ニジマス用飼料におけるエクストルーダー処理コーングルテンミールおよび大豆油粕の利用性に関する研究

Doctoral Dissertation

STUDIES ON THE UTILIZATION OF EXTRUDED CORN GLUTEN AND SOYBEAN MEALS IN RAINBOW TROUT *Oncorhynchus mykiss* DIET

September 2020

Graduate School of Marine Science and Technology

Tokyo University of Marine Science and Technology

Doctoral Course of Applied Marine Biosciences

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Abstract

Nutritional value of fish feed ingredient could be improved through adequate processing of the raw material. Even in aquafeed, heat treatment is one of the common processing techniques used to improve nutritional value of plant ingredients. However, excess heat processing can also negatively affect the overall quality of the raw material for instance, protein availability or digestibility may be reduced and the amino acids including lysine, arginine and cysteine may be destroyed. These unwanted effects could be avoided if the raw material is processed to a high temperature (200°C) for only a short time (30-45 s). This heat processing technique that utilizes high temperature in a short time (HTST) is called extrusion cooking. For rainbow trout and many other carnivorous finfish, fishmeal is normally an important protein source in their diet because of its high protein quality that could give the fish optimal growth, high feed efficiency, and good product quality. However, demand for fishmeal is continually rising thus making its availability limited and its price is getting more expensive. With this issue in the aquafeed industry, there is a need to replace fish meal with other less expensive and sustainable protein sources. Plant-based protein sources such as soybean meal (SBM) and corn gluten meal (CGM) are considered candidate ingredients to replace fish meal. To further improved the nutritional value of SBM and CGM, they may undergo extrusion cooking before utilizing it as fish feed ingredients. With this, three studies were conducted with the general objective to investigate the effects of extrusion cooking temperature on soybean meal and corn gluten meal as combined ingredients in the

diet for juvenile rainbow trout, in terms of nutritional value, fish growth performance, feed utilization, and apparent digestibility.

In the first experiment, the objective was to determine the nutritional values of soybean and corn gluten meals extruded at 100°C (low temperature, LT) or 150°C (high temperature, HT). SBM and CGM were sourced from Nihon Nosan Kogyo and each ingredient underwent extrusion at 100°C or 150°C. The proximate composition of the four extruded ingredients were obtained and results shows that the crude protein of extruded SBM increased but for CGM, it decreased. It could be because CGM usually contains 12-15% starch which is bound tightly together with protein gelatinized. On the other hand, for the crude lipid, higher values were obtained with ingredients extruded at high temperature, which is true for both SBM and CGM. Four isonitrogenous (41%, CP) and isolipidic (14%, CL) diets were made to proceed for the 12-week feeding trial. Control group is fishmeal based, NE diet contains non-extruded SBM and CGM, LT diet is composed of low temperature extruded SBM and CGM while HT diet is composed of high temperature extruded SBM and CGM. 240 individuals of rainbow trout with initial average body weight of 12.5 g were randomly distributed to 12 60L aquaria and fed two times a day at satiation for six days a week. Result of the feeding trial shows that the control group had the highest value for final body weight and weight gain which is significantly different ($P<0.05$) with NE and HT group but not with the LT group. Specific growth rate (SGR) of HT group had the lowest value and is significantly different with that of the control. Daily feed intake was not affected by the dietary treatments. Feed conversion ratio (FCR) of the control group is significantly different among all treatments and trend for the protein efficiency ratio (PER) was the same with that of the SGR. The result of the total amino acid composition of the fish body is noteworthy because the methionine level of both the LT and HT group are low which reflects that of the methionine level of the respective diets. Moreover, the methionine level of the diets is below the requirement of rainbow trout to support optimum growth of the fish. The digestibility data had promising result as the protein digestibility was found to be highest for LT and HT group and phosphorus and manganese absorption was also found to be highest in HT group.

The second experiment was done with the same dietary treatments but with methionine supplementation at 0.3%. Result for the digestibility study shows that protein digestibility of LT and HT group were comparable with the control group and significantly different ($P<0$) with the NE group. For the growth performance, final weight and weight gain, values were found to be highest in HT group which is comparable with the control group and significantly higher than NE group. SGR of NE group was significantly lower among other treatments. FCR and daily feed intake were not affected by dietary treatments. Likewise, PER was found to be highest in HT group and significantly lower than NE group. For the nutrient retention, protein retention was found to be significantly higher in LT, HT and control group than in NE group. Values for lipid retention was significantly higher in LT and HT groups than in control and NE groups which is also reflective of the result for the fish body composition. Levels of phosphorus and manganese were still higher in HT group. It seems that remarkable result was observed for the HT group.

In the third experiment, we would like to find out which among the high temperature extruded ingredients (HT SBM or HT CGM) gives good result for fish growth, feed efficiency, digestibility and body composition. Five isonitrogenous and isolipidic diets were formulated. Control is still fishmeal based; NE contains non-extruded SBM and CGM; HTS has HT SBM + NE CGM; HTC has HT CGM+ NE SBM; HTSC has both HT SBM and HT CGM. ADC for dry matter, crude protein and crude lipid of control group and HTSC group are the same and significantly higher than others. Moreover, the phosphorus absorption of HTSC group is significantly higher among all treatments. Growth performance in terms of final weight, weigh gain and SGR shows that control group had significantly higher values among others followed by HTSC group. For feed efficiency, NE group had significantly lower values among others. For nutrient retention, it is noteworthy to mention that HTSC group had highest values for both protein and lipid retention. This study demonstrated that combined high temperature(150ºC) extruded SBM and CGM resulted to improved growth, feed efficiency, nutrient utilization, digestibility and fish body composition. Also, the performance of fish groups fed HTS or HTC are the same.

In general, these studies demonstrated that extrusion cooking could improve nutritional value of SBM and CGM. Diets containing high temperature (150ºC) extruded SBM and CGM enhanced fish growth, feed utilization, nutrient retention, body composition and digestibility in rainbow trout. Extrusion temperature (150°C) in this study is suitable for the processing of SBM and CGM as feed ingredients for rainbow trout.

LIST OF ABBREVIATIONS

ANFs	anti-nutritional factors
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ADC	apparent digestibility coefficient
CL	crude lipid
CGM	corn gluten meal
CP	crude protein
DFI	daily feed intake
FCR	feed conversion ratio
HT	high temperature
HTC	high temperature extruded corn gluten meal
HTS	high temperature extruded soybean meal
HTSC	high temperature extruded SBM and CGM
LT	low temperature
NE	non-extruded
PER	protein efficiency ratio
SEM	standard error of the mean
SBM	soybean meal
SGR	specific growth rate

CHAPTER 1

LITERATURE REVIEW AND GENERAL INTRODUCTION

Globally and most especially in Asia region, aquaculture which is the farming of aquatic animals and plants continues to dominate aquatic food production (FAO, 2019). It is still rapidly growing fish farming industry. According to Tacon, (2020) over 91% of global aquaculture production are being produced within the Asian region. Moreover, in the current situation, the total global aquaculture production exceeds production from global capture fisheries by over 18.32 million tonnes. The growing aquaculture industry has been a success and one of the greatest contributors is the aquafeed sector which supplies good quality feeds to the cultured fish species. The fast growth in aquaculture was mainly driven by the high demand for fish and shellfish as food worldwide. Some fish species are consumed locally while others are processed and exported as high value products (Olsen & Hasan, 2012). In aquaculture, about 70% of crustacean and fish including Chinese carps, tilapia, shrimp, catfish, salmon, marine fish, and other miscellaneous freshwater and diadromous fishes, freshwater crustaceans, milkfish and eel are direct-fed species. Of the direct-fed species production, about 68% are dependent on the use of commercially manufactured feeds (Tacon & Metian, 2015). The largest consumers of commercial aquaculture feeds were the omnivorous and herbivorous carp at 11.03 million tonnes, tilapia at 6.67 million tonnes, shrimp at 6.18 million tonnes, catfish at 4.27 million tonnes, salmon at 2.98 million tonnes and marine fish at 2.98 million tonnes (Tacon & Metian, 2015). There are still rooms for considerable potential for increased efficiency and efficacy of aquaculture through developing highly nutritious and cost-effective alternatives to the traditional marine protein feedstuffs including fishmeal. According to Costello et al. (2020), one way to sustainable increase mariculture production is through advances in finfish feeds. The use of fish meal and fish oil must be considerably reduced while increasing the use of alternative feed ingredients.

Fishmeal

In the past decades, the world food fish production has increased significantly. According to the current report of FAO (2020), There is an increase of about 14% in the global capture fisheries production; from 1990-2018, global aquaculture production increased at 527% while the total food fish consumption increased up to 122%. In 2018, global fish production was about 179 million tonnes and out of this, 156 million tonnes were used for human consumption while the remaining 22 million were utilized mainly to produce fish meal and fish oil. Of the total production, 46% comes from the produce of aquaculture. With this trend, the pressure in aquaculture production has also increased as capture fisheries are facing a lag in growth in terms of production. This situation puts a lot of pressure also in the production of fish meal as this is normally the main source of protein in the aquafeed industry especially for carnivorous species. According to Hardy (2006), the amount of fish meal used in fish feeds will continue to increase but the rate will be slower than the rate of increase in fish feed production. Tacon and Metian (2008) have reported the estimated global use of fish meal in a compound aquafeed for several major aquatic animal; in shrimp diet fish meal is included at 27%, 18% for marine finfish, 15% for salmon, 11% for Chinese carps, 6% for trout, 6% for eel, 5% for catfish, 5% for tilapia, 4% for freshwater crustaceans and 3% for other freshwater carnivore fish and milkfish.

Fish meal is a generic term for a nutrient-rich feed ingredient that is primarily used in diets for domestic animals including in aquafeed industry. It is generally made from small marine fish that are wild caught and not suitable for direct human consumption. It usually contains high percentage of bones and oil and they are normally considered ‘industrial’ because most of them are obtained for the sole purpose of fishmeal and fish oil production. According to Miles & Chapman (2006), for the nutritionist, they recognized fishmeal as feed ingredient to have high-quality and high digestibility that is highly favored to be included in the diet of most farm animals especially fish and shrimp. It is an excellent source of protein, lipid, minerals and vitamins. By weight, fishmeal normally contains 60% - 70% crude protein and its amino acid profile is outstanding.

In some form or the other, pelagic fish have been used for the production of fish protein and fish oil (Olsen & Hasan, 2012). Based on the report by Huntington & Hasan (2009), in European aquaculture, the main species used to produce fish meal are capelin *Mallotus villosus*, blue whiting *Micromesistius poutassou* and small sand eel *Ammodytes tobianus*; moreover, they also use Peruvian anchovy *Engraulis ringens* and Chilean jack mackerel *Trachurus murphyi* as well as Antarctic krill. In American aquaculture on the other hand, they mainly use Peruvian anchovy and Chilean jack mackerel, Alaska pollock *Theragra chalcogramma*, Argentine hake *Merluccius hubbsi* and southern blue whiting *Micromesistius australis*. African and Near East aquaculture utilizes European pilchard *Sardina pilchardus*, sardines *Sardinella* spp. and European anchovy *Engraulus encrasicolus* and horse mackerel *Trachurus trachurus*. In contrast, Asia aquaculture mainly depends on low value fish or trash fish as fish meal source. Fish meal is normally an important protein source for rainbow trout and other carnivorous finfish commercial feeds because of its high protein quality that gives optimal growth, high feed efficiency and good product quality (Yigit et al., 2006; Drew et al., 2007; Gaylord et al., 2010). However, the global fishmeal supply remains static, but the demand and price are continually increasing. Thus, fishmeal is a raw material that cannot be relied upon for the expansion of aquaculture (Suarez et al., 2013). According to overall demand for fish meal continued to grow thus fish meal price are expected to remain high in the long term. Other than the increasing demand for fish meal, El Niño also contributed to the increase in fish meal price globally. During the period 2000-2005, fish meal price ranged between US $500- US $700 per tonne (FAO, 2008). From 2006 to 2013, fish meal prices increased significantly with the highest value of US $1747 per tonne in 2013 (FAO, 2016). In comparison, the prices of some plant protein ingredients such as soybean meal is US $357 per tonne; corn gluten meal is US $407 per tonne; cotton seed meal is US $270 per tonne; linseed meal is US $250 per tonne; sunflower meal is US $215 per tonne; distillers dried grain is US $155 per tonne and dehydrated alfalfa is US $307 (USDA Market News Service, 2020).

Antinutritional factors

The presence of anti-nutritional factors (ANFs) limits the inclusion of plant ingredients in aquafeed despite of being rich in protein. They are mainly alkaloids that affect palatability but they can be washed out using water of through extrusion (Caruso, 2015). Other ANFs include oligosaccharides, phytate, saponin, and protease inhibitors (Rajeev & Bavitha, 2015). These ANFs are known to adversely affect the digestive and metabolic activity of the cultured species (Chakraborty et al., 2019).

Oligosaccharide

The presence of oligosaccharide in fish feed could increase viscosity of the chime in the digestive tract and interfere with the uptake of nutrients particularly minerals and fat (Refstie et al., 2005). It could reduce fish growth performance (Refstie et al., 1997) and induced enteritis in several salmonid fish species (Bureau et al., 1998; Van den Ingh et al., 1996).

Lectins

Lectins are the bioactive group of glycoproteins (Francis et al., 2001). They have the ability to bind reversibly and specifically to carbohydrates and glycoconjugates thereby interfering with nutrient absorption. It could also increase permeability of membranes to other proteins causing the increase in the incidence of allergic reaction (Gatlin et al., 2007).

Phytic acid

Phytic acid (phytate), myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) is often present in legume seeds. It is a phosphorus storage in many seeds as well as serve as chelating agent for cations and a form of cations. Majority of the phosphorus in plant legume seeds is represented by phytic acid and its salt and monogastric species have limited ability to hydrolyze phytates and release phosphate for absorption (Urbano et al., 2000). The antinutritional effects of phytate is related to the strong chelating ability associated with its six reactive phosphate groups and also its protein binding ability. Particularly, multivalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} are susceptible and form insoluble, indigestible complexes (Couzy et al., 1998). Phytates negatively affect enzyme activity for key digestive enzymes such as amylase, pepsin and trypsin. Moreover, the interaction of Ca^{2+} and phytate facilitates further binding to cations in the diet thus, reducing solubility and nutrient availability (Urbano et al., 2000). About two-thirds of the total phosphorus in oilseed meals or grains and their by-product meals is present as phytic acid (phytate). It lowers the bioavailability of phytate phosphorus and certain divalent cations such as zinc thus reducing the apparent digestibility of protein (Gatlin, 2007).

Phytases (myo-inositol (1,2,3,4,5,6) hexakisphosphate phosphohydrolases) represent a subgroup of phosphatases that are able to initiate the stepwise dephosphorylation of phytate. Bound phytate-P can be effectively converted to available-P by phytase. Currently, commercial phytases obtained from bacteria are available for administration in the preparation of monogastric animal feeds (Sanz-Penella & Haros, 2014). Moreover, in aquafeed, phytase has been used to enhance the growth performance, nutrient utilization and bioavailability of macro and micro minerals in fish as well as to reduce the P pollution into the aquatic environment (Kumar et al., 2011).

Saponins

Presence of saponin in fish feed could cause significant intestinal damage and higher saponin concentrations could depress growth of chinook salmon and rainbow trout (Gatlin, 2007)

Tannins

Tannins are secondary compounds of different chemical structures widely occurring in the plant kingdom. It could interfere with digestive process and diminishes the absorption of Vitamin B12 and could demonstrate toxicity in kidney and liver (Hajra et al., 2013).

Gossypol

Gossypol could cause growth depression, intestinal and other internal organ abnormalities and anorexia. It could also form gossypol-protein complex and may form deficiency of methionine and lysine (Hajra et al., 2013).

Phytic acid and trypsin inhibitors among other ANFs, are known to adversely major issues on cultured species. The level of these ANFs may be lowered if the raw material will undergo further processing before utilizing as feed ingredient in fish feeds. The presence of ANFs and imbalanced amino acid profile limits the incorporation of these plant ingredients in aquafeeds (Hardy, 2010). A summary of common ANFs found in plant ingredients is presented in Table 1 of Appendix 1, its plant derived nutrient source, and the means to lower its presence in the plant ingredients (Kumar & Barman 2012; Francis et al., 2001). Considering cost and availability, plant protein sources are superior to fish meal and this advantage could be allotted for further processing of raw materials to improve their nutritional value (Drew et al., 2007).

Alternative plant protein ingredients

The price of fish meal will continue to rise, and this give opportunity to produce alternative ingredients from recovered seafood processing waste or from grains, oilseed, legumes, and other agricultural products to be utilized specifically for aquafeeds (Hardy, 2006). If alternative sources have equal or superior in nutritional and economic value compared to fishmeal, it would give them potential to be widely used in aquafeeds. Though the characteristics of common alternative protein sources are inferior to fishmeal, research are continually done to develop ways in improving nutritional value of these alternative feed ingredients to support the growing need of the aquafeed industry. Moreover, despite of being inferior to fishmeal, these alternative feed ingredients are still considered to be use in the industry because it complements with fishmeal thus lowering feed cost. Many authors have recommended the plant-based protein ingredients specifically regarding the cost as they seem to be cheaper compared to fish meal (Daniel, 2018). Before an ingredient to be considered as potential alternative to fish meal, it must possess certain characteristics which includes, nutritional suitability, readily available, ease of handling, shipping, storage, and use in feed production. They could be selected through the basis of fish health, performance, consumer acceptance, minimal pollution, human health benefits and competitive pricing (Naylor et al., 2009).

According to Hardy (2006), with respect to apparent protein and amino acid digestibility, plant protein sources are similar to fish meal. However, the amino acid profiles of plant protein sources are inferior than the dietary requirements of carnivorous fish species. Generally, plant protein sources are easily available and have lower market price compared to fish meal. According to several authors, plant ingredients have potential in replacing fishmeal in animal diet if the subject animal showed no difference in the overall performance in the duration of the feeding trial using feed plant (Espe et al., 2007; Hansen et al., 2011; Lund et al., 2011; Yun et al., 2012; Valante et al., 2016; Daniel, 2017). Other researchers have supported this claim showing in the results of their work that plant proteins did not affect the performance of the rainbow trout, tilapia, catfish, and shrimp (Merrifield et al., 2010; Sheikhzadeh et al., 2012; Kpundeh et al., 2015; Guo et al., 2016; Li et al., 2016). Nevertheless, there are also authors that have found that, aquatic animals fed with less fish meal diets generally have a decreased feed intake and growth performance. This effect has been reported in rainbow trout (Gomes et al., 1995; Adelizi et al., 1998; De Francesco et al., 2004; Snyder et al., 2012), European sea bass (Dias et al., 1997), shrimp, *Penaeus monodon* (Sudaryono et al., 1999), turbot (Fournier et al., 2004), Atlantic salmon (Berge et al., 1998; Sveier et al., 2001; Espe et al., 2007), gilthead sea bream (Gomez-Requeni et al., 2004), turbot (Bonaldo et al., 2011), black tiger shrimp (Richard et al., 2011), and in eel, *Anguilla australis australis* (Engin et al., 2005). These results could have been due to the nature of the plant proteins which has less apparent digestibility coefficient (Gatlin et al., 2007), intestinal damage induced by ANFs (Yu et al., 2015) and the deficiency of one or more essential amino acids (Bautista-Teruel et al., 2003), less palatable (Torstensen et al., 2008) and the presence of ANFs (Welker et al., 2016). On the contrary, there are also studies that substantially found that plant proteins can potentially substitute fish meal in the diet of fish without having negative effect on growth and feed intake.

There are several types of plant-based ingredients that have been assessed in nutritional studies for aquaculture. The most frequently used plant protein sources are legumes including soybean, pea and lupin (Kaushik et al., 2004; Dias et al., 2005; Pereira et al., 2002; Glencross et al., 2004); corn gluten meal, cereal concentrates, and wheat have also been tested as feed ingredient for European sea bass, turbot, Atlantic salmon and carp. Corn gluten meal is a good source of leucine however, it is deficient in lysine and arginine. Wheat gluten meal is a good source of cystine but it is deficient in lysine. Canola meal and rapeseed meal on the other hand has a well-balanced essential amino acid profile but contains some ANFs including glucosinolates, protease inhibitors, phytic acid, tannins, and non-starch polysaccharide, oligosaccharides. Copra meal is a rich source of arginine but it is very low in lysine and methionine and contains several ANFs such as phytic acid, tannins, and non-starch polysaccharides, oligosaccharides. Cottonseed meal contains good level of arginine but low in methionine and lysine and also contains several ANFs such as phytic acid, estrogenic factors, gossypol, anti-vitamin E factor and cyclopropenoic acids. Although palm oil is also rich in arginine, however it is deficient in lysine and contains high levels of non-starch polysaccharides, oligosaccharides. Peanut meal is rich in arginine but low in lysine and methionine and contains protease inhibitors, phyto-haemagglutinins, phytic acid, saponins and estrogenic factors. Sunflower seed meal is a rich source of tryptophan and arginine but deficient in lysine and tyrosine and contains protease inhibitors, tannins and arginase inhibitor. Lupin seed meal is a rich source of arginine but deficient in methionine and lysine, moreover it contains protease inhibitors, phytohaemagglutins, cyanogens, phytic acid, tannins and allergens. Pea seed meal on the other hand has low methionine and contains protease inhibitors, phyto-haemagglutins, cyanogens, phytic acid, saponins and anti-vitamin E factor. Soybean meal is known to be rich in tryptophan but low in methionine and lysine and contains several ANFs including protease inhibitors, phytic acid, saponins, estrogenic factors, flatulence factor, anti-vitamin E/A/D/B12 factors, allergens, and non-starch polysaccharides, oligosaccharides (Tacon et al., 2009). Plant based protein sources including soybean meal (SBM) and corn gluten meal (CGM) are considered candidate ingredients as fish meal replacements (Gatlin et al., 2007). Among plant protein sources, soybean meal is already used as replacement for fish meal and some other possible alternative ingredients including corn gluten meal, canola meal, cottonseed meal, palm kernel meal and peanut meal can also be considered (Chakraborty, 2019).

Soybean meal

Globally, soybeans are the fourth leading crop produced by volume and more than 85% is further processed into soybean meal and soybean oil. Soybean meal is the by-product after soybean oil extraction. Due to its good protein content, soybean meal is typically used as an animal feed while soybean oil is mostly utilized for human food and biodiesel production. Soybean meal production have satisfied the global demand for protein and an annual increase of 1.6% on its production is expected until 2027. Most of the production originates from the USA, Brazil and Argentina. Mainly, the increase have been driven by the increasing demand in China (OECD/FAO, 2018). In 2014, soybean production reached up to 190 million tons wherein 54 MT were produced by China, the lead producer followed by USA which produces 37 MT, then 29 MT from Argentina, 27 MT from Brazil, and 10 MT from European Union (Gyan et al., 2019). The international price of soybean meal in year 2004-2016 ranges between US $259- US $563 per tonne. The price of soybean meal in China is US $ 437 per tonne, US $485 per ton in India and US $400 per tonne in Zimbabwe (FAO, 2018). Soybean meals are considered as one of the largest volumes of both plant protein meals and feed ingredients resources available in the world.

A range of processed soybean products such as protein concentrates (SPC) and soy protein isolates (SPI) have also been utilized in the aquaculture feed sector for several fish species (Glencross, 2016). Soy protein concentrates are produced by removing the soluble carbohydrate fraction and some flavor compounds from the defatted meal through acid leaching, aqueous ethanol extraction and moist heat-water leaching. These processes makes protein insoluble while preserving some portion of the carbohydrates soluble in order to separate the two during centrifugation (Guo, 2009). SPC has protein content up to 65% and reduced concentration of ANFs (NRC, 2011). It has good amino acid levels but deficient in methionine and lysine. Moreover, the extraction process significantly lowers the lectins, trypsin inhibitor, saponin and oligosaccharides (Drew et al., 2007). For aquaculture applications, the soybean industry has been promoting the inclusion of SPC in aquaculture feed (USSEC, 2008) although its price (US $1000 per tonne) is a little expensive than SBM (USDA, Gro Intelligence). In 2018, the global soy protein market was valued at US $9.7 billion and by the year 2026, it is expected to reach US $16.6 billion as a compound annual growth rate of 6.9% (Globe Newswire, 2019). About 500,000 mt of SPC was made and about 70% of the produce was utilized for human food application (Hardy, 2010). In aquafeed, SPC have been successfully included as replacement for fish meal at 50% level in rainbow trout and Atlantic salmon (Medale et al., 1998; Refstie et al., 2001). Another processed soybean product is SPI which is the end product when the protein is extracted and precipitated from the defatted flakes, which contains 90% protein (Peisker, 2001). The process of producing SPI enables the removal of heat-stable oligosaccharides and antigens (Cromwell, 2000). SPI is highly refined and purified form of soy protein, most of its non-protein components, fats and carbohydrates were removed (Kalma, 2014). The price of SPI ranges US $1000-1500 per tonne and by year 2026, it is expected that the soy protein isolate segment will reach up to US $5.3 billion (Globe Newswire, 2019).

Particularly SBM is extensively used as protein source in animal diets as its amino acid profile is good, highly available and low cost (Carter & Hauler, 2000; Cheng & Hardy, 2003). Normally, SBM contains 48% crude protein, however it contains ANFs such as trypsin inhibitor, saponins, lectins and oligosaccharides which could have negative impact on nutrient digestibility and fish growth performance. The relatively low protein content of SBM limits its use in high-energy diets because very little room in the formulations for ingredients that are not somewhat purified. Defatted soybean meal is one of the common alternatives to fish meal due to its well-balanced amino acid profile, moderately high protein content, consistent quality, relatively low cost and high domestic availability (Sales, 2009). The first limiting amino acid in SBM as fish feed ingredient is methionine (Storebakken et al., 2000) and also lysine and threonine (Gatlin et al. 2007). SBM has been utilized as fish feed ingredients in several fish species. For salmonids, the use of SBM is limited because of its relatively low protein content and it causes intestinal enteritis in cases where prolonged use of feeds containing 30% SBM (Krogdahl et al., 2003). In order to increase protein content, reduce antinutritional substances, reduce indigestible components or improve digestibility, soy bioprocessing technologies are being optimized (Refstie et al., 2005).

Corn gluten meal

The world production for corn in 2018 is 1.14 MT and the highest producer by region are from Americas (52.5%), followed by Asia (29.1%), Europe (11.2%), Africa (7.1%) and Oceania (0.1%). The top 10 corn producers of corn in year 1994-2018 are, USA, China, Brazil, Argentina, Mexico, India, Indonesia, France, Ukraine and South Africa. (FAOSTAT, 2020). The average price for corn from China in 2018 is US $250 per tonne (FAO, 2018). It is expected that corn consumption will increase by 16% by 2027 because of the increasing demand from animal feed due to the expanding livestock sectors in developing countries which holds the largest share of total utilization, 56% (OECD/FAO, 2018).

Corn gluten meals (CGM) are co-products of the corn wet-milling industry which produces products including corn meal, defatted corn germ protein and corn protein isolate (Colmenero, 2014). CGM is a dried residue generated from corn after the larger part of the starch and germ have been removed and the bran separated by the process of wet milling during the manufacture of corn starch or syrup. It is considered to be cost-effective alternative protein source for aquafeed due to its high available protein (60-70%, dry matter), low fiber content and anti-nutritional factors, steady supply and competitive price (Glencross, 2016). CGM production remains relatively constant as ethanol is mainly produced by dry-milling which produces corn distillers rather than corn gluten meal. The average cost of corn gluten meal in 2017 was US $466 per tonne (Feed Outlook, 2017). In salmon and several marine species including sea bass and sea bream, CGM is widely used. Though it is highly digestible, it is deficient in lysine thus generally it can only be included in the diet in 10-15% inclusion level (Gatlin et al., 2007). According to Hardy (2010), it is necessary to supplement feeds containing high amounts of corn gluten meal with synthetic lysine or to blend corn gluten meal with soy or wheat protein concentrates to produce an improved amino acid profile.

Corn gluten meal is broadly used for salmon and marine fish feeds (Gatlin et al., 2007). CGM has also high protein value, acceptable amino acid profile and is commercially available (Pereira & Oliva-Teles, 2003). According to Morales et al. (1994) corn gluten is an excellent protein source for aquafeed with at least 60% protein content. Sugiura et al. (1998) reported that corn gluten is 97% digestible in trout. Nevertheless, in trout generally corn gluten meal can be added up to 10% but the works of Hardy (2000) and Morales et al. (1994) shows that corn gluten meal can replace 25-40% of fish meal without compromising growth performance of the fish. In gilthead seabream juveniles, CGM can replace up to 60% fish meal protein without any negative effects on fish growth performance (Pereira and Oliva-Teles, 2003). Moreover, in juvenile Japanese flounder, CGM can also replace fishmeal up to 40% (Kikuchi, 1999). Likewise, the work of Robinson et al. (2001) showed that CGM can be included in the diet of channel catfish up to 50% without having a negative effect on feed palatability, fish growth and feed efficiency.

When using corn gluten meal at higher inclusion levels, it tends to cause yellowish color to the flesh of the fish. However, if canthaxanthine or astaxanthine will be added to the trout diet which could mask the yellow color of the flesh of the fish, then corn gluten meal could be added up to 22.5% both in salmon and trout commercial diets (Skonberg et al., 1998). However, the effect of the yellow pigments (xanthophylls) present in corn-co products could be different depending on the fish species. In tilapia, the study of Herath (2016) have shown that no color change was observed in the fillet of the fish given diets containing corn co-products.

Other corn derived protein sources includes distiller dried grain with solubles (DDGS) and high protein distiller dried grains (HPDDG). DDGS is the co-product of the ethanol industry and commonly used as fed for ruminants because of its high fiber content and variability of nutrients (Singh et al., 2005). Distillers dried grains and distillers dried grains with solubles are rich in nutrient content compared to whole corn. During ethanol making process the residue remaining after distillation is separated into ethanol and stillage. The stillage contains a solid portion known as distillers grains and soluble portion known as solubles. The soluble portion also contains a lot of nutrient. The crude protein content of the conventional DDGS ranges 28-32% which is insufficient to be a protein concentrate. However, technologies have been developed to increase its protein content to 40% by removing fiber in order for DDGS to be a suitable ingredient to be incorporated in the feeds for omnivorous fish species (Hardy, 2010). Ethanol production are increasing exponentially thus DDGS production is also expected to increase proportionately (Kim et al., 2008). In 2019 the production of DDG from ethanol reaches 38 million MT of which 28 million MT were utilized for feed and residual purposes (USDA, 2020). The average cost of DDGS in 2017 was US $99 per tonne (Feed Outlook, 2017). The success in the application of DDGS in aquafeed was reported by Herath et al. (2016) wherein they found out that it could fully replace fish meal in Nile tilapia diets by improving growth performance and proximate composition of the fish. HPDDG on the other hand is produced by removing the nonfermentable fractions, bran, pericarp fiber, and germ before fermentation so that the modified product will contain higher protein levels and decreased levels of fiber without solubles (Gibson & Karges, 2006). Its nutritional value is more consistent than that of DDGS (Robinson et al., 2008).

Another gluten meal that is also a prominent alternative to fish meal is wheat gluten which is a proteinaceous material from wheat after starch extraction. In extruded feed, it can act as pellet binder, it has high quality protein source, highly digestible, has good amino acid profile; however, it is low in lysine, tryptophan and arginine (Apper-Bossard et al., 2013). Its cost in 2005 is US $ 1022 per tonne (Hasan et al. 2007). Unlike soy products, wheat gluten contains less carbohydrate and anti-nutrients that could limit its use in feeds. (Hardy, 2010). Although wheat gluten is expensive, there are studies in salmonids supporting that it can replace fish meal when the diets are supplemented with free lysine (Pfeffer et al, 1995; Davies et al., 1997).

Blend of plant ingredients

In rainbow trout, mixture of pea, horse bean and rapeseed can replace 44% of the total dietary protein fish meal without causing any negative effect on fish performance (Lund et al., 2011). Also, according to Lee et al. (2010), plant based diets consisting of corn gluten, yellow soy protein concentrate and wheat gluten meal supplemented with limiting amino acids and highly available inorganic phosphate when fed to rainbow trout replaced 100 % of fish meal without affecting the growth performance and feed utilization. Comparably, in Senegalese sole, Valente et al. (2016) found that fish growth performance was not impaired when fed with diet containing the blend of plant proteins such as soybean meal, peas, corn gluten and wheat. The work of Bonaldo et al. (2011) reported that the mixture of soybean meal, wheat gluten meal and corn gluten meal fed up to 39% levels did not show any negative effect on growth and nutrient utilization. Hansen et al. (2011) worked on Atlantic cod and they have discovered that with or without the addition of lysine and methionine, fish meal can be replaced by the mixture of plant proteins up to 65% as supported by the no significant effect on the growth rate of the fish. Total replacement of fish meal is also possible as revealed in the work of Ahmed et al. (2018) wherein diet containing plant protein mixture (soybean meal, cottonseed meal, canola meal and peanut meal) supplemented with essential amino acids (L-lysine, DL-methionine and L-threonine) totally replace fish meal in the practical diet of blunt snout bream. Moreover, the work of Gomez-Requeni et al. (2004) also showed a success in total replacement of fish meal in the diet of gilthead sea bream with mixture of corn gluten meal, wheat gluten, extruded peas and rapeseed meal with balanced essential amino acids. In rainbow trout, mixture of corn gluten, yellow soy protein concentration and wheat gluten meal supplied with limiting essential amino acids and inorganic phosphate replaced fish meal at 100% level of inclusion (Lee et al., 2010).

Processing techniques

Plant protein ingredients replacing fish meal has also concerns because the quality and concentration of plant proteins are generally inferior to fish meal and its palatability is also relatively lower than fish meal. However, their cost and availability possess advantage over fish meal, and this may allow plant ingredients to undergo further processing to improve their nutritional value (Drew et al., 2007). Below are some of the processing techniques that can be used to improve nutritional value of plant protein ingredients.

Protein concentrate

According to Hardy (2010), the most promising alternative protein source that are used in aquafeeds are high-protein concentrates produced from soy, wheat and other grains or oilseeds. In the case for soy protein concentrate, it is produced from a high-quality soybean, selected, cleaned, dehulled, then oil is extracted to produce the residue, defatted white flakes. The soy white flakes will be subjected to aqueous alcohol extraction to remove the soluble carbohydrates and lower the levels of ANFs including lectins, trypsin inhibitors, glycinin, B-conglycinin, saponins and oligosaccharides (USSEC, 2008). Kaushik et al. (1995) reported that soy protein concentrate did not cause intestinal enteritis in salmonids.

However, soy protein concentrates contain much higher phytic acid than soybean meal (Hardy, 2010). Other plant-derived protein ingredients including lupin and rapeseed protein concentrates have been developed and researched as potential fish meal replacement (Hardy, 2010). Lupin protein concentrate contains 61% crude protein and its amino acid is comparable with that of soybean (Booth et al., 2001). According to Drew et al. (2007), it has a great potential as an aquafeed ingredient. Protein concentration also improved the protein content in peanut as reported in the study of Yu et al., (2007) wherein, peanut protein concentrate contains 85% protein while only 50% protein in the defatted peanut flour that is used as raw material for peanut protein production.

Fermentation

Another viable processing technique in reducing the undesired substances and to enrich the nutritional value of plant ingredients is through fermentation (Shi et al., 2015). It is based on utilizing microorganisms that can exhibit its beneficial role if reared under specific conditions (Siddik et al., 2018; Sugiharto & Ranjitkar, 2019). Fermentation could increase the content of crude protein and decrease the content of crude fiber, ANFs and toxic contents in feed ingredients (Drew et al., 2007; Imourou et al., 2008; Jakobsen et al., 2015). According to Refstie et al. (2005), fermentation by either bacterial or fungal organisms may be used to reduce the negative effects of soybean oligosaccharides on nutrient digestibility and growth performance in fish. Moreover, yeast, bacterial and fungal fermentation have been investigated and demonstrated potential for removing antinutrients and adding essential nutrients such as protein and amino acids (Bairagi et al., 2004; Mukhopadhyay & Ray 1999). The type of microorganisms and plant substrates used in the fermentation will determine the nutrient and antinutrient profile of the product (Gatlin, 2007). According to Jannathulla et al. (2017), fungal fermentation is more suitable for improving the nutritional value of plant protein sources such as soybean meal, groundnut oil cake, rapeseed meal, sunflower oil cake and guar meal.

Enzyme supplementation

The use of enzyme as supplement is also another potential aspect to increase the nutritional value of alternative ingredients. One category of enzyme supplement is those that break down fiber and certain carbohydrates found in protein sources from grains and oilseeds (Hardy, 2000). Debnath (2003) reported that apparent net protein utilization and digestibility in Atlantic salmon were improved by enzyme supplementation. Also, studies on catfish and trout have demonstrated the effectiveness of phytase in increasing phosphorus availability (Li & Robinson, 1997; Vielma, 2000). Moreover, Cao et al. (2007) mentioned that supplemental phytase can enhance digestibility and bioavailability of phosphorus, nitrogen and other minerals, reduce the amount of inorganic-P supplement to maximize growth and bone mineralization, and markedly decrease P load to aquatic environment. Phytase is an enzyme which is microbial in nature and as most monogastric animals like fish cannot produce phytase, thus it must be supplemented by inclusion in the formulated diet of plant origin (Gabriel et al., 2007). However, unlike fermentation and extrusion cooking process, by nature, enzyme supplementation is heat sensitive thus it cannot be included before pelleting but must be supplied only after pelleting.

Extrusion cooking

The technology of feed manufacturing is one of the important factor in the production of a good quality aquafeed. Extrusion cooking technology has been used both in food and feed industries. In the food industry, extrusion technology has a huge impact towards shaping and deriving ready to eat products (Fellows, 2009). Due to its versatility, cost effectiveness, environmental friendliness and better production output, the use of extrusion in food processing has relatively increased (Guy, 2001). In the aquafeed industry, extrusion cooking has also been used to improve the nutritional value of the feed material. The major advantages of extrusion processing involves, energy efficiency, continuous high throughput, improved textural and flavor characteristics and expanded products form. In determining the quality output of the extruded product, processing parameters play a very significant role. Feed rate, screw speed, barrel temperature, water content, feed formulation, screw and die configuration covers the primary process parameters while secondary process parameters include die, temperature, pressure and torque (Chessari & Sellahewa, 2001). Moreover, according to Bailey et al. (1995), pre-conditioning treatment of the raw materials using hot water or 4-5 minutes of steaming helps in gelatinization of starch and protein denaturation of the raw materials during extrusion processing.

Heat treatment commonly reduces ANFs and gelatinizes carbohydrates resulting to increased digestibility (Gomes & Kaushik, 1995; Altan et al., 2009). In Coho salmon diet Arndt et al. (1999) reported that heat treatment of defatted soy flour (SF) using autoclave lowered trypsin inhibitor to 1.8 from 181 (trypsin unit inhibited, TIU) and fish feed diet with heat-treated SF had higher weight gain than those fed untreated SF. Likewise, in common carp using soaked and soaked + autoclaved Sesbania seeds resulted to improved fish growth and feed utilization compared to untreated seeds (Hossain et al., 2001). But excess heat processing can also negatively affect the quality of the raw material; protein availability or digestibility may be reduced and amino acids, such as lysine, arginine and cysteine may be destroyed (Barrows et al., 2007; Zhang & Parsons, 1996). These unwanted denaturation effects on proteins, amino acids, starch and vitamins can be avoided if the raw material is processed at high temperature (200°C) for only a short time (30 – 45 seconds) (Mościcki & Zuilichem, 2011).

Extrusion cooking is a high-temperature short-time (HTST) process of heating, mixing, shearing, and forcing material through a die (Kokini et al., 1992; Cheng & Hardy, 2003). The principle of this processing technique involves, loading the raw material in the feeding hopper where the screw conveys through the raw materials. The volume will be reduced when the raw material pass down the barrel, thereby the food is compressed under pressure into a semi-solid, plasticized mass (Fellows, 2009). Before entering the extruder, the material could be partially or completely cook in a conditioner which is an assembly that adjusts moisture content and temperature of the ingredients (Riaz, 2000). Also for drying purposes, extrudates are dried to a moisture content of 6-8% and a simple rotating drums with electric heater or with a gas operated hot air installation working at temperatures above 100°C can be used. Then, cooling takes place at ambient temperature of 15-20°C (Mościcki & Zuilichem, 2011) . A photo of extruder is shown in Figure 1 and sample extrudates in Figure 2.

In aquafeed, there are two types of extruders that have been used: the single screw extruder and the twin screw extruder. The latter is a better design because one screw wipes out the cavity of the other screw ensuring positive displacement of feed materials through the barrel thus preventing the burning out of products that is prevalent in single screw extruder. In single screw extruder, it requires elaborate drying thus utilizing higher energy. In contrast, in twin screw extruder, lower moisture content of the extrudates requiring no or short time in drying (Vijayagopal 2004). Generally, single screw extruder is cheaper because of its simple construction that twin screw extruder but they are more likely to block than twin screw extruders (Senanayake & Clarke, 1999). In contrast, twin screw extruder is able to process materials including high lipid content thus in application, this type of extruder enables to produce high energy extruded pellet for marine fish and salmon beyond 40% crude lipid content. In addition, the differences between single screw and twin screw extruder was summarized in Table 2 of Appendix 1 according to Ilo et al. (2000).

This processing technique can enhance the nutritive value of several plant ingredients and even reduce or inactivate antinutritional substances (Hardy, 2010; Nwabueze, 2007; Bhandari et al., 2001; Carvalho & Mitchell, 2000). Increased digestibility value for proteins, amino acids and nitrogen may be achieved through extrusion cooking (Sagum & Arcot, 2000; Al-Marzooqi & Wiseman, 2009). The process may cause modification of the side chains of amino acids, denaturation of protein and inactivation of antinutritional substances (tannin, phytate and trypsin inhibitor) which could lead to increased digestibility of the raw material (Bhandari et al., 2001; Carvalho & Mitchell, 2000). With these beneficial effects of extrusion cooking, several studies were conducted to apply the process in feed industry.

Extrusion of soy white flakes improved the digestibility of protein and all amino acids due to the reduction of trypsin inhibitor (Romarheim et al., 2005). Likewise, the nutritive value of canola meal fed to chinook salmon was improved through extrusion cooking by increasing protein and lipid levels while reducing phytic acid concentration (Satoh et al., 1998). According to Singh et al. (2007), with respect to the nutritional content of the final product the advantages of extrusion cooking involves the inactivation of antinutrients, destruction of aflatoxins and increasing digestibility of fibers. In terms of mineral utilization, SBM extruded at 145°C is a suitable ingredient to substitute fish meal in rainbow trout diet (Satoh, et al., 1997). Furthermore, extruded SBM can potentially replace fish meal in kuruma shrimp, *Penaeus japonicus* diet up to 67% without any negative effect on growth, feed intake and digestibility (Saitoh et al., 2000).

Effect of extrusion on raw material composition

Protein

Protein undergo many changes during extrusion process and the most important change is the denaturation (Camire, 2000). The structure of proteins influences enzyme accessibility for protein digestion (Salazar-Villanea et al., 2016). During denaturation, the hydrophobic groups are uncovered resulting in a decreased solubility of the proteins in aqueous solution. The unfolding of protein molecules during denaturation renders them more susceptible to digestion by proteolytic enzyme. According to the work of Singh et al. (2000), after extrusion cooking, the enzyme hydrolysis of protein is improved as a result of the inactivation of antitrypsin activity in extruded products suggesting that extrusion cooking considerably improved the nutritive value of proteins. Furthermore, the effects of extrusion processing on soybeans and oilseeds or legumes includes improved protein digestibility and bioavailability of sulphur amino acids through thermal unfolding of the major globulins, and thermal inactivation of trypsin inhibitors and other growth-retarding factors including lectins (Serrano, 1997).Extrusion of field peas (*Pisum sativum*) at 75, 115 and 155°C increased the standardized ileal digestibility of crude protein from 81 % in untreated field peas to 89, 94 and 92 % for the extrusion treatments, respectively (Stein & Bohlke, 2007). The work of Drew et al. (2000) showed that, in rainbow trout, the ADC of protein of extruded lupin is significantly higher than in fish meal group. In addition, for turbot, the highest ADC values of protein were also recorded for extruded lupin.

Lipid

During extrusion processing, lubrication effects has been exhibited in lipid resulting to a reduced friction between particles in the mix as well as between the barrel surfaces, the screw and the fluid melt (Guy, 2001). Lin et al. (1997) found that lipid with higher fat content has a lubricating effect that could reduce the friction with higher screw speed, and, therefore, resulted in a lower degree of starch gelatinization. While lipids can act as lubricants during extrusion, the amount of lipid content can affect the properties of the extrudates. The presence of lipids in less than 3% does not affect extrusion, however, in quantities above 5% can reduce expansion rate, and above 10% they reduce slip within the extruder barrel, making extrusion difficult (Camire, 2002).Extrusion process can also disrupt the cell walls which could increase the migration of oil outside from the raw material thus producing meal samples with less oil contents (De Pilli et al., 2005). Furthermore, when utilizing high fat material such as whole soy, some lipid might be lost at the die as free oil thus obtaining a lower lipid content of the final material. This lower lipid content could be attributed to the formation of complexes with amylose or protein (Camire, 2000).

Fiber

In terms of dietary fibers, extrusion cooking could improve the total dietary fiber of barley flours as a result of an increase in soluble dietary fiber which could be attributed primarily to a shift from insoluble dietary fiber to a soluble dietary fiber as well as the formation of resistant starch and enzyme-resistant indigestible glucans formed by transglycosidation (Singh et al., 2007). Extrusion process does not significantly change the dietary fiber content rather it solubilizes some fiber component.

Vitamins, carotenoid and mineral

The work of Sing et al. (2007) reported that heat-sensitive vitamins were lost during extrusion. Vitamins A, C, D, and E are also sensitive to oxidation, so these vitamins have minimum retention during storage of extruded food (Riaz et al. 2009). In the presence of oxygen and heat, Vitamin A and related carotenoids are not, therefore, they are particularly vulnerable during extrusion (Verma et al. 2018). Li et al (2009) reported that Vitamin A stability generally declined as post extrusion storage temperature increased. Moreover, Vitamin E is also unstable during extrusion processing and even in storage of extruded food. Ascorbic acid directly added or coated with fat and then added to feed during extrusion is also very unstable (Riaz et al. 2009). The typical solution to potential losses of vitamin C during extrusion is through surface application and according to Sriburi et al. (2000) Ascorbic acid added to cassava starch was retained by at least 50%. In contrast, the study of Anderson & Sunderland (2001) ascorbyl-2-monophosphate (ROVIMIX® STAY-C® 35) and astaxanthin (CAROPHYLL® Pink 8%) were found to be fairly stable during extrusion. Astaxanthin stability in the final product was found to be most dependent on extruder discharge moisture and dryer processing temperature. However it has also been reported that, during post extrusion storage after 3 month, 10% of astaxanthin was lost (EFSA, 2005). Furthermore, Athar et al. (2006) reported that the retention of B vitamins after extrusion processing of cereals was 44-62% and the stability of riboflavin (Vitamin B_2) and niacin (Vitamin B_3) were improved.

Minerals are heat stable and unlikely to become lost in the steam distillate at the die. Generally, extrusion cooking could affect the macro minerals though micro minerals can also be affected by either the extrusion process itself or by changes in larger molecules (Singh et al., 2000). According to Alonso et al. (2001), extrusion can improve the absorption of minerals by reducing other factors that inhibit absorption. Phytate may form insoluble complexes with minerals and eventually affect mineral absorption.

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Appendix 1

Table 1. Common anti-nutritional factors present in plant ingredients for aquafeed.

Anti-nutritional factors	Plant-derived-source	Effects in fish	Means of alleviation
Phytic acid	soybean, pea seed meal, cottonseed meal, sesame meal	Interact with mineral availability and decrease protein digestibility	Use of phytase, heat treatment
Protease inhibitors	soybean, rapeseed meal, lupin seed meal, sesame meal, alfalfa leaf meal	Interact with protein, hindering feed protein utilization	Heat treatment and autoclaving
Saponin	soybean meal, pea seed meal, lupin seed meal, sunflower oil meal	inhibit nutrient transport, depress growth performance, damage intestinal mucosa	aqueous extraction
Lectins	soybean meal, pea seed meal, lupin, faba beans	disruption of the small intestinal metabolism and morphological damage to the villi	aqueous heat treatment or autoclaving
Antivitamins	soybean meal, alfalfa leaf meal, pea seed meal, cotton seed meal	affect vitamin efficiency	heat treatment
Glucosinolates	rapeseed meal, mustard oil cake	thyroid dysfunction, growth depression	heat treatment, extracting with water
Gossypol	cottonseed meal	growth depression and internal organ abnormalities	pre-pressed and solvent extraction
Tannins	rapeseed meal, pea seed meal, mustard oil cake	binds with protein and minerals, reduce absorption of vitamin B_{12}	dehulling of seeds, autoclaving, alkali-treatment
Oligosaccharides and non-starch polysaccharides	soybean meal, sunflower meal	obstructing digestive enzymes and movement of substrates in the intestine	steam cooking, extrusion cooking
Alkaloids	Lupin seed meal	interfere with nerve functioning, lower feed intake	aqueous extraction, high temperature (145°C) extraction

Table 2. Differences between single screw and twin screw extruder.

Parameters	Single screw extruder	Twin Screw Extruder
Moisture content of ingredient (%)	11-28	11-35
Temperature (°C)	70-200	80-200
Lipid (%)	5-6	25
Screw speed (rpm)	40-200	70-500
Mixing efficiency	medium	medium-excellent
Productivity	medium	high
Total energy (kWhr/mt)	86.7	125.6
Energy cost ($/mt)	5.3	8.07
Wear cost ($/mt)	2.1	3.41
Total cost ($/mt)	7.4	11.48

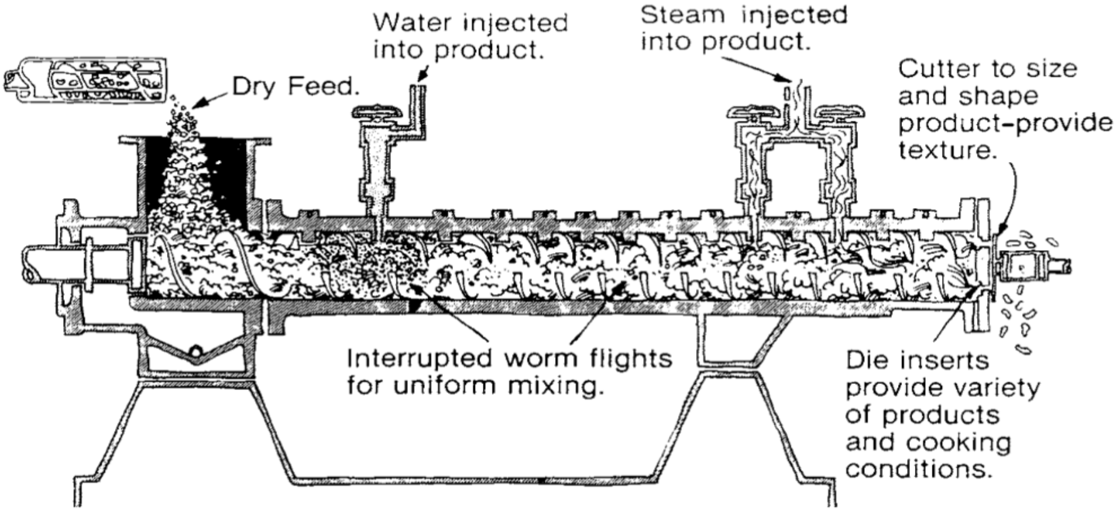

Figure 1. Cross section of extruder (Courtesy of Anderson International Corp., Cleveland, OH in: Extruders in Food Applications, 2000 (Eds) Mian N. Riaz).

Figure 2. Final product after extrusion, extrudates A (SBM, 100°C), B (SBM, 150°C), C (CGM, 100°C), D (CGM, 150°C)

CHAPTER 2

Effect of different thermal extrusion processing of soybean and corn gluten meals on their nutritional value for rainbow trout, *Oncorhynchus mykiss* diet

Abstract

Feeding experiment and digestibility trial were conducted to evaluate the effect of thermal extrusion processing of soybean and corn gluten meals on the nutritional value for juvenile rainbow trout diet. SBM and CGM were extruded at low temperature (100°C, LT) or high temperature (150°C, HT) for 30 seconds. Four isonitrogenous (41%, crude protein) and isolipidic (14%, crude lipid) diets were formulated. Control diet is fish meal based while a combined (1:1) non-extruded SBM and CGM for NE diet, LT SBM and LT CGM for LT diet and HT SBM and HT CGM for HT diet. Two hundred forty rainbow trout juveniles (12.5 g average body weight) were randomly divided into 12 rectangular 60 l glass aquaria and respectively fed with four different diets in triplicate. Fish were fed at satiation twice a day, six days a week for 12 weeks. Result of the feeding trial shows that the control group had the highest value for final body weight and weight gain which is significantly different ($P<0.05$) with NE and HT group but not with the LT group. Specific growth rate (SGR) of HT group had the lowest value and is significantly different with that of the control. Daily feed intake was not affected by the dietary treatments. Feed conversion ratio (FCR) of the control group is significantly different among all treatments and trend for the protein efficiency ratio (PER) was the same with that of the SGR. The result of the total amino acid composition of the fish body is noteworthy because the methionine level of both the LT and

HT group are low which reflects that of the methionine level of the respective diets. Moreover, the methionine level of the diets is below the requirement of rainbow trout to support optimum growth of the fish. Furthermore, the digestibility data had promising result as the protein digestibility was found to be highest for LT and HT group and phosphorus and manganese absorption was also found to be highest in HT group.

Background of the Study

The utilization of sustainable plant-based ingredient has become a research priority in trout production to decrease the reliance on fish meal as main protein source. Thus, this study was conducted to investigate the effects of extrusion cooking temperature on soybean and corn gluten meals as combined ingredients in the diet for juvenile rainbow trout, in terms of nutritional value, fish growth performance, feed utilization and apparent digestibility. Though effects of extruded SBM has been studied in rainbow trout, there is still no study examining the effect of the combination of extruded SBM and CGM in the fish species.

Material and Methods

Extruded ingredients and experimental diets

Extruded soybean and corn gluten meals were sourced from Nihon Nosan Kogyo, Japan. SBM and CGM were extruded each at 100°C or 150°C for 30 seconds making it a total of 4 extruded ingredients to be used in these studies.

Four isonitrogenous (44% crude protein) and isolipidic (14% crude lipid) experimental diets were prepared for the feeding trial. Control diet is fish meal-based while the main protein sources of the other three diets are combination (1:1) of non-extruded SBM and CGM (NE diet), LT SBM and LT CGM (LT diet) and HT SBM and HT CGM (HT diet). In addition, NE, LT and HT diets contained 15% fish meal. Moreover, chromic oxide was added to all diets at 0.5% as inert marker for the digestibility experiment following the method described by Cho & Watanabe (1985). Diets were prepared at the Fish Nutrition Laboratory, Tokyo University of Marine Science and Technology (TUMSAT, Shinagawa Campus), Tokyo, Japan. Prior to diet preparation, all dry ingredients including the extruded SBM and CGM were grinded using a centrifugal mill (ZM 200 model; Retsch GmbH, Haan, Germany) with 0.25-mm mesh size screen. Pre-weighed dry ingredients were mixed through a horizontal mixer (ACM-50 LAT model; Aikoh Saitama, Japan) for 20 min. Lipid sources were then added to the mixed dry ingredients and mixed again for 40 min. Then, a dough was formed by adding water at 25% and mixed for another 10 min. The dough was then subjected to a meat chopper (LCM22 model; Hitachi, Tokyo, Japan) in 3 repetition using a 3 mm die having a spaghetti-like noodles as an outcome. Then, manual sieving was done using 2.83 mm sieve to make it into a diet shaped fish feed. The diets were then freeze-dried in a freeze-dryer (REL 206 model; Kyowa Vacuum Tech., Tokyo, Japan) for 18 h, packed and stored at 4°C until use. Chemical analyses were conducted for both ingredients and all diets.

Chemical composition of ingredients and diets

The diet formulation is displayed in Table 3 of Appendix 2. The proximate composition of both the non-extruded SBM and CGM and the extruded ingredients (LT/HT SBM and LT/HT CGM) were analyzed and results are shown in Table 1 of Appendix 2. The crude protein content of the extruded SBM increased but in CGM it decreased. As for the crude lipid content, higher values were obtained by ingredients extruded at higher temperature (150°C). Values of the dry matter of extruded ingredients were slightly higher than the non-extruded ingredients. Ash content were similar for both extruded and non-extruded plant ingredients. Essential amino acid composition of extruded ingredients were slightly higher than non-extruded ingredients except methionine of SBM. Moreover, the fatty acid profile of the plant ingredients were shown in Table 2.

In Table 4, proximate and amino acid composition of the four experimental diets were displayed. The crude protein of the diets ranges 41% to 43.3% while crude lipid is 14%. For essential amino acid content of the diets, methionine level of the diets ranges between 0.45 to 0.62 and these are lower than the requirement of rainbow trout (0.81) to support normal growth and other metabolic activities with reference from the work of Ogino (1980). Moreover, the lysine level of NE and HT diets (1.71) were also lower than the requirement (2.10). Fatty acid profile of the diets was shown in Table 5 and the extrusion cooking temperature somehow improved the fatty acid level of the extruded ingredients.

Feeding trial

Feeding trial was performed with juvenile rainbow trout *Oncorhynchus mykiss* obtained from Oizumi Research Station, TUMSAT, Yamanashi, Japan and trouts were transported to the fish rearing room of the Laboratory of Fish Nutrition (TUMSAT, Shinagawa Campus), Tokyo, Japan. Before the onset of the growth trial experiment, fish were acclimatized to the rearing condition for 2 weeks and fed with rainbow trout commercial diet (Trout Feed 5P, Feed One Co., Yokohama, Japan). During stocking, all fish were pooled and placed in a circular 100-l fiberglass tank. 240 individuals with average initial weight of 12.5 g were selected and randomly distributed to twelve 60-l experimental tanks in a thermoregulated recirculating water system thermoregulated recirculating water system equipped with six sedimentation tanks (Bio-Clean 1000TR, Ｌ1050×W600× Ｈ750), sand filter, active carbon filter, and heat exchange devise (M6-MFML, Plate Heat Exchanger, Alpha Laval Co., Tokyo) with chiller unit (R407C, Hitachi Co., Tokyo) at 0.5 l min^{-1} flow rate. The water source is dechlorinated city water with finely ground active carbon. Sample of 30 fish were separated from the initial population for proximate body composition. Each diet was fed accordingly to triplicate group of fish twice daily at 10:00 h and 16:00 h to apparent satiation for six days a week. Continuous aeration (500 ml min^{-1}) in each tank was secured, photoperiod was operated on 12:12 h (light: dark) cycle and water temperature was maintained at 16 ± 0.06°C and pH level at 7.5 in the whole duration of the experiment. Also, NH_3 and NO_2 levels are weekly monitored by colorimetric kit and confirmed within safe levels for rainbow trout. To monitor growth and feed efficiency, sampling was done every 4 weeks by individually weighing all the fish in each tank after a 24 h fasting. At the end of the 12-week feeding trial, seven fish were sampled from each experimental tank and frozen (-30°C) for whole body composition analysis.

The following parameters were calculated for each treatment over a period of 12 weeks:

Survival (%) = $\frac{final\ number\ of\ fish}{initial\ number\ \ of\ fish}\ x\ 100$

Weight gain (WG) (%) = $\frac{final\ body\ weight\ (g) - initial\ body\ weight\ (g)}{initial\ body\ weight\ (g)}\ x\ 100$

Specific growth rate (SGR) (% day^{-1}) =

$$\frac{ln\ final\ body\ weight\ (g) - ln\ initial\ body\ weight\ (g)}{days\ of\ feeding}\ x\ 100$$

$$\text{Daily feed intake (DFI)} = \left[\frac{Total\ feed\ consumed\ (g)}{days\ x\ \frac{(initial\ body\ weight\ (g) + final\ body\ weight\ (g))}{2}}\ x\ 100\right]$$

$$\text{Feed conversion ratio (FCR)} = \frac{feed\ given\ (g)}{weight\ gain\ (g)}$$

$$\text{Protein efficiency ratio (PER)} = \frac{weight\ gain\ (g)}{protein\ intake\ (g)}$$

$$\text{Nutrient retention (\%)} = \frac{nutrient\ gain}{nutrient\ intake}$$

Phosphorus excretion (kg t^{-1}) = [FCR x P in diet (g) – P retained in fish (g)/production (t)] x 1000

Digestibility and Phosphorus Absorption

Apparent digestibility coefficients (ADC) for dry matter (DM), protein and lipid of the experimental diets were carried out two weeks before the end of the feeding trial. Feces were collected using the Tokyo University of Fisheries (TUF) column system as described by Satoh et al. (1992). The TUF column measures to be 5 cm in diameter and 30 cm high. Fish were fed the same experimental diets as described in the feeding trial as they already contain Cr_2O_3 as inert marker. After the first feeding time, every tank was cleaned from feces and uneaten feeds, then TUF columns were attached to each tank. Feces settled in TUF columns were collected 3 h and 6 h after attachment and frozen hereafter. Fecal collection was done for two weeks, then the collected feces were pooled per treatment, freeze dried, ground and stored in -30 °C freezer until analyzed. The apparent digestibility coefficients for DM, crude protein and crude lipid were computed according to Cho & Kaushik (1990).

$$\text{ADC of dry matter (\%)} = \left(1 - \frac{Chromium\ in\ feed\ (\%)}{Chromium\ in\ feces\ (\%)}\right)\ x\ 100$$

$$\text{ADC of nutrient (\%)} = \left[1 - \left(\frac{Chromium\ in\ feed\ (\%)}{Chromium\ in\ feces\ (\%)}\right) x \left(\frac{Nutrient\ in\ feces\ (\%)}{Nutrient\ in\ feed\ (\%)}\right)\ x\ 100\right]$$

Absorption of phosphorus was also measured using the following formula:

$$\text{Absorption (\%)} = 100 - \left[100\ x\ \frac{\text{Chromium in diet (\%)}}{\text{Chromium in feces (\%)}}\ x\ \frac{Element\ in\ feces\ (\%)}{Element\ in\ diet\ (\%)}\right]$$

Chemical analyses

Proximate composition of test ingredients, experimental diets, feces and whole fish body were determined in triplicate following the standard methods of AOAC (2005). Samples were oven dried to constant weight in an oven at 105°C to determine the dry matter content. Ash was measured through incineration in a muffle furnace at 600°C for 8 hr. Kjeldahl method was used to measure crude protein (N x 6.25) using a Kjeldahl analyzer (Kjeltec ™ 2400 model, FOSS Analytical AB, Höganäs, Sweden). Total amino acid was determined through an automatic amino acid analyzer (JLC-500/v; JEOL Co., Tokyo, Japan) after digestion of samples with 4M methanesulphonic acid (Sigma-Aldrich, St. Louis, MO, USA) at 110°C for 22 hr. Crude lipid was determined gravimetrically following the method of Folch, Lees & Sloane-Stanley (1957), to ensure sufficient separation of lipid, distilled water was added for the dry pellet. Phosphorus was analyzed in a UV-VIS spectrophotometer (UV-2550, Shimadzu) observing the method of Lowry & Lopez (1946) at 750 nm. Chromic oxide concentration was determined by acid digestion (Furukawa & Tsukahara, 1966) then absorbance was read using UV-VIS spectrophotometer (UV-2550, Shimadzu) at 350 nm. Phytate phosphorus content of plant ingredients was measured using the supernatant difference method described by Thompson & Erdman (1992) for phytic acid then absorbance was also measured using UV-VIS spectrophotometer. For mineral analysis, samples were first digested using $HN0_3$ adnH_2O_2 in Start D Microwave Digestion System then concentration was measured through ICP (Inductively Coupled Plasma) using SPS 7800 Plasma Spectrometer.

Statistical analyses

Results are presented as mean ± SEM (*n* = 3). Data were statistically evaluated using one-way analysis of variance (ANOVA) by means of SPSS software version 20. Significant differences (*P*<0.05) among treatment means were further compared using Tukey's Test.

Results

Growth trial

Result of the growth trial showed 95% to 100% survival and the final body weight and weight gain of the control group had the highest value and is significantly different (P<0.05) with HT and NE group but not with the LT group (Table 5). HT group is significantly different(P<0.05) from that of the control. On the other hand, daily feed intake was not influenced by dietary treatment. Furthermore, feed conversion ratio (FCR) was found to be best in control group and is significantly different (P<0.05) among all treatments. The protein efficiency ratio (PER) of the control group has significantly higher value than that of the HT group.

Apparent digestibility

Result of apparent digestibility coefficient (ADC), mineral (P, Zn, Mn) absorption, nutrient retention and phosphorus excretion is shown in Table 6 of Appendix 2. ADC for protein is already high (91.9% - 95.4%) and the highest value was obtained in LT group (95.4%) which is significantly different (P<0.05). Moreover, ADC for dry matter and lipid was not influenced by dietary treatments as no significant difference was not observed among all treatments. Findings of mineral absorption also shows noteworthy results. Phosphorus and manganese absorption were found to be highest in HT group and significantly different than those of the control group (P<0.05). Zinc absorption however was not affected by the dietary treatments. Nutrient retention in terms of protein was found to be significantly (P<0.05) different in NE group among others. Lipid retention was not affected by dietary treatments. Phosphorus retention of control and NE group were significantly different (P<0.05) than LT and HT groups. Phosphorus excretion of control and NE group were also significantly higher (P<0.05) than those of LT and HT groups.

Proximate body composition

Proximate body composition of rainbow trout in terms of crude protein, crude lipid and ash showed no significant differences among treatment means as shown in Table 7 of Appendix 2. However, moisture content of control and HT group had significantly higher values than the LT group. Fatty acid composition of the fish body is shown is Table 8 and the linolenic and linoleic acid of LT and HT group were a little higher than those of the control.

Discussion

Extrusion processing had some effects on the individual chemical composition of the ingredients in terms of protein and lipid and it also improved the ADC for protein as well as phosphorus and manganese absorption for rainbow trout. In soybean meal, crude protein of extruded SBM (49%, CP) was higher than non-extruded (44%, CP) and this trend is the same with that of the total essential amino acid. However, in the case of corn gluten meal, though the crude protein of extruded CGM were lower than the non-extruded CGM, the value for the total amino acid for extruded CGM was still higher than the non-extruded CGM. In this case, the changes of crude protein may not be due to changes of protein but to changes of non-protein N compounds. The lipid content on the plant ingredient was also affected by extrusion cooking temperature. Increased in lipid content was exhibited in high temperature extruded CGM and its linoleic acid also improved. According to Yagci & Gögus (2009), high temperature extrusion cooking could increase the content of total phenolics by releasing the bound phenolics from the cell matrix. The unsaturated fatty acids levels of the plant ingredients extruded at high temperature were improved in this study. This is in accordance to the work of Grela et al. (1999) wherein they studied the effect of high extrusion temperature on the content of unsaturated fatty acids in extrudates of green peas. They reported an increase in temperature from 100 to 160°C caused an increase in the content of linoleic and linolenic acid while there was slight difference in the content of oleic acid towards high temperature extrusion.

It is likely that extrusion temperature improved protein digestibility and mineral absorption in LT and HT diets. According to Areas (1992) and Kitabatake & Doi (1992), protein denatures during extrusion process which causes inactivation of antinutrients such as lectins and antitrypsin inhibitors resulting in the increase of protein digestibility. During the process, disulphide bond break and reunite while the high molecular proteins dissociate into smaller subunits (Guy, 2001). Furthermore, in this study, extrusion process can also enhanced absorption of mineral which is also evident in the work of Alonso et al. (2001) wherein they reported that the phytates and tannins form complexes with the minerals that inhibit mineral absorption. Extrusion cooking has resulted in breaking down the complex through hydrolysis thereby increasing mineral availability in the extrudates. In this study, Phosphorus and manganese absorption were improved in HT group but zinc absorption was not improved at all though Mn and Zn are both cations. This could be due to the lower binding affinity of manganese to phytate compared with zinc (Lönnerdal, 2002). It is essential to keep the diets environment friendly. In this study, extrusion cooking improved phosphorus retention thereby reducing P discharge. If nutrients are fed in excess and not retained in fish body, they will be discharged into the environment (Hernández et al., 2004).

The improved phosphorus retention could be attributed to the reduced ANFs and increased amount of available P in the diets that were utilized and retained in fish body.

The effect on chemical composition was found to be affected by the processing of the major protein source ingredient. Though not significant, the ADC for dry matter of LT and HT diets were higher than that of the control and NE groups. This suggest that extrusion processing of feed ingredients have a positive impact on the environment by reducing excretions of solids into the water. Likewise, no significant difference were also observed in ADC for lipid. This findings was comparable with the work of Barrows et al. (2007) wherein ADC for organic matter, lipid, energy or carbohydrates were not affected by extrusion cooking. ADC for protein however were also found to be higher in diets containing extruded ingredients, LT and HT. This simply demonstrated that extrusion cooking improved the protein digestibility in rainbow trout. Though was not analyzed in this study, the improved nutrient digestibility can be attributed to a partial degradation of antinutritional substances (Francis et al., 2001). According to Satoh et al. (1997), extrusion cooking at high temperature (150°C) decreases the phytic acid level in SBM from 1.4% to 1.0%. The value for ADC for protein obtained in this study was higher than obtained by Cheng & Hardy (2003) for extruded corn gluten meal, 75% for rainbow trout. Likewise, their work also shows that ADC of crude protein and the availabilities of amino acids in SBM were already high for rainbow trout. In contrast, according to Sørensen et al. (2002), extrusion cooking temperature alone had no effect on ADC for protein or energy in rainbow trout which could be due to endogenous losses of protein.

In rainbow trout, methionine and lysine are essential amino acids and lack of these amino acids could result to growth retardation due to inefficient protein synthesis. Moreover, methionine is important because it is involve in post-translational modification of gene via histone methylation. In this study, extrusion cooking of SBM and CGM at low temperature (100°C) improved fish growth while those extruded at high temperature (150°C) did not. This could be due to lower methionine and lysine levels of the diets which are lower than the requirement of the rainbow trout to support optimum growth and other metabolic activities. Fish feeds that is mostly composed of plant ingredients are typically low in methionine (Belghit et al., 2014). Methionine is an essential amino acid to support normal growth of most animals. It has been reported in chicks and mammals that methionine-free diets resulted to lower rates of whole-body protein synthesis associated with lower mRNA translation efficiency (Kino & Okumura, 1986). It is known that methionine plays a very important role in mRNA translation as the primary amino acid that is needed as initiation signal for protein synthesis (Bradshaw et al., 1998). Beside their role as building block for protein synthesis, amino acids are also essential as signaling molecules in the regulation of cellular growth and metabolism (Skiba-Cassy et al., 2016). The study of Sèitè et al. (2018) showed a drop of growth performance in rainbow trout fed with a diet deficient in methionine for 6 weeks. This observation is due to a general mitochondrial defect and a decrease of the oxidative status in the liver. The authors have demonstrated that methionine availability in rainbow trout diet is essential for mitochondrial integrity. Skiba-Cassy et al. (2016) also suggest that addition of crystalline amino acids is necessary in fish diet when utilizing plant proteins in order to meet the essential amino acid requirements of the fish. Likewise, several authors recommend adjusting methionine supplementation to meet the requirement of the fish and to avoid disturbance of metabolism, feed intake and growth and to reduce nitrogen losses in fish (Tulli et al., 2010; Saravanan et al., 2013; Figueiredo-Silva et al., 2015). In addition, although phosphorus availability was not quantified in this study, fish growth may also be affected due to insufficient dissolved phosphorus in the diet since no supplementation in the form of tri-calcium phosphate was supplied in the diets. According to Ogino & Takeda (1978), the available phosphorus level required to maintain a normal growth for rainbow trout is 0.7 to 0.8% of their diet. It could be possible that fish diets in this study contained lower available phosphorus than the required amount.

In the present study, as feeding rate was not affected by the dietary treatments, palatability could not be the reason on the reduced growth of the fish. Although ADC for protein was higher in LT and HT groups, SGR and PER of HT group were found to be lowest while highest also for FCR. Feed efficiency had similar changing trend with growth performance as expected as growth is significantly driven by protein deposition. Likewise, the trend of change in the body proximate composition (Table 8) of rainbow trout also reflects that of the nutritive value of the dietary treatments. Methionine level of fish carcass reflects the methionine level of the dietary treatments.

Conclusion

Extrusion processing is widely used in several production industries including aquafeed industry. However, thermal condition during extrusion cooking had an effect on the nutritional value of SBM and CGM in relation to growth and feed efficiency in rainbow trout. In this study, it was demonstrated that extrusion of soybean and corn gluten meal could be helpful for improving feed performance of rainbow trout as extrusion cooking of SBM and CGM at low temperature improved fish growth. Moreover, phosphorus and manganese absorption were also improved by extrusion cooking. However, it is recommended to supplement limiting amino acid including methionine to support optimal growth of the fish.

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APPENDIX 2

Table 1. Proximate and amino acid composition (% dry matter) of non-extruded or extruded SBM and CGM

Measured composition	SBM	LT SBM	HT SBM	CGM	LT CGM	HT CGM
Dry matter	91.0	95.5	95.4	91.8	97.2	93.7
Crude protein	44.3	49.1	48.0	65.7	61.8	61.7
Crude lipid	1.93	1.75	3.39	4.72	1.95	5.10
Ash	6.43	6.00	6.32	1.24	2.31	2.33
Essential amino acids						
Threonine	1.23	1.44	1.64	1.14	1.62	1.62
Valine	1.40	1.40	1.86	1.26	2.04	1.91
Methionine	0.34	0.29	0.19	0.28	0.31	0.53
Isoleucine	1.36	1.84	1.95	1.15	2.01	2.29
Leucine	2.68	4.69	3.14	4.64	7.19	8.69
Phenylalanine	1.71	2.26	2.00	1.78	2.80	3.35
Histidine	0.90	0.94	1.03	0.58	0.91	1.05
Lysine	2.30	1.90	2.59	0.60	1.16	1.23
Tryptophan	0.14	0.11	0.19	0.10	0.14	0.11
Arginine	2.64	2.40	3.03	0.93	1.68	1.98
Non-essential amino acids						
Aspartic acid	3.41	3.55	4.59	1.59	2.91	4.59
Serine	1.77	2.00	1.99	1.53	2.24	1.98
Glutamic acid	3.19	7.27	7.41	1.66	8.42	7.42
Glycine	1.59	1.12	1.76	0.89	1.00	1.76
Alanine	1.54	2.17	1.79	2.55	2.74	1.79
Cysteine	0.37	0.40	0.39	0.31	0.45	3.91
Tyrosine	1.36	1.83	1.57	1.52	2.37	1.58
Proline	1.93	1.89	2.09	2.61	3.99	2.09
Total amino acid	29.86	37.5	39.21	25.12	43.98	47.88

Table 2. Fatty acid composition (% dry matter) of non-extruded or extruded SBM and CGM

Fatty acid	SBM	LT SBM	HT SBM	CGM	LT CGM	HT CGM
14:0	0.0	0.3	0.4	0.4	0.3	0.2
16:0	6.1	14.8	14.9	8.4	9.7	15.1
18:0	0.0	3.7	4.9	1.9	2.1	3.9
18:2n-6	47.5	50.3	48.1	52.2	52.6	48.8
18:3n-3	8.3	8.8	12.1	1.8	2.7	5.2
∑ PUFA	56.4	60.6	60.7	54.7	56.5	54.6
∑ n-6 PUFA	47.5	50.3	48.1	52.2	52.6	48.8
∑ n-3 PUFA	8.9	10.3	12.7	2.6	4.0	5.8
n-3:n-6	0.19	0.20	0.26	0.05	0.07	0.12

Table 3. Formula (% dry matter) of experimental diets fed to rainbow trout juveniles

Ingredients	Control	NE	LT	HT
Peruvian anchovy meal	55	15	15	15
Soybean meal	0	25	0	0
HT SBM	0	0	0	25
LT SBM	0	0	25	0
CGM	0	25	0	0
HT CGM	0	0	0	25
LT CGM	0	0	25	0
Defatted rice bran	10	3.3	2.5	3.7
Wheat flour	10	10	10	10
Cod liver oil	5	6.6	7.4	6.2
Rapeseed oil	5	5	5	5
Pre-gelatinized starch	5	5	5	5
Mineral mix[a]	1	1	1	1
Vitamin mix[b]	3	3	3	3
Choline chloride	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1
Chromium oxide (50%)	0.5	0.5	0.5	0.5
Cellulose	4.9	0	0	0

[a] Mineral pre-mix (g kg $^{-1}$): sodium chloride, 50; magnesium sulfate, 745; iron (III) citrate *n*-hydrate, 125; cellulose, 30; trace element mixture, 50. The trace element mixture contains (g kg^{-1}) zinc sulfate heptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminum chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose 440.

[b] Vitamin pre-mix (unit kg $^{-1}$): Vitamin A, 2, 400,000 IU; Vitamin D3, 2,400,000 IU ; Vitamin K3, 6.05 g; thiamine, 3.025 g; riboflavin, 3.63 g; pyridoxine, 2.42 g; cyanocobalamin, 0.006 g; L-ascorbic acid, 368.902 g; nicotinic acid, 24.2 g; D-pantothenic acid, 6.05 g; inositol, 121 g; D-biotin, 0.363 g; folic acid, 0.908 g; *para*-amino benzoic acid, 3.025 g.

Table 4. Proximate, amino acid and fatty acid composition (% dry matter) of experimental diets fed to rainbow trout juveniles

Measured composition	Control	NE	LT	HT	
Dry matter	96.9	94.1	94.9	96.0	
Crude protein	43.4	41.5	41.0	41.1	
Crude lipid	14.5	14.1	14.1	14.2	
Ash	9.60	5.40	5.50	5.80	
Essential amino acids					EAA requirement of rainbow trout*
Threonine	2.05	2.64	1.82	1.58	1.44
Valine	2.07	2.83	1.93	1.72	1.26
Methionine	0.62	0.46	0.47	0.45	0.81
Isoleucine	1.63	2.31	1.65	1.46	0.98
Leucine	3.04	4.15	4.04	3.49	1.75
Phenylalanine	1.65	1.95	2.09	1.81	1.26
Histidine	1.26	1.18	1.00	0.83	0.63
Lysine	3.37	1.71	2.10	1.71	2.10
Tryptophan	0.17	0.20	0.22	0.21	0.21
Arginine	2.54	2.31	2.44	2.11	1.40
Fatty acids					
14:0	4.50	2.34	3.97	2.57	
16:0	11.1	10.9	11.8	11.3	
18:0	2.90	2.39	2.52	2.24	
18:2n-6	11.1	18.5	14.4	15.5	
18:3n-3	4.31	3.81	3.367	4.07	
20:5n-3 (EPA)	5.46	4.53	4.55	5.33	
22:5n-3 (DPA)	1.01	0.59	0.62	0.57	
22:6n-3 (DHA)	4.97	3.50	3.82	3.71	
∑ PUFA	30.2	33.2	29.1	31.3	
∑ n-6 PUFA	11.1	18.5	14.4	15.5	

* Ogino (1980)

Table 5. Growth performance, survival rate, feed efficiency and nutrient utilization of juvenile rainbow trout fed experimental diets for 12 weeks

Parameters	Control	NE	LT	HT
Initial weight (g)	12.57	12.42	12.52	12.51
Final weight (g)	91.23[a]	70.14[b]	77.76[ab]	64.87[b]
Weight gain (%)	626.32[a]	464.79[b]	519.62[ab]	418.65[b]
DFI (g/fish/day)	1.12	0.94	1.14	0.94
SGR (% day $^{-1}$)	2.79[a]	2.43[ab]	2.55[ab]	2.31[b]
FCR	1.03[a]	1.20[b]	1.26[b]	1.27[b]
PER	2.31[a]	2.01[ab]	2.05[ab]	1.96[b]
Survival (%)	98.0	98.0	100	95.0

Values in the same row with different uppercase letters are significantly different (P<0.05).

Abbreviations: DFI, daily feed intake; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio

Table 6. Mineral absorption, nutrient retention, phosphorus excretion and apparent digestibility coefficients of dry matter, crude protein and crude lipid of experimental diets fed to rainbow trout

ADC (%)	Control	NE	LT	HT
Dry matter	78.7	81.9	85.6	83.5
Crude protein	81.9	91.9 [b]	95.4 [a]	94.0 [ab]
Crude lipid	95.9	95.3	96.3	95.8
Nutrient retention (%)				
Protein	37.8 [a]	25.4 [b]	34.5 [a]	36.4 [a]
Lipid	67.7	69.9	63.7	62.9
Phosphorus	7.08 [b]	9.97 [b]	15.15 [a]	15.33 [a]
*P excretion (kg t^{-1})	19.78 [a]	16.2 [a]	12.72 [b]	11.67 [b]
Mineral absorption (%)				
P	63.5 [b]	65.5 [b]	69.3 [ab]	72.6 [a]
Zn	46.4	53.5	52.3	54.5
Mn	27.7 [b]	42.8 [ab]	37.8 [ab]	50.0 [a]

Values in the same row with different uppercase letters are significantly different (*P*<0.05).

*Computed based on production of 1000 kg fish

Table 7. Proximate and essential amino acid composition (% wet weight basis) of rainbow trout fed experimental diets

Measured composition	Control	NE	LT	HT
Moisture	70.2[a]	69.3[ab]	68.4[b]	70.0[a]
Crude protein	16.0	15.2	14.9	15.8
Crude lipid	11.3	13.5	12.5	13.0
Ash	2.12	1.84	1.70	1.64
Essential amino acid				
Threonine	2.33	2.72	2.04	2.33
Valine	2.32	1.74	2.10	2.28
Methionine	0.73	0.92	0.47	0.59
Isoleucine	1.85	2.11	1.08	1.70
Leucine	3.43	3.90	2.54	3.39
Phenylalanine	1.90	2.21	1.74	1.90
Histidine	1.17	1.35	1.06	1.12
Lysine	4.24	4.86	3.76	4.15
Tryptophan	0.33	1.95	0.31	0.36
Arginine	2.81	2.40	2.65	2.79

Values in the same row with different uppercase letters are significantly different (P<0.05).

Table 8 . Fatty acid composition (% dry matter) of rainbow trout.

Fatty acid	Control	NE	LT	HT
14:0	2.32	1.83	1.60	1.71
16:0	11.73	10.42	10.06	11.33
18:0	3.77	2.35	3.58	3.20
18:2n-6	8.19	12.98	11.22	11.95
18:3n-3	2.38	1.81	2.21	2.47
20:5n-3 (EPA)	1.90	1.35	1.52	1.46
20:4n-6 (ARA)	0.56	0.48	0.53	0.51
22:5n-3 (DPA)	0.91	0.69	0.67	0.67
22:6n-3 (DHA)	7.78	7.12	5.76	5.25
∑ PUFA	21.8	24.5	22.0	22.5
∑ n-6 PUFA	8.2	13.0	11.2	11.9
∑ n-3 PUFA	13.6	11.5	10.7	10.6

CHAPTER 3

Utilization of combined extruded soybean and corn gluten meals as feed ingredients for juvenile rainbow trout, *Oncorhynchus mykiss* diet

Abstract

In this study, feeding experiment and subsequent digestibility trial were performed to investigate the utilization of extruded soybean meal (SBM) and corn gluten meal (CGM) as feed ingredients for juvenile rainbow trout. Plant ingredients have undergone extrusion at low temperature (100°C, LT) or high temperature (150°C, HT) for 30 seconds. Four isonitrogenous (44%, crude protein) and isolipidic (14%, crude lipid) diets were formulated. Control diet is fish meal based while a combined (1:1) non-extruded SBM and CGM for NE diet, LT SBM and LT CGM for LT diet and HT SBM and HT CGM for HT diet. Moreover, NE, LT and HT diets were supplemented with 0.3% D-L methionine. Two hundred forty rainbow trout juveniles (7.8 g average body weight) were randomly divided into 12 rectangular 60 l glass aquaria and offered four different diets in triplicate. Fish were fed at satiation twice a day, six days a week for 12 weeks. Phytate phosphorus level of extruded ingredients decreased through extrusion cooking. Final weight, weight gain, SGR and PER of fish fed HT diet were significantly (p<0.05) higher than those fed with NE diet. Apparent digestibility coefficient for protein of LT and HT diets are significantly higher than of NE diet. The results of this study demonstrated that HT extruded SBM and CGM are suitable feed ingredients for rainbow trout diet without compromising fish growth, feed utilization and fish body composition.

Background of study

The objective of this study is to investigate the effects of extrusion cooking temperature on soybean and corn gluten meals as combined ingredients in the diet for juvenile rainbow trout with methionine supplementation, in terms of nutritional value, fish growth performance, feed utilization and apparent digestibility. According to Belghit et al., (2014), fish feeds that is mostly composed of plant ingredients are typically low in methionine. The essential amino acid, methionine is very important as it supports normal growth to most animals as it serves as building block for protein synthesis. It plays a very significant role in mRNA translation as the primary amino acid that is needed to initiate protein synthesis (Bradshaw et al., 1998). Thus, the difference between experiment 1 and experiment 2 is the supplementation of 0.3% methionine in NE, LT and HT diets for the latter.

Material and Methods

Diet preparation

Four isonitrogenous (41%, CP) and isolipidic (14%, CL) diets were prepared in this study. Control diet is fish meal-based while the main protein sources of the other three diets are combination (1:1) of non-extruded SBM and CGM (NE diet), LT SBM and LT CGM (LT diet) and HT SBM and HT CGM (HT diet). In addition, NE, LT and HT diets contained 15% fish meal. Moreover, to meet the requirement of rainbow trout for methionine, 0.3% D-L methionine was added to NE, LT and HT diets. The formulation of the diets is shown in Table 3 of Appendix 3. Diets were prepared in the same way as described in the previous chapter.

Feeding trial

Feeding trial was conducted for 12 weeks using 240 individuals of rainbow trout juveniles with initial average body weight of 7.8g. Twenty fish were put in each of the twelve 60-l aquaria. They were acclimatized to the rearing conditions and fed with commercial rainbow trout diet before the onset of the feeding trial. The same rearing conditions and maintenance were observed in this experiment as described in the previous chapter.

Chemical analysis

Chemical analysis in terms of proximate, amino acid, fatty acid and mineral analysis were done for feed ingredients, diets and fish samples based on the methods described in the previous chapter.

Results

Nutritional value of plant ingredient

The proximate composition, phytate phosphorus, amino acid and mineral levels of non-extruded and extruded (LT & HT) SBM and CGM are presented in Table 1. The phytate phosphorus content of SBM and CGM decreased from 0.14% to 0.08% (LT) & 0.05% (HT) and 0.06% to 0.03% (LT and HT), respectively. In terms of crude protein content, values for LT and HT SBM were similar (CP, 50%), and 2% higher than non-extruded SBM. Conversely, the crude protein content of LT (CP, 64%) and HT (CP, 66%) CGM were lower than that of the non-extruded CGM (CP, 72%). Lipid content of SBM were not affected by extrusion temperature; for CGM however, the lipid content of HT CGM almost doubled of that of LT CGM. It is also noteworthy that the essential amino acid content of LT and HT SBM were significantly higher than those of the non-extruded SBM. In CGM, essential amino acid levels between LT and HT were also the same but their values were lower than those of the non-extruded CGM. Mineral composition of extruded ingredients increased except for zinc in LT and HT CGM.

Fish growth performance

Fish groups readily accepted the respective diets given. In the whole duration of the feeding trial, there were no diseases or mortalities observed caused by any nutritional factors. At the end of the 12-week feeding trial, the fish survival rate ranges from 95% to 98%. The values for final weight, weight gain and SGR of fish fed HT diet were significantly higher ($P<0.05$) than those of fish fed NE diet as shown in Table 7.

Feed efficiency and nutrient utilization

Result for PER follows the same trend with that of the growth parameters. Values for feed efficiency and intake however showed no significant differences ($P>0.05$) among all groups. Although not significant, feed intake of NE was high and low in HT group, while similar feed intake was exhibited by control and LT group.

Values for protein retention was found to be highest (38%) in fish feed HT diet which was significantly different ($P<0.05$) with those fed NE diet. Moreover, values for lipid retention in fish feed LT or HT diets were significantly higher ($P<0.05$) than those fed control or NE diets.

In vivo digestibility, phosphorus absorption and excretion

Both ADC for dry matter and lipid were not significantly different among all treatment groups (Table 8). ADC for protein however was found to be significantly lower ($P<0.05$) in fish fed NE diet compared to other treatment groups. Protein retention of NE group was found to be lowest and is significantly different ($P<0.05$) among all treatment groups. Furthermore, lipid and phosphorus retention of LT and HT group were significantly higher ($P<0.05$) than those of control and NE group. Likewise, values for phosphorus absorption of LT and HT diets were significantly higher ($P<0.05$) than those of control and NE diets. Furthermore, phosphorus excretion was found to be significantly higher in ($P<0.05$) in NE and control groups than those of LT and HT group.

Fish body composition

Final fish body composition in terms of nutritional value of crude protein were not affected by dietary treatment (Table 9). Nonetheless, crude lipid content of fish fed control diet had significantly (*P* <0.05) lower value than those fed LT diet. The mineral composition of the fish body was not affected by extrusion cooking except zinc wherein, value for zinc in control diet was significantly different (*P*<0.05) among all treatments. Moreover, although not significant, manganese and phosphorus level were found to be improved in HT diet.

Discussion

Adequate processing of plant material is beneficial in improving their nutritional value as fish feed ingredient. In heat-treatment processing of plant ingredients, temperature is one of the factors to consider as excessive heat would denature protein and cause destroy amino acids especially lysine and arginine (Saitoh et al., 2000; Barrows et al., 2007). However, according to Mościcki & Zuilichem (2011), exposure to a high temperature (200°C) for only a short time (30 – 45 sec) could avoid unwanted protein and amino acid denaturation. In the present study, the crude protein content of SBM slightly increased while that of CGM decreased. The result for extruded CGM is rather the same with the work of Zheng et al. (2014) in which, percent crude protein decreased after extrusion because CGM usually contains 12-15% starch which is tightly bound with protein and during extrusion process, starch is gelatinized. Diets containing extruded CGM (LT and HT) in this study are in combination with extruded SBM (1:1) and 15% fish meal, thus the effect of the lower protein in extruded CGM was not negatively manifested in the growth and feed efficiency for rainbow trout. Likewise, the amino acid composition for both LT and HT SBM and CGM were comparable with that of non-extruded SBM and CGM as presented in Table 1. Although methionine appears to be a limiting amino acid in the plant ingredients, the amino acid profile of all diets was consistent with the requirement (Ogino, 1980) for rainbow trout to support optimal growth and metabolic activities. Even when the temperature is as high as 150°C, no negative effect on the amino acid component of the ingredients were observed. This agrees with the findings of Sørensen et al. (2002). Extrusion cooking on chemical composition of SBM and CGM were different. This implies that different physico-chemical property of SBM and CGM may affect effect of extrusion of both ingredients. This time SBM and CGM were extruded individually. However, mixing SBM and CGM before extrusion could provide different result when compared individual extrusion of SBM and CGM. Hence, extrusion temperature (100°C or 150°C) in this study is suitable for the processing of SBM and CGM as feed ingredients for rainbow trout.

In the present study, although the phytate phosphorus level of SBM and CGM are lower than reported, extrusion cooking at 150°C reduced the phytate phosphorus level as much as 65% in SBM and 50% in CGM. Romarheim et al. (2005) reported that trypsin inhibitor activity was reduced by approximately 76% in diets containing soy products for salmonid fish. Unfortunately, effect of extrusion cooking on other anti-nutritional substances were not investigated in this study. Furthermore, availability of phosphorus is an increasing concern in commercial fish production. In freshwater environment, this mineral is considered as source of pollution (Cheng & Hardy, 2003). To make aquaculture more environment friendly, dietary phosphorus level and fecal phosphorus discharged must be reduced.

Extrusion cooking improves nutrient digestibility, palatability, pellet durability, water stability and pellet storage life (Barrows & Hardy, 2000). Hence, growth performance and feed efficiency of the animal fed extruded diet tend to also improve (Cheng & Hardy, 2003). This is in contrast with the findings of Stone et al. (2005) wherein growth of rainbow trout was not improved by extrusion of diets and the presence of indigestible carbohydrate in corn distiller's dried grain limits protein replacement level of fish meal with corn products to 18%. As presented in this study, rainbow trout fed HT diet had the highest growth rate which is significantly higher than those fish fed NE diet and comparable with those fed the control diet. This is likely due to improved nutritional value of extruded SBM and CGM, increased protein digestibility and higher phosphorus availability of HT diet compared with NE diet. When you see EAA content, control diet met 87.6% of requirement level of methionine for rainbow trout. For NE diet, lysine and valine are 95.7% and 96%, respectively. For LT and HT diets, sufficiency of valine were 99.2% and 96.8%, respectively. However, sufficiency of methionine was lower in control diet than HT and LT diets. Also, NE diets did not meet two for the EAAs. Therefore, it could be that the growth of fish in NE and control were slightly lower because of the insufficient dietary EAA than LT and HT groups, eventually appeared to have better performance in LT and HT groups. Furthermore, this study failed to detect tryptophan.

The requirement of fish for dietary phosphorus is higher than any other mineral element as this mineral is very important for optimum growth and metabolism (Satoh et al., 2002). Daily feed intake was similar in all treatment groups indicating that there is no problem on the palatability of the diet as fish in all treatment groups readily fed on the given diets. Despite no significant differences in daily feed intake and FCR, lower growth was exhibited in fish fed NE diet. This could be due to lower protein digestibility, phosphorus absorption and PER of NE diet. In gilthead bream, Venou et al. (2003) reported that raw corn meal diet had a faster rate of passage of digesta into the intestine which led to a less efficient utilization. In this study, extrusion cooking processing appears to improve growth and nutrient utilization in rainbow trout. In comparison, extrusion processing of canola meal at high temperature (150°C) also improved feed intake and growth rate of salmon (Satoh et al., 1998).

In the current study, dry matter, protein and lipid in diets formulated with extruded SBM and CGM appeared to be digested by rainbow trout as efficiently as those of the fish meal-based control diet. Cheng & Hardy (2003) reported that extrusion increased the digestibility of dry matter in soybean meal from 75% to 78% in rainbow trout. According to Allan & Booth (2004), extruding soybean meal could increase dry matter and energy digestibility between 3% and 14% in silver perch. Likewise, in this study, the dry matter digestibility of diets containing extruded SBM and CGM increased from 80% to 84%. This positive result could mean reduction of solids excreted into the water (Cheng & Hardy 2003). Nevertheless, findings from other studies have reported that extrusion cooking temperature alone had no effect on ADC for protein or energy (Sørensen et al., 2002) and ADC for organic matter, lipid, energy or carbohydrate (Barrows et al., 2007) in rainbow trout which could be due to endogenous losses of protein and amino acids. Furthermore, Barrows et al. (2007) mentioned that pre-cooking of SBM did improve ADC for organic matter, energy and carbohydrate in rainbow trout therefore, extrusion cooking alone was not adequate enough to provide all the energy available to the fish. Cheng & Hardy (2003) also reported a decreased value on ADC for protein of CGM but for SBM, its ADC for protein was already high as 98.1%. In extruded lupin, its dry matter, protein and energy digestibility in trout were reported to be higher (Burel et al., 2000). Comparably, for saltwater fish, extrusion also improved the protein and lipid digestibility in gilthead sea bream fed diet containing extruded wheat and corn (Venou et al., 2003). In corn gluten meal, Bu et al. (2018) reported that no significant differences were found in ADC of dry matter and protein when substitution level was 40% suggesting that CGM could replace 40% of fishmeal protein in *Pseudobagrus ussuriensis*. In the present study, ADC for protein of LT and HT diets are significantly higher than that of NE diet demonstrated that extrusion cooking improved the protein digestibility in rainbow trout diet. Improved nutrient digestibility by extrusion cooking can also be attributed to a partial degradation of antinutritional substances (Francis et al., 2001). Soybean meal contains about 1.4% phytic acid and Satoh et al. (1997) reported that extrusion cooking at high temperature (150°C) decreases the phytic acid level in SBM from 1.4% to 1.0%. Furthermore, it is known that excessive heat treatment may reduce protein digestibility however, the current experiment demonstrated that increasing extrusion temperature from 100°C to 150°C did not surpass the limit that could reduce protein digestibility in rainbow trout. This is likely due to the short retention time in the extruder (Sørensen et al., 2002). In the review article of Sørensen (2012), it was mentioned that extrusion process will not negatively affect the nutritional value of the feed as long as the temperature does not exceed 150°C. Moreover, dietary phosphorus absorption increased from 62% (NE) to 75% (HT) which agrees with the findings of Satoh et al. (2002) wherein extrusion cooking at 150°C improved the availability of phosphorus in SBM. Burel et al. (2000) also reported that the availability of phosphorus in extruded lupin was high for both trout and turbot. Findings from this study would suggest that extrusion cooking enhanced the digestibility, phosphorus absorption and retention of SBM and CGM in rainbow trout diet. Furthermore, an improved in phosphorous absorption and retention in LT and HT diets in this experiment lowered phosphorus excretion suggesting an improved in phosphorus bioavailability for rainbow trout.

The value for the whole-body crude lipid content of fish fed control diet were lower than those of the other groups, especially those fed LT diet. This result is somewhat similar with the findings of Satoh et al. (1998). Although not estimated in this study, the digestible energy or higher concentration of carbohydrates available for lipogenesis in LT and HT diets could cause the increase in whole body lipid of the fish (Higgs et al., 1995; Venou et al., 2003). Nevertheless, diet containing combination of extruded SBM and CGM (1:1) at high temperature (HT) enhanced growth rate and protein efficiency ratio in rainbow trout without compromising feed utilization and fish body composition.

Conclusion

In conclusion, extruded SBM and CGM at 150°C can be included in the diet for rainbow trout without adversely affecting fish performance, feed utilization, nutrient retention and fish body composition. Furthermore, it is recommended to evaluate extruded SBM and CGM at different inclusion levels to partially or totally replace fish meal in rainbow trout diet.

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APPENDIX 3

Table 1. Proximate, phytate in phosphorus , and mineral composition (% dry matter) of non-extruded or extruded SBM and CGM

Measured composition	SBM	LT SBM	HT SBM	CGM	LT CGM	HT CGM
Dry matter	86.25	93.35	94.43	89.91	96.05	93.46
Crude protein	47.97	50.96	50.02	72.45	64.25	66.57
Crude lipid	2.21	2.19	2.21	5.89	4.42	7.97
Ash	5.81	6.65	5.57	2.59	2.35	2.27
Phytate in phosphorus	0.14	0.08	0.05	0.06	0.03	0.03
Minerals						
Ca (mg g^{-1})	2.97	3.65	3.52	0.47	2.06	2.89
Cu (mg kg^{-1})	17.01	19.68	18.74	13.19	17.48	18.75
Fe (mg kg^{-1})	132.81	159.85	153.94	132.68	154.30	158.94
K (mg g^{-1})	11.48	11.23	11.78	13.85	15.36	14.80
Mg (mg g^{-1})	3.62	3.90	3.91	0.56	0.79	0.69
Mn (mg kg^{-1})	38.63	41.33	40.51	10.99	15.15	14.66
P (mg g^{-1})	6.65	6.65	6.81	4.54	6.01	5.69
Zn (mg kg^{-1})	42.01	69.67	74.74	35.88	32.21	30.82

Abbreviations: Ca, calcium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; P, phosphorus and Z, zinc.

Table 2. Essential and non-essential amino acid composition (% dry matter) of non-extruded or extruded SBM and CGM

Measured composition	SBM	LT SBM	HT SBM	CGM	LT CGM	HT CGM
Essential amino acids						
Arginine	2.42	3.46	3.51	2.52	2.07	1.92
Histidine	0.80	1.13	1.16	1.19	1.09	1.30
Isoleucine	1.37	1.83	1.87	2.35	2.23	2.28
Leucine	2.44	3.34	3.37	10.27	8.72	10.59
Lysine	2.14	2.95	2.93	1.18	1.34	1.28
Methionine	0.08	0.24	0.24	0.60	0.47	0.56
Phenylalanine	1.64	2.26	2.28	3.98	3.50	3.77
Threonine	1.47	1.92	1.91	2.50	2.33	2.44
Tryptophan	0.17	0.15	0.14	0.22	0.17	0.17
Valine	1.52	2.12	2.04	2.79	2.50	2.64
Non-essential amino acids						
Alanine	1.51	1.97	2.01	5.80	4.88	5.28
Aspartic acid	3.93	5.07	5.13	4.71	3.93	4.65
Cystine	0.28	0.45	0.44	0.62	0.40	0.46
Glutamic acid	6.19	8.43	8.49	14.22	12.67	13.51
Glycine	1.50	1.93	1.97	1.79	1.70	1.77
Serine	1.74	2.30	2.39	3.38	3.04	3.65
Tyrosine	1.37	1.77	1.85	3.40	3.01	3.21

Table 3. Formula (% dry matter) of experimental diets fed to rainbow trout juveniles

Ingredients	Control	NE	LT	HT
Peruvian Anchovy meal	55	15	15	15
Soybean meal	-	25	-	-
HT Soybean meal	-	-	-	25
LT Soybean meal	-	-	25	-
Corn gluten meal	-	25	-	-
HT Corn gluten meal	-	-	-	25
LT Corn gluten meal	-	-	25	-
Defatted rice bran	9.7	1.0	0.2	1.4
Wheat flour	10	10	10	10
Cod liver oil	5.0	6.6	7.4	6.2
Rapeseed oil	5.0	5.0	5.0	5.0
Pre-gelatinized starch	5.0	5.0	5.0	5.0
Mineral mix[a]	1.0	1.0	1.0	1.0
Vitamin mix[b]	3.0	3.0	3.0	3.0
Choline chloride	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1
D-L Methionine	-	0.3	0.3	0.3
Chromic oxide (50%)	0.5	0.5	0.5	0.5
Calcium monophosphate	-	2.0	2.0	2.0
Cellulose	5.2	-	-	-

[a] Mineral pre-mix (g kg $^{-1}$): sodium chloride, 50; magnesium sulfate, 745; iron (III) citrate *n*-hydrate, 125; cellulose, 30; trace element mixture, 50. The trace element mixture contains (g kg^{-1}) zinc sulfate heptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminum chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose 440.

[b] Vitamin pre-mix (unit kg $^{-1}$): Vitamin A, 2, 400,000 IU; Vitamin D3, 2,400,000 IU ; Vitamin K3, 6.05 g; thiamine, 3.025 g; riboflavin, 3.63 g; pyridoxine, 2.42 g; cyanocobalamin, 0.006 g; L-ascorbic acid, 368.902 g; nicotinic acid, 24.2 g; D-pantothenic acid, 6.05 g; inositol, 121 g; D-biotin, 0.363 g; folic acid, 0.908 g; *para*-amino benzoic acid, 3.025 g.

Table 4. Proximate and mineral composition of experimental diets fed to rainbow trout juveniles

Measured composition (% dry matter)	Control	NE	LT	HT
Dry matter	96.78	97.35	98.19	98.73
Crude protein	44.75	44.62	44.03	44.73
Crude lipid	14.40	13.95	13.60	13.33
Ash	13.82	7.34	7.75	8.65
Minerals				
Ca (mg g^{-1})	33.19	14.44	16.31	15.03
Cu (mg kg^{-1})	1.89	4.67	4.92	5.19
Fe (mg kg^{-1})	351.08	306.14	328.97	328.70
K (mg g^{-1})	8.8	9.22	10.10	11.14
Mg (mg g^{-1})	2.53	2.62	2.66	2.73
Mn (mg kg^{-1})	28.51	21.82	21.40	22.94
P (mg g^{-1})	26.51	21.56	22.48	21.94
Zn (mg kg^{-1})	75.35	59.18	57.95	58.08

Table 5. Amino acid composition of experimental diets fed to rainbow trout juveniles

Essential amino acids					Rainbow trout EAA requirement*
Arginine	2.84	2.05	2.15	2.23	1.4
Histidine	1.39	1.00	0.94	0.97	0.63
Isoleucine	1.70	1.04	1.09	1.11	0.98
Leucine	3.26	4.25	4.40	4.23	1.75
Lysine	3.97	2.01	2.12	2.15	2.10
Methionine	0.73	1.12	1.09	1.14	0.81
Phenylalanine	1.80	2.04	2.13	2.08	1.26
Threonine	2.06	1.47	1.54	1.53	1.44
Valine	2.11	1.21	1.25	1.22	1.26
Non-essential amino acids					
Alanine	2.79	2.82	2.87	2.87	
Aspartic acid	4.07	3.47	3.77	3.82	
Cystine	0.22	0.35	0.33	0.30	
Glutamic acid	6.31	7.80	8.27	7.56	
Glycine	3.00	1.80	1.89	1.97	
Serine	1.85	2.22	2.34	2.30	
Tyrosine	1.48	1.64	1.72	1.68	

*Ogino (1980)

Table 6. Fatty acid composition (% dry basis) of experimental diets fed to rainbow trout

Fatty acid	Control	NE	LT	HT
14:0	4.51	2.82	3.96	4.07
16:0	11.05	10.94	11.8	11.32
18:0	2.62	2.36	2.52	2.25
18:2n-6	11.11	18.80	14.41	15.50
18:3n-3	4.32	3.87	3.68	4.07
20:5n-3 (EPA)	6.09	4.54	4.55	5.33
22:5n-3 (DPA)	1.01	0.60	0.62	0.62
22:6n-3 (DHA)	5.47	3.49	3.82	3.71
∑ PUFA	30.2	33.2	29.1	31.1
∑ n-6 PUFA	11.1	18.8	14.4	15.5
∑ n-3 PUFA	14.8	10.5	11.0	11.7
n-3:n-6	1.33	0.56	0.76	0.75

Table 7. Growth performance, survival rate and feed efficiency of juvenile rainbow trout fed experimental diets for 12 weeks

Parameters	Control	NE	LT	HT
Initial weight (g)	7.83 ± 0.0	7.86 ± 0.0	7.81 ± 0.0	7.86 ± 0.0
Final weight (g)	68.92 ± 2.8 ^{b}	52.33 ± 0.11 ^{a}	65.31 ± 1.4 ^{ab}	71.11 ± 6.2 ^{b}
Weight gain (%)	780.06 ± 33.1 ^{b}	565.67 ± 1.6 ^{a}	736.53 ± 18.3 ^{ab}	804.01 ± 75.5 ^{b}
SGR (% day $^{-1}$)	3.40 ± 0.1 ^{b}	2.96 ± 0.0 ^{a}	3.32 ± 0.03 ^{b}	3.43 ± 0.22 ^{b}
FCR	1.01 ± 0.0	1.19 ± 0.1	1.02 ± 0.0	0.96 ±0.0
PER	2.30 ± 0.1 ^{ab}	1.96 ± 0.1 ^{a}	2.28 ± 0.0 ^{ab}	2.38 ± 0.1 ^{b}
DFI	2.50 ± 1.2	2.75 ± 1.6	2.54 ± 0.0	2.40 ± 1.5
Survival (%)	97 ± 1.7	95 ± 2.9	98 ± 1.7	95 ± 2.9

Values are expressed as mean ± SEM (*n* = 3). Values in the same row with different uppercase letters are significantly different (*P*<0.05).

Abbreviations: SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; DFI, daily feed intake

Table 8. Apparent digestibility coefficients of dry matter, crude protein, crude lipid, nutrient retention, phosphorus absorption and excretion of experimental diets fed to rainbow trout

ADC (%)	Control	NE	LT	HT
Dry matter	81.92 ± 1.3	80.33 ± 1.7	83.68 ± 0.9	84.22 ± 0.8
Crude protein	93.00 ± 0.5[b]	89.02 ± 0.8[a]	93.60 ± 0.3[b]	92.70 ± 0.4[b]
Crude lipid	94.36 ± 0.9	93.87 ± 0.8	94.71 ± 0.4	94.36 ± 0.3
Nutrient retention				
Protein	36.11 ± 3.0 [b]	26.22 ± 1.8 [a]	34.42 ± 0.1[b]	37.02 ± 3.7[b]
Lipid	64.59 ± 3.0 [a]	67.16 ± 0.2 [a]	88.94 ± 3.7 [b]	88.97 ± 11.8[b]
Phosphorus	11.99 ± 0.8 [a]	9.88 ± 1.2 [a]	14.88 ± 0.9 [b]	16.20 ± 0.8 [b]
P absorption	66.63 ± 1.9[a]	62.26 ± 2.1[a]	76.19 ± 0.1[b]	75.57 ±1.4[b]
*P excretion (kg t^{-1})	29.5[b]	33.2[b]	18.9[a]	18.4[a]

Values are expressed as mean ± SEM (*n* = 3). Values in the same row with different uppercase letters are significantly different (P<0.05).

*Computed based on a production of 1000kg fish.

Table 9. Proximate and mineral composition (% wet weight basis) of rainbow trout fed experimental diets

Measured composition	Control	NE	LT	HT
Moisture	71.25 ± 0.30	71.07 ± 0.63	69.33 ± 0.46	70.48 ± 0.58
Crude protein	15.87 ± 0.44	14.23 ± 0.10	16.08 ± 1.06	15.27 ± 0.29
Crude lipid	9.10 ± 0.72[a]	11.24 ± 0.1[ab]	12.11 ± 0.30[b]	11.02 ± 0.73[ab]
Ash	1.72 ± 0.03	1.67 ± 0.07	1.80 ± 0.18	2.06 ± 0.18
Minerals				
Ca (mg g^{-1})	4.02 ± 0.14	3.90 ± 0.06	4.05 ± 0.03	4.20 ± 0.07
Cu (mg kg^{-1})	1.06 ± 0.15	1.55 ± 0.13	1.84 ± 0.10	1.43 ± 0.06
Fe (mg kg^{-1})	9.96 ± 0.05	9.28 ± 0.04	8.47 ± 0.10	9.29 ± 0.26
K (mg g^{-1})	3.56 ± 0.13	3.32 ± 0.04	3.73 ± 0.11	3.71 ± 0.09
Mg (mg g^{-1})	0.36 ± 0.01	0.33 ± 0.01	0.39 ± 0.01	0.39 ± 0.02
Mn (mg kg^{-1})	1.36 ± 0.08	0.94 ± 0.06	1.23 ± 0.05	1.56 ± 0.13
P (mg g^{-1})	3.79 ± 0.19	3.81 ± 0.14	4.72 ± 0.06	6.20 ± 0.22
Zn (mg kg^{-1})	11.86 ± 0.20[a]	7.56 ± 0.18[b]	7.74 ± 0.27[b]	7.66 ± 0.14[b]

Values are expressed as mean ± SEM (*n* = 3). Values in the same row with different uppercase letters are significantly different ($P<0.05$).

Table 10. Fatty acid composition (% wet weight basis) of rainbow trout fed experimental diets

Fatty acid	Control	NE	LT	HT
14:0	4.0	1.8	3.3	3.2
16:0	13.2	8.0	11.0	11.0
18:0	3.1	2.7	2.4	2.4
18:2n-6	9.6	14.5	14.1	15.1
18:3n-3	3.2	3.3	3.5	4.3
20:5n-3 (EPA)	5.9	4.6	5.3	5.3
20:4n-6 (ARA)	0.60	0.45	0.55	0.57
22:5n-3 (DPA)	0.9	0.7	0.8	0.7
22:6n-3 (DHA)	4.2	3.3	5.3	5.3
∑ PUFA	23.8	26.4	29.0	30.7
∑ n-6 PUFA	9.6	14.5	14.1	15.1
∑ n-3 PUFA	14.2	11.9	14.9	15.6
n-3:n-6	1.48	0.82	1.05	1.03

CHAPTER 4

Effect of high temperature extrusion of soybean and corn gluten meals as feed ingredients for juvenile rainbow trout, *Oncorhynchus mykiss* diet

Abstract

Five isonitrogenous and isolipidic diets were formulated. Control is fishmeal based; NE contains non-extruded SBM and CGM; HTS has HT SBM + NE CGM; HTC has HT CGM+ NE SBM; HTSC has both HT SBM and HT CGM. ADC for dry matter, crude protein and crude lipid of control and HTSC groups are comparable and are significantly ($P<0.05$) higher among other groups. Phosphorus absorption of HTSC group is significantly ($P<0.05$) higher among all treatments. Growth performance in terms of final weight, weight gain and SGR shows that control group had significantly higher ($P<0.05$) values among others followed by HTSC group. Protein and lipid retention of HTSC group had the highest value among others. In terms of proximate body composition, HTS group had the lowest crude protein level which is significantly different with the control group. Likewise, for crude lipid content, control group had the lowest value which is significantly different ($P<0.05$) with HTC group. Mineral composition of the fish body in terms of iron, manganese and phosphorus were improved in diets containing high temperature extruded ingredients. Total essential amino acid composition of fish fed control diet and HTSC diet had the same values. Thus, this study demonstrated that combined high temperature (150°C) extruded SBM and CGM (HTSC) resulted to improved growth, feed efficiency, nutrient utilization, digestibility and fish body composition.

Background of the Study

Corn gluten meal is relatively high protein source, (Gatlin et al., 2007) highly digestible for aquafeeds and has minimal concerns in terms of ANFs (Lim et al., 2008). Corn gluten meal has been known to have relatively lower ANFs than SBM. Extrusion process is known to have been effective in decreasing or eliminating the antinutritional factors present in plant ingredients. Moreover, the production of corn and its co-products are higher than other plant proteins including oilseed meals and grains (Belyea et al., 2004) making it more available to be used for aquafeed. Thus, this study was conducted to determine if the combination of extruded SBM and non-treated CGM could give acceptable feed performance in rainbow trout

Material and Methods

Diet preparation

In this study, five experimental diets were formulated. Control is fishmeal based; NE contains non-extruded SBM and CGM; HTS has HT SBM + NE CGM; HTC has HT CGM+ NE SBM; HTSC has both HT SBM and HT CGM. Diets are isonitrogenous (43%, CP) and isolipidic (13%, CL). Diet formulation is shown in Table 3 of Appendix 4. Diets were prepared in the same way as described in the Chapter 2.

Feeding trial

Feeding trial was conducted for 12 weeks using 300 rainbow trout juveniles with initial average body weight of 6.95g. Twenty fish were stocked to each of the fifteen, 60-l aquaria. The same rearing conditions and maintenance were observed in this experiment as described in Chapter 2.

Chemical analysis

Chemical analysis in terms of proximate, amino acid, fatty acid and mineral analysis were done for feed ingredients, diets and fish samples based on the methods described in the previous chapter.

Results

Apparent digestibility, mineral and nutrient retention

ADC for dry matter, crude protein and crude lipid and phosphorus absorption are shown in Table 8 of Appendix 3. ADC for dry matter, protein and lipid of control and HTSC group are the same and significantly higher among others. Moreover, the phosphorus absorption of HTSC group is significantly higher among all treatments. Manganese absorption also tends to improve by extrusion cooking. Likewise, for zinc absorption, values tend to increase in diets containing extruded SBM or CGM.

For nutrient retention, HTSC group had highest values for both protein and lipid retention. Protein retention was found to be lowest in NE group and is significantly different ($P<0.05$) among other treatments. Lipid retention was found to be lowest in the control group which is only significantly different ($P<0.05$) with HTSC group. Phosphorus retention was found to be highest in HTSC group which is significantly different among others ($P<0.05$). Furthermore, P excretion in HTS, HTC and HTSC were significantly lower ($P<0.05$) than those of control and NE group. This study demonstrated that combined high temperature(150ºC) extruded SBM and CGM resulted to improved growth, feed efficiency, nutrient utilization, digestibility and fish body composition. Also, the performance of fish groups fed HTS or HTC are the same.

Growth and feed efficiency

In Table 7 of Appendix 4, growth performance, survival and feed efficiency are shown. In terms of final weight, weight gain and SGR, result shows that control group had significantly higher values among others followed by HTSC group; the lowest growth rate was exhibited in NE group. Though not determined in this study, HTS and HTC diets contain ANFs thus growth of fish were affected. HTSC on the other hand contains extruded SBM and CGM and extrusion is known to lower ANFs to make nutrients available to support fish growth. For feed efficiency, NE group had significantly lower values among others although daily feed intake of NE was found to have the highest value.

Body composition

The proximate and mineral body composition of rainbow trout are shown in Table 9 of Appendix 3. The moisture content of the fish fed control diet was found to have significantly higher value ($P<0.05$) among others. Likewise, the crude protein of control group was found to have higher value than HTS group but not with the other groups. However for lipid content, control group had significantly lower value than HTC group and comparable with other groups. Furthermore, ash content of control and HTS group were significantly higher among others. Iron, manganese and phosphorus content of HTS, HTC and HTSC group were quite higher compared to other treatment groups. It is also noteworthy that the amino acid composition of the control group and the HTSC group are comparable.

Discussion

Dry matter apparent digestibility coefficient of different protein ingredients gives an estimate of overall digestibility thus a low value for dry matter ADC signifies presence of high level indigestible material present in the feedstuff (Li et al., 2013). Furthermore, Luo et al. (2008) reported, dry matter ADC provides better estimate of the amount of indigestible material present in feedstuffs in comparison with the digestibility coefficients for individual nutrients. In this study, it was presented that the apparent digestibility coefficient of control and HTSC diets in terms of dry matter were comparable and are significantly higher ($P<0.05$) among other dietary treatments. This could also mean a more environment friendly aquaculture as less solids will be excreted into the water (Cheng & Hardy, 2003). Likewise, according to Barrows & Hardy (2000), extrusion cooking confer important benefits to the physical attributes of pellets including nutrient digestibility. In this study ADC for protein and lipid were found to be higher in HTSC and control group compared to other dietary treatments. The result on apparent digestibility in this study is comparable with the findings of Cho et al. (2006) for olive flounder, *Paralichthys olivaceus* wherein extrusion also improved the digestibility of ingredients and they are generally accepted by the fish. Mineral bioavailability in term of phosphorus, manganese and zinc in this study were also improved by extrusion cooking. This could be due to the reduced factors that inhibit absorption such as phytates. Although phytate was not analyzed in this study, but according to Alonso et al. (2001) and Singh et al. (2007), high temperature extrusion could result in phytate hydrolysis resulting in higher availability of minerals after processing.

As presented in this study, the survival of fish was found to have significant difference wherein HTSC had the highest value (90%) while HTS and HTC had the lowest (81%). This has nothing to do with any nutritional effects but due to human error in the fish rearing room. The feed efficiency of diets containing high temperature extruded ingredients and the control diet were comparable in terms of FCR and PER. Extrusion cooking appears to improve feed efficiency in rainbow trout diet. This is in accordance with the result obtained by Barrows et al. (2007) that extrusion influenced feed intake in rainbow trout. However, result of growth trial shows that rainbow trout fed control diet had the highest growth rate exhibited in final weight and weight gain which is significantly higher among other dietary treatments followed by HTSC group. The combination of high temperature extruded SBM and CGM exhibited an improved growth compared to NE, HTS and HTC diets. This could be due to an improved digestibility and phosphorus availability exhibited in HTSC. In this study, phosphorus absorption was improved by extrusion cooking at high temperature the same as reported by Satoh et al. (2002). Likewise, the work of Barreto-Curiel et al. (2018) suggest that extrusion process clearly improved protein efficiency ratio and apparent digestibility coefficient in juvenile *Totoaba macdonaldi.* Specific growth rate shows a good trend as lowest value was exhibited by NE group which is significantly different among others; followed by the HTS and HTC group, and among dietary treatments containing plant ingredients, HTSC group exhibited the highest value for SGR. This simply reflects the improved nutritional value of the extruded ingredients.

Nutrient retention for protein was found to be highest in HTSC group which is comparable with the control group but higher than NE group. Furthermore, lipid retention was also highest in HTSC group and comparable than the control. The work of Xu, et al. (2017) also showed an increase in protein retention from 35.9 to 39.7% in extruded feeds given to channel catfish, *Ictalurus punctatus* which is due to the reduced feeding rate from satiation (100%) to 70% of satiation. Likewise, in this study daily feed intake value was found to be least in HTSC group but still has the highest protein and lipid retention. Moreover, P retention was improved by extrusion cooking as higher values were exhibited by diets containing extruded SBM or CGM. As P retention was improved, P excretion was reduced making the diets more environment friendly.

P absorption were significantly improved in HTS, HTC, and HTSC than NE. Mn and Zn absorption were also tended to be improved in these groups than NE. Body mineral composition shows increased Cu, Fe, Mg, Mn, P in these groups than NE. K content seems to also be increased in HTC and HTSC groups than NE. In contrast, Zn content in HTC and HTSC decreased compared to that of NE group. Considering that Zn absorption tended to be improved in these two treatments and minerals interact each other, decreased Zn content in these two groups may be due to relative decrease of Zn together with increased other minerals content.

Conclusion

This study demonstrated that combined high temperature (150°C) extruded SBM and CGM resulted to improved growth, feed efficiency, nutrient utilization, digestibility and fish body composition. Moreover, the performance of fish groups fed HTS and HTC diets were just the same.

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Appendix 4

Table 1. Proximate and mineral composition of non-extruded or high temperature extruded SBM and CGM

Measured composition (% dry matter)	SBM	HT SBM	CGM	HT CGM
Dry matter	86.67	94.79	90.09	94.61
Crude protein	41.43	47.91	64.83	61.18
Crude lipid	2.54	1.76	6.34	6.54
Ash	5.92	6.20	1.20	2.17
Mineral composition				
Ca (mg g^{-1})	2.20	3.21	0.41	2.05
Cu (mg kg^{-1})	17.26	18.61	13.68	17.68
Fe (mg kg^{-1})	130.72	151.19	128.67	153.86
K (mg g^{-1})	11.07	11.00	13.06	14.15
Mg (mg g^{-1})	3.16	3.18	0.59	0.79
Mn (mg kg^{-1})	38.77	40.69	10.67	15.27
P (mg g^{-1})	6.65	7.86	4.39	6.68
Zn (mg kg^{-1})	42.69	79.12	33.32	29.08

Abbreviations: Ca, calcium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; P, phosphorus and Z, zinc.

Table 2. Amino acid composition (%) of non-extruded or high temperature extruded SBM and CGM.

Essential amino acid	SBM	HT SBM	CGM	HT CGM
Arginine	1.30	2.94	1.14	1.54
Histidine	0.38	0.77	0.65	0.67
Isoleucine	0.44	1.17	0.87	0.91
Leucine	1.22	3.16	6.38	6.23
Lysine	1.09	2.57	0.69	0.93
Methionine	0.14	0.42	0.95	0.85
Phenylalanine	0.86	2.07	2.63	2.58
Threonine	0.66	1.45	1.43	1.44
Tryptophan	0.06	0.32	0.17	0.22
Valine	0.47	1.28	1.26	1.27
Non-essential amino acid				
Alanine	0.85	1.66	4.48	4.22
Aspartic acid	2.24	2.97	3.06	3.28
Cystine	0.13	0.41	0.43	0.24
Glutamic acid	3.61	7.60	10.47	10.43
Glycine	0.84	1.65	1.53	1.44
Serine	1.00	2.24	2.57	2.57
Tyrosine	0.57	1.54	2.30	2.17

Table 3. Formula (% dry matter) of experimental diets fed to rainbow trout juveniles

Ingredients	Control	NE	HTS	HTC	HTSC
Peruvian Anchovy meal	55.0	15.0	15.0	15.0	15.0
Soybean meal	-	25.0	-	25.0	-
HT Soybean meal	-	-	25.0	-	25.0
Corn gluten meal	-	25.0	25.0	-	-
HT Corn gluten meal	-	-	-	25.0	25.0
Defatted rice bran	9.70	0.80	0.80	1.60	1.40
Wheat flour	10.0	10.0	10.0	10.0	10.0
Cod liver oil	5.00	6.80	6.80	6.00	6.20
Rapeseed oil	5.00	5.00	5.00	5.00	5.00
Pre-gelatinized starch	5.00	5.00	5.00	5.00	5.00
Mineral mix[a]	1.00	1.00	1.00	1.00	1.00
Vitamin mix[b]	3.00	3.00	3.00	3.00	3.00
Choline chloride	0.50	0.50	0.50	0.50	0.50
Vitamin E (50%)	0.10	0.10	0.10	0.10	0.10
D-L Methionine	-	0.30	0.30	0.30	0.30
Chromium oxide (50%)	0.50	0.50	0.50	0.50	0.50
Calcium monophosphate	-	2.00	2.00	2.00	2.00
Cellulose	5.20	-	-	-	-

[a] Mineral pre-mix (g kg^{-1}): sodium chloride, 50; magnesium sulfate, 745; iron (III) citrate *n*-hydrate, 125; cellulose, 30; trace element mixture, 50. The trace element mixture contains (g kg^{-1}) zinc sulfate heptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminum chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose 440.

[b] Vitamin pre-mix (unit kg^{-1}): Vitamin A, 2, 400,000 IU; Vitamin D3, 2,400,000 IU ; Vitamin K3, 6.05 g; thiamine, 3.025 g; riboflavin, 3.63 g; pyridoxine, 2.42 g; cyanocobalamin, 0.006 g; L-ascorbic acid, 368.902 g; nicotinic acid, 24.2 g; D-pantothenic acid, 6.05 g; inositol, 121 g; D-biotin, 0.363 g; folic acid, 0.908 g; *para*-amino benzoic acid, 3.025 g.

Table 4. Proximate and mineral composition of experimental diets fed to rainbow trout juveniles

Measured composition (% dry matter)	Control	NE	HTS	HTC	HTSC
Dry matter	98.4	97.1	97.6	98.0	98.5
Crude protein	43.6	43.9	42.9	42.3	42.3
Crude lipid	14.5	13.2	13.7	13.3	14.3
Ash	11.9	6.10	6.78	7.07	6.74
Mineral composition					
Ca (mg g^{-1})	33.76	14.55	13.59	13.59	15.62
Cu (mg kg^{-1})	1.9	4.27	4.3	4.3	5.8
Fe (mg kg^{-1})	357.8	306.8	329.6	326.9	329.7
K (mg g^{-1})	8.25	9.17	10.21	10.21	11.22
Mg (mg g^{-1})	2.16	2.10	2.11	2.11	2.12
Mn (mg kg^{-1})	28.5	21.3	20.3	22.3	23.8
P (mg g^{-1})	23.87	22.42	21.53	21.65	22.65
Zn (mg kg^{-1})	71.4	59.4	57.8	57.2	58.7

Table 5. Essential amino acid composition (% dry matter) of experimental diets fed to rainbow trout juveniles

Essential amino acid	Control	NE	HTS	HTC	HTSC	EAA requirement of rainbow trout*
Arginine	2.36	5.04	1.94	1.85	3.41	1.4
Histidine	1.03	0.65	0.82	0.66	0.74	0.63
Isoleucine	1.37	0.82	1.15	0.97	1.17	0.98
Leucine	2.93	3.04	4.32	3.67	3.96	1.75
Lysine	3.17	1.59	1.89	1.62	1.76	2.10
Methionine	0.91	0.85	1.03	0.90	0.85	0.81
Phenylalanine	1.65	1.54	2.10	1.88	1.88	1.26
Threonine	1.82	1.02	1.56	1.42	1.42	1.44
Valine	1.66	1.04	1.32	1.12	1.16	1.26

* Ogino (1980)

Table 6. Fatty acid composition (% dry matter) of experimental diets fed to rainbow trout juveniles

Fatty acid	Control	NE	HTS	HTC	HTSC
14:0	3.80	2.89	3.43	3.41	3.49
16:0	12.6	11.0	12.4	11.9	12.1
18:0	2.69	2.26	2.36	2.35	2.44
18:2n-6	9.88	18.8	17.4	15.8	14.9
18:3n-3	3.33	3.83	3.96	4.08	4.10
20:4n-6 (ARA)	0.41	0.28	0.29	0.28	0.28
20:5n-3 (EPA)	6.97	5.03	5.18	5.11	4.98
22:5n-3 (DPA)	0.91	0.70	0.68	0.68	0.68
22:6n-3 (DHA)	7.26	4.34	4.66	5.22	4.38
∑ PUFA	21.18	27.9	26.9	26.0	24.4
∑ n-6 PUFA	10.3	19.0	17.6	16.0	15.2
∑ n-3 PUFA	11.15	8.87	9.30	9.97	9.16
n-3:n-6	1.12	0.47	0.53	0.62	0.60

Table 7. Growth performance, survival and feed efficiency of juvenile rainbow trout fed experimental diets for 12 weeks

Parameters	Control	NE	HTS	HTC	HTSC
Initial weight (g)	6.86	6.98	7.00	7.00	6.92
Final weight (g)	73.9^{d}	51.0^{a}	57.4^{b}	58.9^{b}	66.2^{c}
Weight gain (%)	977^{d}	631^{a}	720^{b}	742^{b}	857^{c}
SGR (% day $^{-1}$)	3.71^{d}	3.11^{a}	3.29^{b}	3.33^{b}	3.53^{c}
FCR	0.93^{b}	1.08^{a}	0.96^{b}	0.97^{b}	0.93^{b}
PER	2.47^{b}	2.06^{a}	2.44^{b}	2.45^{b}	2.70^{b}
DFI	2.41^{ab}	2.57^{a}	2.35^{ab}	2.40^{ab}	2.22^{b}
Survival (%)	85.0^{ab}	85.0^{ab}	81.7^{a}	81.7^{a}	90.0^{b}

Values in the same row with different uppercase letters are significantly different (P<0.05).

Abbreviations: SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; DFI, daily feed intake

Table 8. Apparent digestibility coefficients of dry matter, crude protein, crude lipid and P, Mn, Zn absorption and nutrient retention of experimental diets fed to rainbow trout

ADC (%)	Control	NE	HTS	HTC	HTSC
Dry matter	82.4^{b}	73.8^{a}	76.1^{a}	76.3^{a}	83.0^{b}
Crude protein	93.4^{b}	88.2^{a}	90.1^{a}	90.4^{a}	92.8^{b}
Crude lipid	95.7^{b}	90.5^{a}	91.0^{a}	91.8^{a}	94.9^{b}
Mineral Absorption (%)					
P	64.1^{b}	53.0^{a}	59.5^{b}	60.1^{b}	80.4^{c}
Mn	41.7	41.4	45.1	53.8	58.4
Zn	76.7	78.9	80.7	82.0	83.1
Nutrient retention (%)					
Protein	35.7^{bc}	29.3^{a}	33.6^{b}	34.6^{bc}	37.8^{c}
Lipid	78.9^{a}	82.9^{ab}	$86.4a^{b}$	90.6^{ab}	93.7^{b}
Phosphorus	10.55	7.36	11.14	11.53	15.36
*P excretion (kg t^{-1})	11.39	16.76	9.16	9.37	5.46

Values in the same row with different uppercase letters are significantly different (*P*<0.05).

*Computed based on production of 1000kg fish

Table 9. Proximate and mineral body composition of rainbow trout fed experimental diets

Measured composition (% wet weight basis)	Control	NE	HTS	HTC	HTSC
Moisture	77.1^{b}	71.0^{a}	71.0^{a}	71.4^{a}	71.1^{a}
Crude protein	14.5^{b}	14.3^{ab}	13.8^{a}	14.2^{ab}	14.0^{ab}
Crude lipid	10.6^{a}	11.4^{ab}	11.5^{ab}	11.7^{b}	11.3^{ab}
Ash	2.86^{b}	1.83^{a}	2.45^{b}	1.79^{a}	1.71^{a}
Minerals					
Ca (mg g^{-1})	3.13	3.23	3.14	3.10	3.12
Cu (mg kg^{-1})	1.02	0.65	0.93	0.82	0.76
Fe (mg kg^{-1})	9.87	12.67	17.60	14.97	14.84
K (mg g^{-1})	3.76	3.75	3.76	3.82	3.87
Mg (mg g^{-1})	0.38	0.34	0.39	0.38	0.39
Mn (mg kg^{-1})	1.34	1.29	1.76	1.76	1.76
P (mg g^{-1})	3.76	3.75	4.76	4.81	5.87
Zn (mg kg^{-1})	9.77	9.45	9.92	8.83	9.02

Values are expressed as mean ± SEM (n = 3). Values in the same row with different uppercase letters are significantly different (P<0.05).

Table 10. Total essential amino acid composition of fish body (wet basis, %)

Essential amino acid	Control	NE	HTS	HTC	HTSC
Arginine	2.76	2.10	2.32	2.41	2.88
Histidine	1.06	0.73	0.99	0.75	0.99
Isoleucine	1.14	0.81	1.52	0.99	1.49
Leucine	3.22	2.57	3.02	2.62	3.44
Lysine	3.97	2.91	3.61	3.32	4.05
Methionine	1.30	1.15	1.24	1.27	1.33
Phenylalanine	1.75	1.33	1.62	1.60	1.74
Threonine	2.05	1.36	2.30	1.88	2.16
Valine	1.43	1.09	2.06	1.68	1.97
Tryptophan	0.32	0.13	0.17	0.18	0.16

Table 11. Fatty acid composition (% wet basis) of fish body diets fed to rainbow trout juveniles

Essential amino acid	Control	NE	HTS	HTC	HTSC
14:0	2.43	1.96	1.23	1.62	1.76
16:0	12.5	9.61	11.1	10.1	14.4
18:0	3.80	2.79	3.30	3.35	3.42
18:2n-6	8.10	12.6	11.3	11.6	12.8
18:3n-3	2.32	2.49	2.67	2.65	2.84
20:5n-3 (EPA)	1.92	1.34	1.61	1.49	1.52
20:4n-6 (ARA)	0.61	0.48	0.52	0.50	0.57
22:5n-3 (DPA)	0.93	0.74	0.73	0.67	0.76
22:6n-3 (DHA)	8.14	6.12	6.11	6.62	6.15
∑ PUFA	21.41	23.29	22.42	23.03	23.97
∑ n-6 PUFA	8.10	12.6	11.3	11.6	12.8
∑ n-3 PUFA	13.31	10.69	11.12	11.43	11.17

CHAPTER 5

GENERAL CONCLUSION & RECOMMENDATION

In general, these studies demonstrated that extrusion cooking could improve nutritional value of SBM and CGM. Diets containing high temperature (150ºC) extruded SBM and CGM enhanced fish growth, feed utilization, nutrient retention, body composition and digestibility in rainbow trout. Extrusion temperature (100°C and 150°C) in this study is suitable for the processing of SBM and CGM as feed ingredients for rainbow trout. Moreover, findings from these studies have potential for applying to the commercial aquaculture feed and enhancing distribution of low fish meal diet. This may lead to the establishment of sustainable aquaculture industry.

For further studies, we recommend to consider total replacement of fish meal with high temperature extruded soybean meal and corn gluten meal. Moreover, extruding SBM and CGM at higher temperature (200°C) and test the effects on the tested parameters. Also, to evaluate the anti-nutritional factors of the extruded ingredients and test the effects of extruded ingredients with other fish species. Combined effect of extruded SBM and CGM on enteritis and intestinal microbiome could also be considered for future study.