ytterbium oxide, yttrium oxide and other rare earth metal oxides. The indirect method gives what so called "apparent digestibility". The apparent digestibility coefficient (ADC) of each specific nutrition is calculated by measuring the percentage of the indicator and nutrient in the feed and faeces (Glencross et al., 2007), based on the following equation:

$$ADC_{Diet} = 1 - \left(\frac{Marker\ in\ diet \times nutrient\ in\ faeces}{Marker\ in\ faeces \times nutrient\ in\ diet} \right)$$

The equation should be multiplied by 100 to achieve apparent digestibility in percentage.

Faeces collection methods have been shown to have significant effects on ingredient digestibility results (Glencross et al., 2007). One of the major error source of under or over estimation of apparent digestibility coefficient is errors in quantitative faecal collection (Belal, 2005; Riche et al., 1995). Dissection, stripping and collection of the faeces deposited in the water are three methods of faecal collection used by the most researchers (Glencross et al., 2007). Stripping and dissection methods have the risk of underestimation of digestibility due to the incomplete digestion and mixing of digesta with endogenous materials (Belal, 2005). In the other hand, collection of faeces from water media has the risk of overestimation of digestibility due to the leaching of the organic matter to the water. Different methods are used to collecting faeces from water media. Cho, used a small settling chamber to collect Rainbow trout faeces (Cho, 1979). This method which known as Guelph system was modified by other authors later to reduce the water contact with faeces and consequently reduce the leaching (Glencross et al., 2007). Cheoubert et al, modified the Guelph system in a way that settled faeces being removed from the water on to a mechanically rotating filter screen and then deposited on to a collection tray (Choubert et al., 1982). In our study a wedge wire screen is used to collect the faeces and uneaten feed. The wire screen is flat-welded stainless steel which is connected to the tank as an angled screen. The outlet water passes through the wire screen which is like a sieve and the uneaten feed and faeces remain on the screen. In this method the water contact with uneaten feed and faeces is low due to the breaking effects of the inbuilt support profiles which are placed under and across the entire screen.

The slop of the screen also helps to the minimum contact of the water and faeces (Shomorin et al., 2019).

1.6 Aims of this study

In the present study we evaluated the apparent nutrient digestibility of three different feeds for Atlantic salmon (Salmo Salar). Three dietary treatments were tested including raw faba beans which milled before used in feed processing, the enzyme treated beans incubated both with a xylanase and phytase, and sprouted beans which were dried before using in the feed. Apparent digestibility coefficient for Kjeldahl and Dumas N, S, P, Mg, Zn, H, carbon, starch, lipid and energy of these three dietary treatments from the faeces collected on a wedge wire screen was compared. In addition, we compared the apparent digestibility for Dumas N, carbon, Mg, sulphur and Zn when faeces collected either using a wedge wire screen or stripping method.

2. Materials and Methods

2.1 Experimental diet

Three experimental diets were formulated and produced at NMBU Center For Feed Technology (FôrTek). Table 2.1 and 2.2 shows the diet formulation and diet proximate composition respectively.

Table 2. 1 Formulation of three experimental diets

Diet	Control diet (Untreated)		Enzyme treated		Sprouted	
Ingredient (kg per treatment)	%	kg per treatment	%	kg per treatment	%	kg per treatment
Fish meal	17.00	7.65	17.00	7.65	17.00	7.65
Soy protein concentrate	17.00	7.65	17.00	7.65	17.00	7.65
Faba bean	25.00	11.25	25.00	11.25	25.00	11.25
Vital wheat gluten	11.55	5.20	11.55	5.20	11.55	5.20
Fish oil	10.00	4.50	10.00	4.50	10.00	4.50
Soybean oil	13.60	6.12	13.60	6.12	13.60	6.12
Choline chloride	0.03	0.01	0.03	0.01	0.03	0.01
MCP	1.82	0.82	1.82	0.82	1.82	0.82
Limestone	0.40	0.18	0.40	0.18	0.40	0.18
L-lys	0.86	0.39	0.86	0.39	0.86	0.39
DL Met	1.75	0.79	1.75	0.79	1.75	0.79
L Thr	0.07	0.03	0.07	0.03	0.07	0.03
L Val	0.31	0.14	0.31	0.14	0.31	0.14
Stay C 35%	0.10	0.05	0.10	0.05	0.10	0.05
Y2O3	0.01	0.0045	0.01	0.0045	0.01	0.0045
Premix	0.50	0.23	0.50	0.23	0.50	0.23
Econase xt 25 L	0.00	0.00	0.01	0.005	0.00	0.00
Quatntum blue 5 L	0.00	0.00	0.03	0.014	0.00	0.00

A basal dry diet was formulated and prepared with fish meal (17% dry matter), soy protein concentrate (17% DM) and vital wheat gluten (11.55% DM) as the primary source of proteins (table 1). Dietary inclusion level of fish oil and soybean oil as the fat source for diet was 10% and 13.6% dry matter respectively. Vital amino acids and vitamins was added to the diet to have a balance diet for fish. Yttrium Oxide (Y2O3) was included in basal diet at 0.01% dry matter basis for nutrient digestibility assessments. Diet 1 serves as a control diet containing 25% whole untreated, milled (Hammer mill Alpine UPZ 160, model 100-1400) faba bean which was added to basal diet. In diet 2 the same amount of faba bean has been pre-incubated with 0.01 % NSPase

(Econase xt 25 L) and 0.03% phytase (Quatntum blue 5 L) for 30 minutes at 37°C and added to dry basal diet mix before extrusion, without prior drying. In diet 3 the same amount of faba bean was sprouted for 5 days and dried over night at 60°C, milled and added to dry basal diet before extrusion. All dry ingredients were mixed in a 60 lit twin shaft mixer (IsDeCa 60 lit. NMBU), and extruded in a twin-screw extruder (Bühler BCTG 62, Switzerland) through a 2.5 mm die. The amount of added water to diet 1 and 3 was 30% and in diet 2 was 25%. The resultant pellet then dried at a batch drier and kept at 2 °C throughout the study. Table 2.3 presents the extrusion processing parameter for three diets.

Table 2. 2 Analyzed composition of the three diets

Diet	Control diet (Untreated)	Enzyme treated	Sprouted
Chemical composition			
Crude protein (N*6.25) (g/kg)	427.46±0.3	429.65±0.21	444.40±0.18
N Dumas %	6.9±0.4	7.2±0.02	7.4±0.01
Crud fat (g/kg)	198.0±9.7	216.5±5.23	201.3±3.2
Starch %	10.7±0.15	10.7±0.01	10.2±0.20
Ash (g/kg)	71.94±0.54	71.27±0.20	70.52±0.24
Total P (mg/g)	12,01±0.09	11,45±0.32	11,57±0.32
Zink (mg/g)	0.13±0.01	0.12±0.00	0.14±0.01
Magnesium (mg/g)	1.8±0.05	1.8±0.03	1.7±0.01
Energy(mj/kg)	22.72±0.06	23.24±0.03	22.74±0.01
Hydrogen %	7.5±0.04	7.8±0.03	7.6±0.06
Carbon %	47.7±2.6	50.5±0.02	49.1±0.02
Sulphur %	0.71±0.04	0.78±0.02	0.79±0.00

All results are presented as mean±SD and n=3.

Table 2. 3 Extrusion parameters of experimental diets

Date	04.02.2019	04.02.2019	04.02.2019
Diet	1	2	3
Die size	2.5	2.5	2.5
Number of dies	6	6	6
Calibration (hz)	6.5	6.5	6.5
Barrel 1 temp. (°C)	43.7	39.1	35
Barrel 2 temp. (°C)	84.7	64.7	55.5
Barrel 3 temp. (°C)	110.9	109.4	108.3
Barrel 4 temp. (°C)	130	130.7	130.2
Barrel 5 temp. (°C)	125	127.4	127
Die temp. (°C)	114	118	121
Die pressure (bar).	13	12.2	12.4
Screw speed (rpm)	400	400	400
Torque (Nm)	188	171	179
Torque (%)	43	39	41
Drive power (kW)	7.8	6.9	7.5
SME (Wh/kg)	591	655,3	568
Extr. water (kg/h)	13.2	11	13.2
Knife speed (rpm)	1000	1000	1000
Number of knifes	6	6	6
Bulk density, 1st	536	543	569
Bulk density, 2nd		510	569

2.2 Fish trial

This project was conducted at fish lab of Norwegian University of Life Sciences from 3rd to 17th of April 2019. Atlantic salmon (*Salmo salar*) eyed egg were obtained from Aquagen and hatched in the fish lab. Salmon fry were fed commercial Skretting feed through rearing period until start of experiment. Fish was transferred to experimental tanks one week prior to start of the experiment to adapt to the experimental condition and fed the commercial feed during acclimation period. They were randomly distributed to 9 fiber glass tanks, with water volume of 230 L and water flow of 6-7 l/min. Each tank contained 10 fish. Average water temperature was 14.3 ± 0.4 ^{o}C, the oxygen concentration at outlet water was approximately 83.5 % of saturation, and fish was exposed to 24h light during the experiment. At the start of the experiment three diets were randomly assigned among nine tanks with each diet having three replicates (3 diets, 3 experimental tanks per diet, 9 tanks in all). The initial weight of fish was 374.1± 2.8 g. Fish was fed 1.5% of their body weight by automatic belt feeder twice a day at 7.00 am and 17.00 pm. Each feeding lasted for one hour.

Faeces were collected by a wedge wire screen (Shomorin et al., 2019) over two days, April 14th to April 16th, following the afternoon feeding.

At the end of trial, on April 17th, faeces was collected by stripping as described by (Austreng, 1978) following anesthetizing with Tricaine mesylate (100 mg/lit). The stripping was done by applying gentle pressure to abdomen and care was taken to avoid contamination of faeces with mucus, urine and water. The stripped faeces from fishes in each tank was pooled together and kept frozen at -20°C prior to freeze drying for chemical analysis.

2.3 Chemical analysis

All chemical analysis was carried out in triplicate at LabTek (Analysis lab for livestock and aquaculture) at NMBU. Three diets were finely ground in an electric blade grinder and analyzed for N (Kjeldahl), C, H, N S (Dumas), lipid, starch, energy, magnesium, zinc, phosphorous and yttrium. Kjeldahl-N was analysed according to the method by Kjeldahl (Kjeldahl, 1883) with some modifications (Thiex et al., 2002). Total carbon, hydrogen, nitrogen and Sulphur (CHNS) was

determined according to the Dumas method (P. ISO, 2008) by a Vario El Cube element analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Fat content was determined by accelerated solvent extraction (Richardson, 2001). Starch was analyzed utilizing a modification of the method described by (McCleary et al., 1994). Phosphorus was determined by a spectrometric method (Daly et al., 1972). Zinc, magnesium and yttrium were analyzed by atomic absorption spectrometry using MP-AES (Microwave Plasma Atomic Emission Spectrometer). Energy was determined by bomb calorimetry method (ISO9831, 1998). Ash was determined gravimetry by combustion at 550 °C for min. 4 hours (ISO, 2002). Fecal sample from wedge wire screen was freeze dried, homogenized and analyzed for the same element as diet and as described for the diet. Stripped fecal samples was analyzed for Dumas N, carbon, sulphur, magnesium and zinc as described for the diets after freeze drying and homogenizing.

2.4 Digestibility calculation

Apparent coefficient digestibility (ADC) of Kjeldahl and Dumas N, lipid, starch, energy, carbon, hydrogen, sulphur, magnesium, zinc, Phosphorous and energy was calculated for all three diets from faeces samples collected from wedge wire screen. Pooled faeces samples from each replicate tank (n= 3 per each diet) was freeze dried and homogenized before analyzing. Yttrium oxide was used as a marker for ADC analysis. Faeces samples from striping method was used to estimate Dumas N, carbon, sulphur, magnesium and zinc apparent digestibilites as we did not have enough samples from stripping methods for all nutrient digestibility estimations.

The apparent digestibility coefficient of the nutrients and minerals was calculated as following:

ADC%=100-((100(%Yt in feed/%Yt in faeces)×(%nutrient in faeces/%nutrient in feed))

2.5 Statistical analysis

Mean values of the 3 replicates for each diet was used for digestibility calculations. The results were analyzed by one-way analysis of variance (ANOVA) to detect statistically significant differences between digestibility of three diets using SAS version 9.4. The significance level was

considered as $p \leq 0.05$ and trend was considered as $0.05 < p \leq 01$. The means were ranked by least square (LS) means and P-diff under the general linear model procedure in SAS.

A two-way ANOVA with interaction was used to analyze the main effect of treatment of the beans and faeces collection method (wedge wire screen collection vs. stripping) and the interaction between diet and collection method. Significant differences were set at $p \leq 0.05$ and ranked by LS means and P-diff. All values are presented as the mean ± s.e.m.

3. Results

3.1 Digestibility experiment

3.1.1 Digestibility estimates obtained from analyses of faeces samples collected on the wedge wire screen

Table 3.1 shows the diet digestibility evaluation of faeces samples collected from wedge wire screen (Shomorin et al., 2019).The raw peas were only milled before used in feed processing, the incubated pea had been incubated both with a xylanase and phytase, while the sprouted peas were leniently dried before in was used in the feed.

Table 3. 1 Apparent digestibility coefficients (ADC) for nutrients in samples that have been collected on the wedge Wire screen

ADC	Raw Bean	Incubated	Sprouted	P(F)[1]
Lipid	96.9±0.30[a]	98.0±0.21[b]	98.1±0.12[b]	0.021
Nitrogen (Kjeldahl)	95.2±0.20[a]	96.1±0.20[b]	96.1±0.23[b]	0.020
Nitrogen (Dumas)	95.0±0.04[a]	96.1±0.33[b]	96.1±0.23[b]	0.018
Starch	80.0±0.73	81.7±0.74	81.4±0.41	0.223
Carbon	85.6±0.30[a]	87.6±0.16[b]	87.2±0.15[b]	0.001
Hydrogen	82.2±2.33	84.4±2.01	84.4±2.02	0.703
Energy	87.9±0.15[a]	89.3±0.17[b]	88.98±0.2[b]	0.003
Sulphur	95.1±0.09	96.0±0.59	94.9±0.50	0.272
Magnesium	67.5±1.50	69.2±0.85	70.4±0.23	0.202
Zinc	27.8±3.21	33.2±3.09	35.0±1.73	0.234
Phosphorous	55.0±0.85[a]	58.0±0.54[b]	59.2±0.70[b]	0.013

[1]Statistically significant (P(F)≤0.05) were ranked by least-square means, and significance is indicated by Superscript letters [a, b]

Lipid digestibility (Table 3.1) was significantly higher in enzyme treated and sprouted faba bean compared to raw faba bean, while the sprouted and enzyme treated diets did not differ significantly in lipid digestion ($p \leq 0.05$).

Kjeldahl and Dumas nitrogen digestibility was significantly improved in enzyme treated and sprouted compared to the raw faba bean. The sprouted and enzyme treated performed at the similar level. Both mean values and standard error of the means (SEM) of nitrogen digestibility obtained by N quantification by Dumas and the Kjeldahl methods were similar.

Starch digestibility did not show significant difference between three diets. Carbon digestibility was significantly higher in enzyme treated and sprouted faba bean compared to untreated faba bean diet. Enzyme treated and sprouted faba bean did not show any significant difference in carbon digestibility. No significant differences were found in hydrogen, sulphur, zinc or magnesium digestibility between three diets.

ADC of hydrogen, zinc and magnesium digestibility showed a high SEM in all three diets.

Energy digestibility was significantly elevated in the enzyme treated and sprouted faba bean compared to untreated faba bean ($P \leq 0.5$). Enzyme treated and sprouted faba bean did not show any differences regarding energy digestibility.

Phosphorous digestibility was significantly higher in enzyme treated and sprouted faba bean than untreated faba bean diet ($P \leq 0.5$). There was no significant differences between enzyme treated and sprouted faba bean for Phosphorous digestibility.

3.1.2 Digestibility estimates obtained from analysis of feces samples collected by stripping faeces

Table 3.2 shows diet digestibility evaluation of stripped faeces samples (Austreng, 1978).

Table 3.2 Apparent digestibility coefficients for nutrients in stripped samples

ADC of	Raw Bean	Incubated	Sprouted	P(F)[1]
Nitrogen (Dumas)	92.6±0.12	92.8±0.61	92.7±0.14	0.941
Carbon	82.9±0.20	83.4±0.89	82.9±0.26	0.788
Sulphur	65.2±1.74	69.79±2.15	70.32±1.78	0.193
Magnesium	72.43±0.07	73.18±2.09	72.45±1.13	0.909
Zinc	18.25±6.59	17.26±2.90	23.24±4.70	0.676

[1]Statistically significant (P(F)≤0.05 were ranked by least-square means, and significance is indicated by superscript letters a, b

ADC of Dumas nitrogen, carbon, sulphur, magnesium and zinc digestibility did not show significant difference between three diets in stripped faeces samples. ADC of Dumas N, carbon and magnesium showed low random variation (SEM) between three diets. ADC of Zn and S showed a high SEM in all three diets.

3.1.3 Comparison of digestibility estimates by analysis of faeces from wedge wire screen and stripped samples

Table 3.3 shows the main effects for faba bean treatment, faecal sample collection method and interaction between diet and collection method.

Table 3. 3 Main effects for faba bean treatment and faecal collection method.

ADC	Diet				Feces collection		P[1](Collection)	P[1](interaction)
	Untreated	Enzyme	Sprouted	P[1](Diet)	Stripping	Screen		Diet*collection
Dumas N	93.8±0.5	94.5±0.8	94.5±0.8	0.088	92.8±0.2[a]	95.8±0.2[b]	0.0001	0.20
Carbon	84.3±0.6[a]	85.5±1.0[b]	85.0±0.9[b]	0.040	83.1±0.2[a]	86.8±0.3[b]	0.0001	0.12
Sulphur	80.1±6.7	82.91±5.9	82.64±5.5	0.131	68.4±1.2[a]	95.36±0.2[b]	0.0001	0.19
Magnesium	69.9±1.2	71.2±1.3	71.43±0.6	0.449	72.6±0.7[a]	69.05±0.6[b]	0.0030	0.49
Zink	23.0±3.9	25.2±4.0	29.1±3.4	0.335	19.5±2.6[a]	32.0±1.7b	0.0025	0.72

[1]Statistically significant differences (P≤0.05) were ranked by least-square means, and the pdiff routine, significance is indicated by superscript letters [a, b].

There were no significant effects of diet on Dumas N, sulphur, magnesium or zink apparent digestibility coefficients of the stripped faeces samples. There was a significant effect of diet type on ADC for carbon. ADC for carbon was significantly higher in enzyme treated and sprouted faba bean diet than the untreated faba bean. However, there was no significant difference between sprouted and enzyme treated diet for carbon ADC.

There was a significant effect of faecal collection type on ADCs for Dumas N, carbon, sulphur, magnesium and zinc. ADCs of these nutrient was significantly higher in the faecal samples collected from wedge wire screen compared to the stripped faecal samples.

There was no significant interaction between different diets and faecal collection types.

4. Discussion

ADC of Kjeldahl and Dumas N of three diets estimated in the faeces samples from wedge wire screen was generally high (Table 3.1). ADC of Nitrogen in present study was comparable with the ADC of crude protein (96.5±0.3) in a study on the Juvenile Meagre fed 30% faba bean meal (Oliveira Filho et al., 2006) and higher than the crude protein digestibility in a study by (Carla Patrícia et al., 2012) which reported crude protein digestibility of 85.2±0.8 in the feed containing 25% faba bean meal for Nile tilapia.

ADCN from both Kjeldahl and Dumas method evaluated in faeces collected from wedge wire screen was significantly higher in enzyme treated and sprouted faba bean compared to untreated faba bean diet. This suggests that the N digestibility in specific and protein digestibility in general improved by enzyme treating and germination.

Alonso et al. reported that in vitro protein digestibility of faba bean was increased significantly after 72h of germination compared to raw faba bean (Alonso et al., 2000). They also showed that phytic acid, condensed tannins, polyphenol, trypsin and chymotrypsin inhibitors contents decreased significantly after 24-72h of germination. The authors concluded that increasing protein digestibility could be due to the decreasing activity of phytic acid, condensed tannins and

polyphenols. These antinutrients could bond with proteins and form complex and consequently reduce the protein solubility and susceptibility to proteases enzymes. Increasing in protein digestibility also could be attributed to the decreasing activity of trypsin and chymotrypsin inhibitors. Trypsin and chymotrypsin are important enzymes in protein digestion process in fish (Rungruangsak-Torrissen et al., 2006). Trypsin and chymotrypsin inhibitors reduce the protein availability and digestibility by binding to the trypsin and chymotrypsin and restriction the binding of proteins to these enzymes. However, heat processing during extrusion which is used in this project in all three diets could inactivate significant amount of trypsin and chymotrypsin inhibitors. Enhanced protein digestibility in germinated faba bean diet could also be attributed to the proteolysis, partial degradation and solubilization of complex storage proteins during germination process (Mbithi-Mwikya et al., 2000; Nkhata et al., 2018).

Higher nitrogen digestibility in xylanase and phytase enzyme treated faba bean compared to untreated faba bean diet could be attributed to the hydrolyzation of non-starch polysaccharides and phytate by xylanase and phytase enzymes respectively and probably their synergic effects on antinutrients. Xylanase hydrolyze the NSPs to smaller molecules, restrict their ability to form highly viscose digesta in gut, and increase the digesta passage rate (Dalsgaard et al., 2016). This could improve the protein digestibility and availability in fish probably by increasing the interaction of proteases enzymes with proteins (Sinha et al., 2011).

Phytase could improve protein digestibility by catalysis the hydrolyses of phytate (IP_6) and breakdown the IP_6-protein bonds. Phytate could bond with proteins and protease enzymes and decrees the protein digestibility and availability probably by restrict the protein availability for proteases enzymes (V. Kumar et al., 2012). Nevertheless, the results of protein digestibility in enzyme treated faba bean diet in current study is in contrast with the study on Nile tilapia where enzyme treatment (phytase and xylanase) of the sunflower, wheat barn and citrus pulps diets showed no effects on crude protein digestibility (Roel M. Maas et al., 2019). In an another study on salmon, crude protein digestibility was not significantly different between soybean meal pretreated with a mixed enzyme containing phytase, xylanase, protease and soy bean meal diet without enzyme treatment (Jacobsen et al., 2018). The contradicting results could be related to the use of different diets, enzyme types and complexes, methods of adding enzyme to the feed and rearing condition of the trial period.

N digestibility results from Dumas and Kjeldahl methods in faeces from wedge wire screen was very similar to each other (Table 3.1), however Dumas method showed slightly higher variation in standard error of mean. A study compared the Dumas and Kjeldahl method for total nitrogen in various meat products and concluded that both methods had high and same precision and similar results (Mihaljev et al., 2015). In another review study, Kjeldahl and Dumas N of some food stuffs evaluated and demonstrated that Dumas method gave slightly higher results compared to Kjeldahl due to the conversion of nonprotein forms of nitrogen into elemental nitrogen in the Dumas method (Mihaljev et al., 2015). The authors also demonstrated that the Dumas results showed higher standard error than Kjeldahl results. One possible explanation is that higher amounts of sample are weighed in for Kjeldahl than Dumas N analysis, stressing the need for homogeneity of the samples that are analyzed by Dumas' method.

ADC of lipid estimated from the faeces collected from wedge wire screen in three diets was generally high and ranged from 96.2±0.30 to 98.1±0.12. The results are comparable with the lipid ADC of 95.18±0.37 in a study on rainbow trout fed a diet containing 30% faba bean (Ouraji et al., 2013).

ADC of lipids was significantly higher in enzyme treated and sprouted faba bean diet compared to the untreated diet. This result is in agreement with a study on Nile tilapia which lipid digestion improved by adding xylanase and phytase enzymes to a control diet (Roel M Maas et al., 2018). Xylanase and phytase supplementation effects on the lipid digestibility could be due to the increasing the access of digestive enzymes such as lipase to the lipids as a result of hydrolyzing antinutrients by xylanase and phytase (Roel M Maas et al., 2018). Phytate can complex with cation groups of lipids along with other nutrients (Ca and Mg) and form a complex referred as "lipophytin" which has a reduced digestibility (V Kumar et al., 2012).

ADC of Carbon was also generally high and significantly higher in enzyme treated and sprouted faba bean diet compared to untreated faba bean diet (Table 3.1). Carbon digestibility gives an overall estimates of organic matter digestibility. Complex carbohydrates such as starch and fibers reduces the fish capacity for nutrient digestion (Gaylord et al., 1996). So, one explanation for

improved carbon digestibility in sprouted and enzyme treated faba bean diet could be the hydrolyzing effects of germination and xylanase enzyme on the complex carbohydrate molecules such as fibers, cellulose and hemicellulose and reducing the fiber content of bean. Another explanation could be the higher protein and lipid digestibility in these diets as explained above which gives a higher carbon digestibility in treated diet compared to untreated.

The significantly higher energy digestibility in enzyme treated and sprouted faba bean compared to untreated faba bean could be attributed to the higher protein and lipid digestibility in those diets compared to untreated diet. Since energy digestibility is the sum of digested energy from crude protein, crude fat and total carbohydrates, based on the higher lipid and protein digestibility in treated faba bean diets, greater energy digestibility coefficient would be expected. Increased energy ADC results in current study in treated faba bean diets is in the agreement with the study on Nile tilapia which incorporation of a mixed enzymes of xylanase and phytase resulted in a higher energy digestibility compared to control untreated diets (Roel M. Maas et al., 2019).

Starch digestibility results is generally high ranged from 80.0±0.73 to 81.7±0.74 for untreated and germinated faba bean diets respectively. The results are higher than the ADC starch digestibility of 70.5±0.1 in a faba bean diets for juvenile meagre (Carla Patrícia et al., 2012) and ADC starch digestibility of 49.6 in whole faba bean meal for Atlantic salmon (Aslaksen et al., 2007). However, starch digestibility did not show significant difference among the three diets.

ADC of phosphorous was 55.0±0.85, 58.0±0.54 and 59.2±0.70 for untreated, enzyme treated and germinated faba bean diets respectively. It significantly improved by enzyme treated and sprouted diets compared to the untreated diet. ADCs of P for all three diets in current study is higher than the P ADC of 36% in faba bean diet for Atlantic salmon (Aslaksen et al., 2007).
Higher P digestion in germinated faba bean diet could be due to the reducing of phytate content during seed germination due to activation of phytase (Goyoaga et al., 2011; V Kumar et al., 2012). Phosphorous bond to the phytate is not available for fish. Degradation of phytate during germination increase the bioavailability of P for the animal. Improved ADC of P in enzyme treated faba bean could be due to the hydrolyzing phosphomonoester bonds from phytate by phytase and consequently liberating inorganic phosphate (Gupta et al., 2015). It has been claimed that xylanase

could also improve the P digestibility, probably due to the liberation of encapsulated nonphytate P from fibrous material (Cowieson et al., 2005).

In a study by (Roel M Maas et al., 2018) incorporation of phytase to the diet for Nile tilapia significantly increased the P digestibility compared to untreated diet while the xylanase did not show any improvement in P digestibility. A complex enzyme of phytase and xylanase improved the P digestibility compared to untreated diet but no synergic effects was found by using both enzymes in diet.

The P digestibility results in this study is in contrast with the results of P digestibility in Atlantic salmon where the ADC of P have not been improved by using a mix enzyme of phytase, protease, xylanase and cellulase in a soy bean meal diet (Jacobsen et al., 2018).

ADCs of zinc, magnesium, sulphur and hydrogen did not show any differences among the three diets. The ADC results of these elements showe a high and variable SEM. These high and variable SEMs could be due to the non-homogeneity of sample materials or volatilization loss during repetition (Malomo et al., 2017). So, the insignificant difference of ADC of these elements between treated and untreated faba bean diet could be due to the sampling and analytical errors and not a lack of response to the treatment.

In contrast to the fecal samples collected from wedge wire screen, the samples collected by stripping (Table 3.2) did not show differences between the diets in Dumas N and carbon digestibility. The current experiment does not reveal the reason for this difference.

The significantly higher ADCs of Dumas N, sulphur, zinc, magnesium and carbon in faeces samples collected from wedge wire screen compared to stripped samples (Table 3.3) is consistent with previous findings which shows higher ADC estimates from faeces collected from water medium compared to stripping method (Hajen et al., 1993; Spyridakis et al., 1989; Vandenberg et al., 2001). One study on rainbow trout have also shown that ADC of nutrients was significantly higher in faeces samples from wedge wire screen compared to the stripped faeces samples (Shomorin et al., 2019). The higher ADC estimates in faeces collected from screen compared to stripped faeces samples could be attributed to the leaching of nutrients from faeces on the screen to the water. It could be also attributed to the ADC's underestimation of stripping method due to the contamination with digesta. However, Shomorin et al., evaluted the wedge wire screen as a

valid and useful method for faecal collection as they observed a low rate of nutrient leaching at different time intervals (Shomorin et al., 2019) .

5. Conclusions

The apparent digestibility values obtained in this study are generally high specially for nitrogen, carbon, lipid and phosphorous which are of great importance in fish growth and performance. Germination and enzyme treatment with xylanase and phytase significantly improved nitrogen, lipid, carbon, phosphorous and energy digestibility compared to raw, untreated faba bean in faeces samples collected from wedge wire screen. These improvement in digestibility could probably be due to the reduction of antinutrients as well as structural polysaccharides presented in faba bean. Improved digestibility is important for fish growth and welfare. Besides, improved phosphorous digestibility is also important for reducing P emission and pollution in rearing water. Apparent digestibility coefficient of Zn, Mg, S, and H did not show any improvement in treated diets compared to untreated diet in faeces samples collected from wedge wire screen. As, these elements also showed a high and variable standard error mean, it could be concluded that this results probably are related to the error in sampling or analytical error and not lacking of response to treatment. The recommendation to have more accurate results could be minimize the variability of samples and consider adequate replication of samples.

The digestibility evaluation from the faeces collected on wedge wire screen was significantly higher than those collected by stripping method, probably due to nutrient leaching from the feces collected on the wedge wire screen. It is well known that faeces collection methods can affect the digestibility estimates of nutrients. However, it is difficult to select the most appropriate method as each method has its advantages and drawbacks. But as the Dumas N and carbon digestibility results gained from faeces on wedge wire screen could identified the significant differences between untreated and treaded diets it could be concluded that wedge wire screen is more efficient method in current study.

In conclusion, enzyme treated and sprouted faba bean (25% of diet dry matter) can probably be a potential plant ingredient in salmon diets as they showed improved nutrient digestibility compared to raw faba bean. However, it should be considered that raw faba bean also showed a high nutrient digestibility.

6. References

Adeola, O., & Cowieson, A. (2011). Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *Journal of animal science, 89*(10), 3189-3218.

Adsule, R. N., & Akpapunam, M. (1996). Faba bean (Vicia faba L.). In E. Nwokolo & J. Smartt (Eds.), *Food and Feed from Legumes and Oilseeds* (pp. 197-202). Boston, MA: Springer US.

Ai, Q., Mai, K., Zhang, W., Xu, W., Tan, B., Zhang, C., & Li, H. (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, Lateolabrax japonicus. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147*(2), 502-508.

Alonso, R., Aguirre, A., & Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry, 68*(2), 159-165.

Angel, R., Tamim, N., Applegate, T., Dhandu, A., & Ellestad, L. (2002). Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. *Journal of Applied Poultry Research, 11*(4), 471-480.

Aslaksen, M., Kraugerud, O., Penn, M., Svihus, B., Denstadli, V., Jørgensen, H., Hillestad, M., Krogdahl, Å., & Storebakken, T. (2007). Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, Salmo salar, fed diets with legumes, oilseeds, or cereals. *Aquaculture, 272*(1-4), 541-555.

Austreng, E. (1978). Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture, 13*(3), 265-272.

Baruah, K., Sahu, N. P., Pal, A. K., Debnath, D., Yengkokpam, S., & Mukherjee, S. C. (2007). Interactions of dietary microbial phytase, citric acid and crude protein level on mineral utilization by rohu, Labeo rohita (Hamilton), juveniles. *Journal of the World Aquaculture Society, 38*(2), 238-249.

Bate-Smith, E. (1954). Flavonoid compounds in foods. In *Advances in food research* (Vol. 5, pp. 261-300): Elsevier.

Becker, K., & Makkar, H. (1999). Effects of dietary tannic acid and quebracho tannin on growth performance and metabolic rates of common carp (Cyprinus carpio L.). *Aquaculture, 175*(3-4), 327-335.

Belal, I. E. (2005). A review of some fish nutrition methodologies. *Bioresource technology, 96*(4), 395-402.

Benincasa, P., Falcinelli, B., Lutts, S., Stagnari, F., & Galieni, A. (2019). Sprouted Grains: A Comprehensive Review. *Nutrients, 11*(2). doi:10.3390/nu11020421

Bohn, L., Meyer, A. S., & Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science B, 9*(3), 165-191.

Booth, M. A., Allan, G. L., Frances, J., & Parkinson, S. (2001). Replacement of fish meal in diets for Australian silver perch, Bidyanus bidyanus: IV. Effects of dehulling and protein concentration on digestibility of grain legumes. *Aquaculture, 196*(1-2), 67-85.

Brown, E., & Roberts, F. (1972). Formation of vicine and convicine by Vicia faba. *Phytochemistry, 11*(11), 3203-3206.

Cain, K. D., & Garling, D. L. (1995). Pretreatment of soybean meal with phytase for salmonid diets to reduce phosphorus concentrations in hatchery effluents. *The Progressive Fish-Culturist, 57*(2), 114-119.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Luo, Z., & Li, D. (2007). Application of microbial phytase in fish feed. *Enzyme and Microbial Technology, 40*(4), 497-507.

Carla Patrícia, & OlimPublished, R. (2012). *Apparent digestibility coefficient of feed ingredients for juvenile meagre*.

Carter, C., Houlihan, D., Buchanan, B., & Mitchell, A. (1994). Growth and feed utilization efficiencies of seawater Atlantic salmon, Salmo salar L., fed a diet containing supplementary enzymes. *Aquaculture Research, 25*(1), 37-46.

Castillo, S., & Gatlin III, D. M. (2015). Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: a review. *Aquaculture, 435*, 286-292.

Chikwati, E. M., Venold, F. F., Penn, M. H., Rohloff, J., Refstie, S., Guttvik, A., Hillestad, M., & Krogdahl, Å. (2012). Interaction of soyasaponins with plant ingredients in diets for Atlantic salmon, Salmo salar L. *British Journal of Nutrition, 107*(11), 1570-1590.

Chilomer, K., Kasprowicz-Potocka, M., Gulewicz, P., & Frankiewicz, A. (2013). The influence of lupin seed germination on the chemical composition and standardized ileal digestibility of protein and amino acids in pigs. *Journal of animal physiology and animal nutrition, 97*(4), 639-646.

Cho, C. (1979). *Apparent digestibility measurement in feedstuffs for rainbow trout.* Paper presented at the Proc. World Symp. on Finfish Nutrition and Fishfeed Technology Vol. II.

Choct, M. (1997). Feed non-starch polysaccharides: chemical structures and nutritional significance. *Feed milling international, 191*(June issue), 13-26.

Choubert, G., De la Noue, J., & Luquet, P. (1982). Digestibility in fish: improved device for the automatic collection of feces. *Aquaculture*.

Chung, K.-T., Wong, T. Y., Wei, C.-I., Huang, Y.-W., & Lin, Y. (1998). Tannins and human health: a review. *Critical reviews in food science and nutrition, 38*(6), 421-464.

Cowieson, A., & Adeola, O. (2005). Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. *Poultry Science, 84*(12), 1860-1867.

Crépon, K., Marget, P., Peyronnet, C., Carrouee, B., Arese, P., & Duc, G. (2010). Nutritional value of faba bean (Vicia faba L.) seeds for feed and food. *Field Crops Research, 115*(3), 329-339.

Dalsgaard, J., Bach Knudsen, K., Verlhac, V., Ekmann, K. S., & Pedersen, P. B. (2016). Supplementing enzymes to extruded, soybean-based diet improves breakdown of non-starch polysaccharides in rainbow trout (O ncorhynchus mykiss). *Aquaculture Nutrition, 22*(2), 419-426.

Dalsgaard, J., Verlhac, V., Hjermitslev, N., Ekmann, K. S., Fischer, M., Klausen, M., & Pedersen, P. B. (2012). Effects of exogenous enzymes on apparent nutrient digestibility in rainbow trout (Oncorhynchus mykiss) fed diets with high inclusion of plant-based protein. *Animal Feed Science and Technology, 171*(2-4), 181-191.

Daly, J. A., & Ertingshausen, G. (1972). Direct method for determining inorganic phosphate in serum with the" CentrifiChem". *Clinical chemistry, 18*(3), 263-265.

De Santis, C., Bartie, K. L., Olsen, R. E., Taggart, J. B., & Tocher, D. R. (2015). Nutrigenomic profiling of transcriptional processes affected in liver and distal intestine in response to a soybean meal-induced nutritional stress in Atlantic salmon (Salmo salar). *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 15*, 1-11.

De Santis, C., Martin, S. A., Dehler, C. E., Iannetta, P. P., Leeming, D., & Tocher, D. R. (2016). Influence of dietary inclusion of a wet processed faba bean protein isolate on post-smolt Atlantic salmon (Salmo salar). *Aquaculture, 465*, 124-133.

De Santis, C., Ruohonen, K., Tocher, D. R., Martin, S. A., Krol, E., Secombes, C. J., Bell, J. G., El-Mowafi, A., & Crampton, V. (2015). Atlantic salmon (Salmo salar) parr as a model to predict the optimum inclusion of air classified faba bean protein concentrate in feeds for seawater salmon. *Aquaculture, 444*, 70-78.

De Santis, C., Tocher, D. R., Ruohonen, K., El-Mowafi, A., Martin, S. A., Dehler, C., Secombes, C. J., & Crampton, V. (2016). Air-classified faba bean protein concentrate is efficiently utilized as a dietary protein source by post-smolt Atlantic salmon (Salmo salar). *Aquaculture, 452*, 169-177.

Denstadli, V., Skrede, A., Krogdahl, Å., Sahlstrøm, S., & Storebakken, T. (2006). Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (Salmo salar L.) fed graded levels of phytic acid. *Aquaculture, 256*(1-4), 365-376.

Duc, G., Marget, P., & Arese, P. (2011). Breeding priorities for improved nutritional value of Vicia faba seeds. *Grain legumes, 56*, 17-18.

Duc, G., Marget, P., Esnault, R., Le Guen, J., & Bastianelli, D. (1999). Genetic variability for feeding value of faba bean seeds (Vicia faba): Comparative chemical composition of isogenics involving zero-tannin and zero-vicine genes. *The Journal of Agricultural Science, 133*(2), 185-196.

Duc, G., Sixdenier, G., Lila, M., & Furstoss, V. (1989). *Search of genetic variability for vicine and convicine content in Vicia faba L.: a first report of a gene which codes for nearly zero-vicine and zero-convicine contents.* Paper presented at the 1. International Workshop on'Antinutritional Factors (ANF) in Legume Seeds', Wageningen (Netherlands), 23-25 Nov 1988.

El-Hack, M. A., Alagawany, M., Laudadio, V., Demauro, R., & Tufarelli, V. (2017). Dietary inclusion of raw faba bean instead of soybean meal and enzyme supplementation in laying hens: Effect on performance and egg quality. *Saudi journal of biological sciences, 24*(2), 276-285.

El-Shemy, H., Abdel-Rahim, E., Shaban, O., Ragab, A., Carnovale, E., & Fujita, K. (2000). Comparison of nutritional and antinutritional factors in soybean and fababean seeds with or without cortex. *Soil science and plant nutrition, 46*(2), 515-524.

Ellingsen, H., Olaussen, J., & Utne, I. (2009). Environmental analysis of the Norwegian fishery and aquaculture industry—A preliminary study focusing on farmed salmon. *Marine Policy, 33*(3), 479-488.

Encarnação, P. (2016). Functional feed additives in aquaculture feeds. In *Aquafeed formulation* (pp. 217-237): Elsevier.

EY. (2017). *the Norwegian aquaculture analysis: an overview*. Retrieved from

FAO. (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals*. Retrieved from Rome:

Fish, B. C., & Thompson, L. U. (1991). Lectin-tannin interactions and their influence on pancreatic amylase activity and starch digestibility. *Journal of agricultural and food chemistry, 39*(4), 727-731.

Francis, G., Makkar, H. P., & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture, 199*(3-4), 197-227.

Frias, J., Miranda, M. L., Doblado, R., & Vidal-Valverde, C. (2005). Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of Lupinus albus L. var. Multolupa. *Food Chemistry, 92*(2), 211-220.

Gaber, M. M. (2006). Partial and complete replacement of fish meal by broad bean meal in feeds for Nile tilapia, Oreochromis niloticus, L., fry. *Aquaculture Research, 37*(10), 986-993.

Gaylord, T. G., & Gatlin III, D. M. (1996). Determination of digestibility coefficients of various feedstuffs for red drum (Sciaenops ocellatus). *Aquaculture, 139*(3-4), 303-314.

Ghavidel, R. A., & Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *LWT-Food Science and Technology, 40*(7), 1292-1299.

Giczewska, A., & Borowska, J. (2003). Nutritional value of broad bean seeds. Part 1: Starch and fibre. *Food/Nahrung, 47*(2), 95-97.

Gillund, F., & Myhr, A. I. (2010). Perspectives on salmon feed: A deliberative assessment of several alternative feed resources. *Journal of agricultural and environmental ethics, 23*(6), 527-550.

Glencross, B. D., Booth, M., & Allan, G. L. (2007). A feed is only as good as its ingredients–a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition, 13*(1), 17-34.

Goldstein, W. S., & Spencer, K. (1985). Inhibition of cyanogenesis by tannins. *Journal of Chemical Ecology, 11*(7), 847-858.

Goyoaga, C., Burbano, C., Cuadrado, C., Romero, C., Guillamón, E., Varela, A., Pedrosa, M. M., & Muzquiz, M. (2011). Content and distribution of protein, sugars and inositol phosphates during the germination and seedling growth of two cultivars of Vicia faba. *Journal of Food Composition and Analysis, 24*(3), 391-397.

Grabner, M., & Hofer, R. (1985). The digestibility of the proteins of broad bean (Vicia faba) and soya bean (Glycine max) under in vitro conditions simulating the alimentary tracts of rainbow trout (Salmo gairdneri) and carp (Cyprinus carpio). *Aquaculture, 48*(2), 111-122.

Grela, E., & Günter, K. (1995). Fatty acid composition and tocopherol content of some legume seeds. *Animal Feed Science and Technology, 52*(3-4), 325-331.

Griffiths, D. W., & Ramsay, G. (1996). The distribution of pyrimidinone glucosides in developing seedlings of Vicia faba and Vicia narbonensis. *Journal of the Science of Food and Agriculture, 72*(4), 469-475.

Griffiths, D. W., & Thomas, T. A. (1981). Phytate and total phosphorus content of field beans (Vicia faba L.). *Journal of the Science of Food and Agriculture, 32*(2), 187-192.

Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of food science and technology, 52*(2), 676-684.

Haidar, M. N., Petie, M., Heinsbroek, L. T., Verreth, J. A., & Schrama, J. W. (2016). The effect of type of carbohydrate (starch vs. nonstarch polysaccharides) on nutrients digestibility, energy retention and maintenance requirements in Nile tilapia. *Aquaculture, 463*, 241-247.

Hajen, W., Beames, R., Higgs, D., & Dosanjh, B. (1993). Digestibility of various feedstuffs by post-juvenile chinook salmon (Oncorhynchus tshawytscha) in sea water. 1. Validation of technique. *Aquaculture, 112*(4), 321-332.

Hajra, A., Mazumder, A., Verma, A., Ganguly, D. P., Mohanty, B. P., & Sharma, A. P. (2013). Antinutritional factors in plant origin fish feed ingredients: the problems and probable remedies. *Advances in Fish Research, 5*, 193-202.

Hardy, R. W. (2000). New developments in aquatic feed ingredients, and potential of enzyme supplements. *Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola*, 19-22.

Hardy, R. W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquaculture Research, 41*(5), 770-776.

Hardy, R. W., & Shearer, K. D. (1985). Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (Salmo gairdneri). *Canadian Journal of Fisheries and Aquatic Sciences, 42*(1), 181-184.

Hegazi, S. M. (1974). Effect of germination on the carbohydrate, protein and amino acid contents of broad beans. *Zeitschrift für Ernährungswissenschaft, 13*(4), 200-203.

Helsper, J., & Van Norel, A. (1991). Antinutritional Factors in Faba Bean and Resistance to Soil-Borne Diseases. In *Developments in Agricultural and Managed Forest Ecology* (Vol. 23, pp. 387-391): Elsevier.

Helsper, J. P., Hoogendijk, J. M., van Norel, A., & Burger-Meyer, K. (1993). Antinutritional factors in faba beans (Vica faba L.) as affected by breeding toward the absence of condensed tannins. *Journal of agricultural and food chemistry, 41*(7), 1058-1061.

Hemre, G.-I., Amlund, H., Aursand, M., Bakke, A. M., Olsen, R. E., Ringø, E., Svihus, B., Bernhoft, A., Jenssen, B. M., & Møretrø, T. (2018). Criteria for safe use of plant ingredients in diets for aquacultured fish. *European Journal of Nutrition & Food Safety*, 240-242.

ISO9831. (1998). Animal feeding stuffs, animal products, and faeces or urine — Determination of gross calorific value — Bomb calorimeter method. In.

ISO. (2002). Animal Feeding Stuffs–Determination of Crude Ash. *International Organization for Standardization*.

ISO, P. (2008). Food products-determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content. In *Oilseeds and animal feeding stuffs* (pp. 16634-16631).

JA, D. (1981). *Handbook of legumes of world economic importance*: Plenum Press,New York.

Jacobsen, H. J., Samuelsen, T. A., Girons, A., & Kousoulaki, K. (2018). Different enzyme incorporation strategies in Atlantic salmon diet containing soybean meal: Effects on feed quality, fish performance, nutrient digestibility and distal intestinal morphology. *Aquaculture, 491*, 302-309.

Jamalian, J., & Ghorbani, M. (2005). Extraction of favism-inducing agents from whole seeds of faba bean (Vicia faba L var major). *Journal of the Science of Food and Agriculture, 85*(6), 1055-1060.

Jones, P., & De Silva, S. (1998). Comparison of internal and external markers in digestibility studies involving the Australian freshwater crayfish, Cherax destructor Clark (Decapoda, Parastacidae). *Aquaculture Research, 29*(7), 487-493.

Kadam, S., & Salunkhe, D. (1989). *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization*: CRC Press.

Khalil, A., & Mansour, E. (1995). The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. *Food Chemistry, 54*(2), 177-182.

Khamassi, K., Jeddi, F. B., Hobbs, D., Irigoyen, J., Stoddard, F., O'Sullivan, D. M., & Jones, H. (2013). A baseline study of vicine–convicine levels in faba bean (Vicia faba L.) germplasm. *Plant Genetic Resources, 11*(3), 250-257.

Khokhar, S., & Apenten, R. K. O. (2003). Antinutritional factors in food legumes and effects of processing. *The role of food, agriculture, forestry and fisheries in human nutrition, 4*, 82-116.

Kim, H. Y., Hwang, I. G., Kim, T. M., Woo, K. S., Park, D. S., Kim, J. H., Kim, D. J., Lee, J., Lee, Y. R., & Jeong, H. S. (2012). Chemical and functional components in different parts of rough rice (Oryza sativa L.) before and after germination. *Food Chemistry, 134*(1), 288-293.

Kjeldahl, J. (1883). Neue methode zur bestimmung des stickstoffs in organischen körpern. *Fresenius' Journal of Analytical Chemistry, 22*(1), 366-382.

Knudsen, K. E. B. (2014). Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. *Poultry Science, 93*(9), 2380-2393.

Kraugerud, O. F., Penn, M., Storebakken, T., Refstie, S., Krogdahl, Å., & Svihus, B. (2007). Nutrient digestibilities and gut function in Atlantic salmon (Salmo salar) fed diets with cellulose or non-starch polysaccharides from soy. *Aquaculture, 273*(1), 96-107.

Kumar, V., Sinha, A., Makkar, H., De Boeck, G., & Becker, K. (2012). Phytate and phytase in fish nutrition. *Journal of animal physiology and animal nutrition, 96*(3), 335-364.

Kumar, V., Sinha, A. K., Makkar, H. P. S., De Boeck, G., & Becker, K. (2012). Phytate and phytase in fish nutrition. *Journal of animal physiology and animal nutrition, 96*(3), 335-364. Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1439-0396.2011.01169.x. doi:10.1111/j.1439-0396.2011.01169.x

Kuz'Mina, V. (1996). Influence of age on digestive enzyme activity in some freshwater teleosts. *Aquaculture, 148*(1), 25-37.

Landry, E. J., Fuchs, S. J., & Hu, J. (2016). Carbohydrate composition of mature and immature faba bean seeds. *Journal of Food Composition and Analysis, 50*, 55-60.

Lawrence, J. M., Lawrence, A. L., & Watts, S. A. (2013). Feeding, digestion and digestibility of sea urchins. In *Developments in aquaculture and fisheries science* (Vol. 38, pp. 135-154): Elsevier.
Laxmi, G., Chaturvedi, N., & Richa, S. (2015). The impact of malting on nutritional composition of foxtail millet, wheat and chickpea. *Journal of Nutrition & Food Sciences, 5*(5), 1.
Lech, G. P., & Reigh, R. C. (2012). Plant products affect growth and digestive efficiency of cultured Florida pompano (Trachinotus carolinus) fed compounded diets. *PLoS One, 7*(4), e34981.
Leenhouwers, J., Adjei-Boateng, D., Verreth, J., & Schrama, J. (2006). Digesta viscosity, nutrient digestibility and organ weights in African catfish (Clarias gariepinus) fed diets supplemented with different levels of a soluble non-starch polysaccharide. *Aquaculture Nutrition, 12*(2), 111-116.
Leenhouwers, J. I., ter Veld, M., Verreth, J. A., & Schrama, J. W. (2007). Digesta characteristiscs and performance of African catfish (Clarias gariepinus) fed cereal grains that differ in viscosity. *Aquaculture, 264*(1-4), 330-341.
Lei, X. G., Porres, J. M., Mullaney, E. J., & Brinch-Pedersen, H. (2007). Phytase: source, structure and application. In *Industrial enzymes* (pp. 505-529): Springer.
Liener, I. (1989). Antinutritional factors in legume seeds: state of the art. *Recent advances of research in antinutritional factors in legume seeds*, 6-13.
Maas, R. M., Verdegem, M. C., Dersjant-Li, Y., & Schrama, J. W. (2018). The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. *Aquaculture, 487*, 7-14.
Maas, R. M., Verdegem, M. C. J., & Schrama, J. W. (2019). Effect of non-starch polysaccharide composition and enzyme supplementation on growth performance and nutrient digestibility in Nile tilapia (Oreochromis niloticus). *Aquaculture Nutrition, 25*(3), 622-632. Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1111/anu.12884. doi:10.1111/anu.12884
Makkar, H. P., Becker, K., Abel, H., & Pawelzik, E. (1997). Nutrient contents, rumen protein degradability and antinutritional factors in some colour-and white-flowering cultivars of Vicia faba beans. *Journal of the Science of Food and Agriculture, 75*(4), 511-520.
Malomo, G. A., & Ihegwuagu, N. E. (2017). Some Aspects of Animal Feed Sampling and Analysis. *Ideas and Applications Toward Sample Preparation for Food and Beverage Analysis*, 77.
Martin, A., Cabrera, A., & Medina, J. L. (1991). Antinutritional. factors in faba bean. Tannin content in Vicia faba: possibilities for plant breeding.
Masey, H. O. N., Smith, J., & Bedford, M. (2014). Multicarbohydrase Enzymes for Non-ruminants. *Asian-Australasian journal of animal sciences, 27*(2), 290-301.
Maynard, L. A., & Loosli, J. K. (1969). Animal nutrition. *Animal nutrition.*(6th ed).
Mbithi-Mwikya, S., Van Camp, J., Yiru, Y., & Huyghebaert, A. (2000). Nutrient and antinutrient changes in finger millet (Eleusine coracan) during sprouting. *LWT-Food Science and Technology, 33*(1), 9-14.
McCleary, B., Solah, V., & Gibson, T. (1994). Quantitative measurement of total starch in cereal flours and products. *Journal of Cereal Science, 20*(1), 51-58.
Mihaljev, Ž. A., Jakšić, S. M., Prica, N. B., Ćupić, Ž. N., & Živkov-Baloš, M. M. (2015). Comparison of the Kjeldahl method, Dumas method and NIR method for total nitrogen determination in meat and meat products. *gas, 2*, 7.
Msangi, S., Kobayashi, M., Batka, M., Vannuccini, S., Dey, M., & Anderson, J. (2013). Fish to 2030: prospects for fisheries and aquaculture. *World Bank Report, 83177*(1), 102.
Multari, S., Stewart, D., & Russell, W. R. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. *Comprehensive Reviews in Food Science and Food Safety, 14*(5), 511-522.

Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition, 6*(8), 2446-2458.
Oghbaei, M., & Prakash, J. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food & Agriculture, 2*(1), 1136015.
Oliva-Teles, A., Enes, P., & Peres, H. (2015). Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In *Feed and feeding practices in aquaculture* (pp. 203-233): Elsevier.
Oliveira Filho, P. R. C. d., & Fracalossi, D. M. (2006). Apparent digestibility coefficients of feed ingredients for jundia juveniles. *Revista Brasileira de Zootecnia, 35*(4), 1581-1587.
Oomah, B. D., Luc, G., Leprelle, C., Drover, J. C., Harrison, J. E., & Olson, M. (2011). Phenolics, phytic acid, and phytase in Canadian-grown low-tannin faba bean (Vicia faba L.) genotypes. *Journal of agricultural and food chemistry, 59*(8), 3763-3771.
Otutu, O., Ikuomola, D., & Oloruntoba, R. (2014). Effect of sprouting days on the chemical and physicochemical properties of sorghum starch. *American Journal of Food and Nutrition, 4*(1), 11-20.
Ouraji, H., Zaretabar, A., & Rahmani, H. (2013). Performance of rainbow trout (Oncorhynchus mykiss) fingerlings fed diets containing different levels of faba bean (Vicia faba) meal. *Aquaculture, 416*, 161-165.
Papatryphon, E., Howell, R. A., & Soares Jr, J. H. (1999). Growth and mineral absorption by striped bass Morone saxatilis fed a plant feedstuff based diet supplemented with phytase. *Journal of the World Aquaculture Society, 30*(2), 161-173.
Prodanov, M., Sierra, I., & Vidal-Valverde, C. (2004). Influence of soaking and cooking on the thiamin, riboflavin and niacin contents of legumes. *Food Chemistry, 84*(2), 271-277.
Qamar, S. Z. (2018). *Extrusion of Metals, Polymers, and Food Products*: BoD–Books on Demand.
Rana, K. J., Siriwardena, S., & Hasan, M. R. (2009). *Impact of rising feed ingredient prices on aquafeeds and aquaculture production*: Food and Agriculture Organization of the United Nations (FAO).
Revilla, I. (2015). Impact of Thermal Processing on Faba Bean (Vicia faba) Composition. In *Processing and Impact on Active Components in Food* (pp. 337-343): Elsevier.
Richardson, R. K. (2001). Determination of fat in dairy products using pressurized solvent extraction. *Journal of AOAC International, 84*(5), 1522-1533.
Riche, M., Trottier, N., Ku, P., & Garling, D. (2001). Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (Oreochromis niloticus) fed phytase pretreated soybean meal diets. *Fish Physiology and Biochemistry, 25*(3), 181.
Riche, M., White, M., & Brown, P. (1995). Barium carbonate as an alternative indicator to chromic oxide for use in digestibility experiments with rainbow trout. *Nutrition Research, 15*(9), 1323-1331.
Rostami, H., & Giri, A. (2013). An overview on microbial phytase and its biotechnological applications. *Intl. J. Adv. Biotech. Res, 4*, 62-71.
Rungruangsak-Torrissen, K., Moss, R., Andresen, L., Berg, A., & Waagbø, R. (2006). Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (Salmo salar L.). *Fish Physiology and Biochemistry, 32*(1), 7.
Sajjadi, M., & Carter, C. (2004). Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (Salmo salar L.) fed a canola-meal-based diet. *Aquaculture, 240*(1-4), 417-431.
Savelkoul, F., Van der Poel, A., & Tamminga, S. (1992). The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibitors in legume seeds during germination. A review. *Plant Foods for Human Nutrition, 42*(1), 71-85.
Schrader, K. K. (2008). Compounds with inhibitory activity against the channel catfish pathogens Edwardsiella ictaluri and Flavobacterium columnare. *North American Journal of Aquaculture, 70*(2), 147-153.

Schwediauer, P., Hagmüller, W., & Zollitsch, W. (2018). Germination of faba beans (Vicia faba L.) for organic weaning piglets. *Organic Agriculture, 8*(3), 249-258.

Selvendran, R. R. (1984). The plant cell wall as a source of dietary fiber: chemistry and structure. *American Journal of Clinical Nutrition (USA)*.

Shi, L., Mu, K., Arntfield, S. D., & Nickerson, M. T. (2017). Changes in levels of enzyme inhibitors during soaking and cooking for pulses available in Canada. *Journal of food science and technology, 54*(4), 1014-1022.

Shomorin, G. O., Storebakken, T., Kraugerud, O. F., Øverland, M., Hansen, B. R., & Hansen, J. Ø. (2019). Evaluation of wedge wire screen as a new tool for faeces collection in digestibility assessment in fish: The impact of nutrient leaching on apparent digestibility of nitrogen, carbon and sulphur from fishmeal, soybean meal and rapeseed meal-based diets in rainbow trout (Oncorhynchus mykiss). *Aquaculture, 504*, 81-87.

Simon, O. (2000). Non starch polysaccharide (NSP) hydrolysing enzymes as feed additives: Mode of action in the gastrointestinal tract. *Lohmann Information, 23*, 7-13.

Sinha, A. K., Kumar, V., Makkar, H. P., De Boeck, G., & Becker, K. (2011). Non-starch polysaccharides and their role in fish nutrition–A review. *Food Chemistry, 127*(4), 1409-1426.

Smeets, N., Nuyens, F., Van Campenhout, L., Delezie, E., & Niewold, T. (2018). Interactions between the concentration of non-starch polysaccharides in wheat and the addition of an enzyme mixture in a broiler digestibility and performance trial. *Poultry Science, 97*(6), 2064-2070.

Spinelli, J., Houle, C. R., & Wekell, J. C. (1983). The effect of phytates on the growth of rainbow trout (Salmo gairdneri) fed purified diets containing varying quantities of calcium and magnesium. *Aquaculture, 30*(1-4), 71-83.

Spyridakis, P., Metailler, R., Gabaudan, J., & Riaza, A. (1989). Studies on nutrient digestibility in European sea bass (Dicentrarchus labrax): 1. Methodological aspects concerning faeces collection. *Aquaculture, 77*(1), 61-70.

Stone, D., Allan, G., & Anderson, A. (2003). Carbohydrate utilization by juvenile silver perch, Bidyanus bidyanus (Mitchell). IV. Can dietary enzymes increase digestible energy from wheat starch, wheat and dehulled lupin? *Aquaculture Research, 34*(2), 135-147.

Storebakken, T., Shearer, K., & Roem, A. (1998). Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, Salmo salar. *Aquaculture, 161*(1-4), 365-379.

Sugiura, S., Gabaudan, J., Dong, F., & Hardy, R. (2001). Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout [Oncorhynchus mykiss (Walbaum)] fed soybean meal-based diets. *Aquaculture Research, 32*(7), 583-592.

Suzuki, U., Yoshimura, K., & Takaishi, M. (1907). About the enzyme “phytase”, which splits “anhydro-oxy-methylene diphosphoric acid”. *Bulletin of the College of Agriculture, Tokyo Imperial University, 7*, 503-512.

Sørensen, M., Berge, G. M., Thomassen, M. S., Ruyter, B., Hatlen, B., Ytrestøyl, T., Aas, T. S., & Åsgård, T. E. (2011). Today’s and tomorrow's feed ingredients in Norwegian aquaculture. *Nofima rapportserie*.

Thiex, N. J., Manson, H., Anderson, S., & Persson, J.-Å. (2002). Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. *Journal of AOAC International, 85*(2), 309-317.

Tian, B., Xie, B., Shi, J., Wu, J., Cai, Y., Xu, T., Xue, S., & Deng, Q. (2010). Physicochemical changes of oat seeds during germination. *Food Chemistry, 119*(3), 1195-1200.

Traoré, T., Mouquet, C., Icard-Vernière, C., Traore, A., & Trèche, S. (2004). Changes in nutrient composition, phytate and cyanide contents and α-amylase activity during cereal malting in small production units in Ouagadougou (Burkina Faso). *Food Chemistry, 88*(1), 105-114.

Vandenberg, G., & De La Noüe, J. (2001). Apparent digestibility comparison in rainbow trout (Oncorhynchus mykiss) assessed using three methods of faeces collection and three digestibility markers. *Aquaculture Nutrition, 7*(4), 237-245.

Vasconcelos, I. M., & Oliveira, J. T. A. (2004). Antinutritional properties of plant lectins. *Toxicon, 44*(4), 385-403.

Vidal-Valverde, C., Frias, J., Sotomayor, C., Diaz-Pollan, C., Fernandez, M., & Urbano, G. (1998). Nutrients and antinutritional factors in faba beans as affected by processing. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 207*(2), 140-145.

Vielma, J., Ruohonen, K., & Peisker, M. (2002). Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, Oncorhynchus mykiss. *Aquaculture, 204*(1-2), 145-156.

Wanasundara, P., Wanasundara, U., & Shahidi, F. (1999). Changes in flax (Linum usitatissimum L.) seed lipids during germination. *Journal of the American Oil Chemists' Society, 76*(1), 41-48.

Wang, P.-X., & Ueberschär, K. (1990). The estimation of vicine, convicine and condensed tannins in 22 varieties of fababeans (Vicia faba L.). *Animal Feed Science and Technology, 31*(1-2), 157-165.

Weerd, J. v. (1999). Balance trials with African catfish Clarias gariepinus fed phytase-treated soybean meal-based diets. *Aquaculture Nutrition, 5*(2), 135-142.

Åsgård, T., & Shearer, K. (1997). Dietary phosphorus requirement of juvenile Atlantic salmon, Salmo salar L. *Aquaculture Nutrition, 3*(1), 17-23.

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