Dietary effects on growth performance and health indices for burbot *(Lota lota maculosa)*

A Thesis

Presented in Partial Fulfilment of the Requirements for the

Degree of Master of Science

Major in Natural Resources

in the

College of Graduate Studies

University of Idaho

by

Sinem Gulen

Major Professor: Kenneth Cain, Ph.D.

Committee Members: Brian Small, Ph.D.; Vikas Kumar, Ph.D.

Department Administrator: Lisette P. Waits, Ph.D.

December 2019

Authorization to Submit Thesis

This thesis of Sinem Gulen, submitted for the degree of Master of Science with a Major in Natural Resources and titled "Dietary effects on growth performance and health indices for burbot *(Lota lota maculosa)*," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: ______________________________ Date: ___________
Kenneth Cain, Ph.D.

Committee Members: ______________________________ Date: ___________
Brian Small, Ph.D.

______________________________ Date: ___________
Vikas Kumar, Ph.D.

Department Administrator: ______________________________ Date: ___________
Lisette Waits, Ph.D.

Abstract

Burbot (*Lota lota maculosa*) are the only freshwater members of the Gadidae family and can be reared in similar water quality parameters as rainbow trout under controlled rearing conditions (Barron et al. 2012). Burbot have desirable fillets for consumers and demonstrate good growth performance in culture (Jensen et al. 2011). As such, burbot are a favorable species for aquaculture and may have potential for polyculture in trout facilities to diversify production. To successfully develop burbot culture on a production scale, nutrient requirements in burbot feed should be evaluated. In this project, we aimed to determine growth performance of fish fed two dietary formulations (marine type or trout type) for economic burbot culture. Additionally, the digestibility of soy protein ingredients for burbot was investigated to address the potential to produce more sustainable diets for burbot production in the future.

To evaluate growth performance with commercial diet blends, a feeding trial was conducted, incorporating a marine-type commercial diet (Europa), marine-type formulated diet (Burbot1), trout-type commercial diet (Oncor) and trout-type formulated diet (Burbot 2). The experiment was conducted as a complete randomized design, with three replicate tanks per diet. No significant differences in growth parameters such as FCR, SGR, RG and K factor ($p>0.05$) were observed for burbot fed the different dietary treatments. There were no statistically significant difference between treatments. Taking these results into account it is suggested that a trout type dies such as Oncor would provide equal growth to a higher cost marine type diet and be a preferred choice for most producers at this life stage. There were significant differences observed between treatments for some organosomatic indices. Fillet yield of fish fed Burbot 2 diet was significantly lower than fish fed other diets

($p<0.05$), and fish fed Oncor diet had the highest fillet yield level. This indicates that fish fed Burbot 2 diet may not have utilized dietary protein as efficiently for muscle creation in fillets, and fish fed the Oncor diet had the most efficient dietary protein usage. Also, gastrointestinal index (GII) was significantly higher in fish that fed with Oncor diet than fish fed Burbot 1 diet ($p<0.05$). Based on economic analyses, feeding this size burbot with Oncor diet much more economical than Europa diet. Producers can save $0.69 from each fish to growth them from 100g to 400g (market size for burbot) by feeding fish with Oncor diet instead of Europa.

For burbot soy digestibility, soybean meal diet (SBM), soy protein concentrate diet (SPC), microbially enhanced soy protein diet (MESBM) and reference diet were used to feed adult burbot. 30% of the reference diet was replaced with dietary soy protein ingredient in experimental diets (SBM, SPC, and MESBM). Also, yttrium oxide, an inert marker was added in all diets at 0.1% ratio in order to calculate the nutrient digestibility. The present study indicates that burbot can digest high soy protein diets, also there is a significant difference between treatment digestibility for burbot. Furthermore, the soy protein ingredient digestibility of burbot was high, and there were significant differences between treatments on soy protein ingredient digestibility. There was no significant difference among the protein ingredient digestibility of the SBM (92.2%) diet and SPC (93.5%) diets for burbot. The trout protein digestibility level for SBM ingredient was 92.1% and for SPC ingredient was 97.9% (Glencross et al. 2005). Atlantic cod protein digestibility for SBM ingredient was 91.5% and for SPC ingredient was 94% (Tibbet et al. 2006). So, burbot had similar ingredient protein digestibility level of SBM with trout and Atlantic cod, and SPC ingredient protein digestibility of burbot and cod were similar and lower than trout. The ingredient MESBM digestibility level (88%) was significantly lower than SBM and SPC in burbot. Therefore, SPC and SBM may serve as better soy protein ingredients compared to MESBM (88%) evaluated for replacing fishmeal in burbot diets, based on higher levels of apparent digestibility coefficient (ADC).

Acknowledgments

I would like to thank Dr. Kenneth Cain for his support, patience and supervising throughout my program. I would like to thank my committee members, Dr. Brian Small and Dr. Vikas Kumar for their help and wisdom in their fields. I thank my funding sources, the Western Regional Aquaculture Center and the U.S. Department of Agriculture (USDA) NIFA, and the Soy Aquaculture Association for providing funding on the research projects. A special thanks to my program sponsor, the Republic of Turkey for funding and providing me this academic opportunity. I also would like to thank to Dr. Tim Bruce and Dr. Jie Ma for their help in my research and support for my graduate school experience. I want to thank to all members of the UI Fish Health laboratory and ARI, along with Moureen Matuha, Joe Evavold, Daniel Korbel, Luke Oliver and Scott Williams for their help and support.

Dedication

This work is dedicated to Mehmet Gulen and Fehime Gulen.

Table of Content

List of Figures

List of Tables

General Introduction

World aquaculture production in 2017 was 80.1 million tons of aquatic animals and 31.8 million tons aquatic plants 2.2 million tons nonfood products (FAO 2019). The production of cold freshwater species in Europe mainly consists of trout (Kucharczyka et al. 2018). Interestingly, commercial trout value of sale has decreased at a rate of 10% from 2017 in the United States (US) (NASS 2019). Supplying an alternative species that lives in a similar water quality with trout may be beneficial for trout facilities and diversify their product offerings.

Burbot (*Lota lota maculosa*) are the only freshwater members of the cod family Gadidae and can be found in both lacustrine and riverine systems (Polinski et al. 2010; Terrazas et al. 2017). Burbot populations have been declining in both North America and Europe (Jensen et al. 2008; Beard 2017) due to anthropogenic pressure and environmental change (Stapanian et al. 2010; Stapanian and Myrick 2015; Harrison et al. 2016; Beard 2017; Stańczak et al. 2017). Burbot conservation aquaculture programs have been developed as a result of such population decreases (Jensen et al. 2008). For juvenile burbot, the optimum rearing water temperature appears to range between 15-20°C (Barron et al. 2012; Terrazas et al. 2017), and burbot grow well under controlled rearing conditions and have desirable fillet quality (Wong 2008). In addition, burbot (a freshwater gadiform species) body proximate composition appears comparable to cod (a saltwater gadiform species) indicating this species provides a high protein food source similar to other marine fish species (Wong 2018). Burbot are highly fecund (Barron et al. 2013) and larvae have been shown to transition from live feeds to commercial diets and perform well with high protein diets (Jensen et al. 2011). Based on work done to date, burbot (*Lota lota maculosa*) appear to be a potential candidate for commercial aquaculture. Although the potential is there for this emerging species, many uncertainties and questions remain. These include the need to identify optimal nutrient requirement that provide adequate growth performance at different life stages, and at a minimum there is a need to identify existing commercial feeds that are most appropriate for burbot production.

Fishmeal from captured wild marine fish is the primary protein source in many commercial aquafeeds (Wacyk et al. 2012; Jobling. 2016). Limited supply of wild marine fish restricts fishmeal production and increases the price of fishmeal (Salze et al. 2010). The limitation restricts the aquaculture feed production and increases production cost (Burr et al. 2012; Biswas et al. 2009). For sustainable aquaculture production, it is crucial to find an alternative protein source for fishmeal in feeds (Wacyk et al. 2012; Biswas et al. 2009). The alternative source should be from sustainable sources, have high protein levels, and be highly digestible for target fish species (Gatlin et al. 2007; Cardinaletti et al. 2019). One such alternative ingredient is soy protein, which is readily available, low in price, and has a balanced nutrient composition and amino acid profile (Cheng and Hardy 2004; Minijarez-Osorio et al. 2016). When Atlantic cod were fed with diet where 24% of fishmeal was replaced by soybean meal, no negative effects were observed (Forde-Skjaervik et al. 2006).

Based on this background and knowledge, we hypothesized that burbot would exhibit similar growth performance as Atlantic cod when fed with marine type diets, and may show high soy protein ingredients apparent digestibility similar with cod. The following objectives were addressed in this research to test the most efficient diet type for burbot growth and health, and to understand whether soy protein ingredients are suitable for replacing with fishmeal in burbot diets in the future:

1. Determine dietary effects on sub-adult burbot growth and health indices.
    - Compare high protein (cod type) commercial and reference diets with lower protein (trout type) commercial and reference diets.
    - Provide recommendation useful for producers interested in cost of burbot production.
2. Determine digestibility of soy protein ingredients for burbot.
    - Currently best protein ingredients in aquafeed.

References

Barron, J.M., Jensen, N.R., Anders, P.J., Egan, J.P., Ireland, S.C., Cain, K.D., 2012. Effect of temperature on the intensive culture performance larval and juvenile North American burbot (*Lota lota maculosa*). Aquaculture 364-365:67-73.

Barron, J.M., Jensen, N.R., Anders, P.J., Egan, J.P., Ireland, S.C., Cain, K.D., 2013. Effects of Stocking Density on Survival and Yield of North American Burbot Reared under Semi-Intensive Conditions. Transactions of the American Fisheries Society 142 (6): 1680-1687.

Beard, Z.S., 2017. Survival, Movement, and Distribution of Juvenile Burbot in a Tributary of the Kootenai River. North American Journal of Fisheries Management 37:1274–1288.

Biswas, B.K., Ji, S.C., Biswas, A.K., Seoka, M., Kim, Y.S., Kawasaki, K., Takii, K., 2009. Dietary protein and lipid requirements for the Pacific bluefin tuna *Thunnus orientalis* juvenile. Aquaculture 288:114–119.

Burr, G.S., Wolters, W.R., Barrows, F.T., Hardy, R.W., 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture 334-337:110–116.

Cardinaletti, G., Randazzo, B., Messina, M., Zarantoniello, M., Giorgini, E., Zimbelli, A., Bruni, L., Parisi, G., Olivotto, I., Tulli, F., 2019. Effects of Graded Dietary Inclusion Level of Full-Fat *Hermetia illucens* Prepupae Meal in Practical Diets for Rainbow Trout (*Oncorhynchus mykiss*). Animals 9:251.

Cheng, Z.J., Hardy, R.W., 2004. Effects of Microbial Phytase Supplementation and Dosage on Apparent Digestibility Coefficients of Nutrients and Dry Matter in Soybean Product-Based Diets for Rainbow Trout *Oncorhynchus mykiss*. Journal of the World Aquaculture Society Vol. 35, No. 1.

Food and Agriculture Organization (FAO) of the United Nation 2019.

Førde-Skjærvik, O., Refstie, S., Aslaksen, M.A., Skrede, A., 2006. Digestibility of diets containing different soybean meals in Atlantic cod (*Gadus morhua*); comparison of collection methods and mapping of digestibility in different sections of the gastrointestinal tract. Aquaculture 261:241–258.

Gatlin III, D.M., Barrows, F.T., Paul Brown, P., Konrad Dabrowski, K., Gaylord, T.G.,Hardy, R.W., Herman, E., Gongshe Hu, G.,Krogdahl, A., Richard Nelson, R., Overturf1, K., l Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., EveWurtele, E., 2007. Expanding the utilization of sustainable plant products in aqua feeds: a review. Aquaculture Research 38:551-579.

Gonzalez, E.B., Boer, F. 2017. The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. Fish Sci 83:661–670.

Grealisa, E., Hynesa, S., O'Donoghueb, C., Vegaa, A., Oscha, S.V., Twomeyc, C., 2017. The economic impact of aquaculture expansion: An input-output approach. Marine Policy 8:29–36.

Harrison, P. M., Gutovsky, L.F.G., Martins, E.G., Patterson, D.A., Cooke, S.J., Power, M., 2016. Burbot and large hydropower in North America: benefits, threats and research needs for mitigation. Fisheries Management and Ecology 23:335–349.

Jensen, N.R., Ireland, S.C., Siple, J.T., Williams, S.R., Cain, K.D., 2008. Evaluation of burbot egg incubation methods and larval feeding regimes for North American burbot. North American Journal of Aquaculture 70:162–170.

Jensen, N.R., Anders, P.J., Hoffman, C.A., Porter, L.S., Ireland, S.C., Cain, K.D., 2011. Performance and Macronutrient Composition of Age-0 Burbot Fed Four Diet Treatments. North American Journal of Aquaculture 73 (3):360-368.

Jobling, M., 2016. Fish nutrition research: past, present and future. Aquacult Int 24:767–786.

Kucharczyka, D., Nowosada, J., Kujawaa, R., Dietricha, G., Biegaja, M., Sikoraa, M., Luczynskib, M.J., 2018. Comparison of spontaneous and hormone-induced reproduction of burbot (*Lota lota L.*) under hatchery conditions. Aquaculture 485:25–29.

Minjarez-Osorio, C., Castillo-Alvarado, S., Gatlin III, D.M., González-Félix, M.L., Perez-Velazquez, M., Rossi Jr, W., 2016. Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*): A comparative study. Aquaculture 453:122-129.

Nadarajah, S., Flaaten, O., 2017. Global aquaculture growth and institutional quality. Marine Policy 84:142–151.

National Agricultural Statistics Service (NASS), Agricultural Statistics Board, United States Department of Agriculture (USDA), 2019. Available at https://usda.library.cornell.edu/concern/publications/t722h882h.

Polinski, M., Fehringer, T., Johnson, K., Snekvik, K., Lapatra, S., LaFrentz, B., Ireland, S., Cain, K., 2010. Characterization of susceptibility and carrier status of burbot, *Lota lota* (L.), to IHNV, IPNV, *Flavobacterium psychrophilum*, *Aeromonas salmonicida*, and *Renibacterium salmoninarum*. Journal of Fish Diseases 90:15–23.

Salze, G., McLean, E., Battle, P.R., Schwarz, M.H., Craig, S.R., 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fishmeal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. Aquaculture 298:294–299.

Stańczak, K., Król, J., Żarski, D., Hliwa, P., 2017. Marking of Burbot (*Lota lota*) with Fluorescent Dyes Using the Per Os Method. Turkish Journal of Fisheries and Aquatic Sciences 17:121-127.

Stapanian, M.A., Myrick, C.A., 2015. Ecology, culture, and management of Burbot: an introduction. Hydrobiologia 757:1–2.

Stapanian, M.A., Paragamian, V.L., Madenjian, C.P., Jackson, J.R., Lappalainen, J., Evenson, M.J., Neufeld, M.D., 2010. Worldwide status of burbot and conservation measures. Fish and Fisheries 11:34–56.

Terrazas, M. M., Adams, J.R., Sudheesh, P.S., Cain, K.D., 2017. Effects of Diel Temperature Fluctuation on Growth, Stress Response, and Immune Function of Burbot. Transactions of the American Fisheries Society [Online serial] 146:5, 996-1007, DOI:10.1080/00028487.2017.1334701. https://doi.org/10.1080/00028487.2017.1334701.

Wacyk, J., Powell, M., Rodnick, K., Overturf, K., Hill, R.A., Hardy, R., 2012. Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets. Aquaculture 356–357:223–234.

Wong, A. 2008. Lipid profiles of tissue and liver oil of burbot *Lota lota* (L.). Acta Ichthyologica Et Piscatoria 38 (1):55–61

Chapter 1: Literature Review

Aquaculture Production

Aquaculture is defined as farming aquatic animals and plants (Young et al. 2019) and is important with respect to food production, long term food security and its contribution to environmental, economic, and social sustainability (Grealis et al. 2017; Guillen et al. 2019; Stoll et al. 2019). Aquaculture has grown continuously in recent decades (Young et al. 2019) increasing annually by an average of 8.8% between 1980 and 2012 (Grealis et al. 2017; Nadarajah and Flaaten. 2017).

The world population is constantly increasing and is expected to reach 8.5 billion by 2030 and 9.7 billion by 2050 (Grealis et al. 2017). The growing world population requires a high amount of protein and seafood plays a crucial role to meet the protein need of the expanded world`s population (Bruce and Brown 2017; Grealis et al. 2017; Mohantya et al. 2019). Fish (finfish, crustacean and molluscs) are healthy and supplies an average of 20% of the protein intake for over a third of the world`s population (Mohantya et al. 2019). Many fish are good source of omega (ω)-3 polyunsaturated fatty acids (PUFAs) and have high levels of micronutrients, such minerals (iodine, selenium, zinc, magnesium and calcium) and vitamin D in oily fish (Nadarajah and Flaaten 2017; Mohantya et al. 2019). Capture fisheries have been declining (Nadarajah and Flaaten 2017) and wild fish stocks cannot satisfy the increased demand for marine food products (Grealis et al. 2017). In order to maintain sustainable seafood production, the aquaculture sector has expanded at rate of 8.8% annually (Grealis et al. 2017). Global aquaculture production remains the fastest-growing animal food production sector (Guillen et al. 2019), and production has increased from 5.2 million tons in 1981 to million tons in 2015 (Young et al. 2019). Cultured food fish production is 44% of total fish supply (capture and culture) and is supposed to increase compared to wild-caught fish (Stoll et al. 2019), so aquaculture is more important for total sea food production than fisheries (Nadarajah and Flaaten 2017). Local and national economies have been influenced by aquaculture expansion (Senten et al. 2018) and the value of global aquaculture production (including aquatic plants) was $243.5 billion in 2016 (Guillen et al. 2019). In addition, aquaculture has improved food security and nutrition in rural communities with limited availability and access to resources (Senten et al. 2018). There are many fish species with a wide price range for selection; therefore, allowing families with low and high incomes to afford fish as a protein source (Mohantya et al. 2019). Global fish consumption per capita annually has been increased from 9 kg in 1961 to 20.2 kg in 2015 (Cordeiro 2019).

Rainbow trout (*Oncorhynchus mykiss*) are a major aquaculture species across the globe, representing 8.5% of the total value at approximately $13 billion (Gonzalez and Boer 2017; Kucharczyka 2018). Although trout are an important aquaculture species globally, in the United States (US) commercial trout sales value has decreased at a rate of 10% since 2017 (NASS 2019). Given the challenges facing trout aquaculture, an alternative fish species that could be produced under similar water quality conditions would be beneficial and allow trout facilities to diversify their products.

Burbot (*Lota lota*) life history

Adult burbot are piscivorous and play an important role among predators in lacustrine and riverine habitats (Gallagher and Dick 2015). Further, burbot have a crucial effect on the dynamics of the deep-water fish communities and can reside in brackish water near river estuaries (Palinska-Zarska et al. 2007; McCullough et al. 2015). Burbot are nocturnal and can live up to 15 years, with adults maturing at 3 to 4 years of age (30 to 50 cm in length and weighing from approximately 1 to 7 kg; Wong 2008). In the wild, burbot spawn from January to March often under ice cover and tend to congregate over sand or gravel bottoms (Wong 2008). The optimal rearing temperature is between 15 and 20˚C during the juvenile life stage (Barron et al. 2012; Terrazas et al. 2017). Burbot populations have been declining in both North America and Europe in recent years (Jensen et al. 2008; Beard 2017). It has been suggested that industrial pollution of aquatic ecosystems and dams have resulted in decreased burbot numbers in the North America and Europe coastal regions (Stapanian et al. 2010; Stapanian and Myrick 2015; Harrison et al. 2016; Beard 2017; Stańczak et al. 2017). These burbot populations are in constant decline within their natural environment because of anthropogenic pressure. Such declines have resulted the development of conservation aquaculture for this species in both North America and Europe (Barron et al. 2013; Barron et al. 2012; Jensen 2008; Egan et al. 2015). Declining burbot populations and small specimen sizes have made the capture of marketable size fish from the wild difficult (Wong 2008). Burbot conservation aquaculture is not only developing in response to declining wild stocks, but also for the immediate restoration of populations in North America and Europe (Jensen et al. 2008). In 2003, the University of Idaho's Aquaculture Research Institute (UI-ARI) initially started an experimental research program to evaluate the feasibility of developing burbot culture as a population restoration measure (Jensen et al. 2011).

Beyond conservation efforts, there is a potential to culture this species commercially for food. Commercial burbot aquaculture interest has increased following population decline in many parts of their geographic range, and development of burbot culture has begun for both North American *Lota lota maculosa* and Eurasian burbot *Lota lota lota* (Barron et al.

2012; Jensen 2008; Egan et al. 2015; Foltz et al. 2012; Barron et al. 2013). Wong 2008 caught burbot from Athapapuskow Lake (Manitoba) and Amisk Lake (Saskatchewan) in Canada and analyzed the lipid content of fish tissue and liver, he reported that burbot tissue had similar lipid level with cod and burbot livers have high levels of HUFA fatty acids (6.4% EPA, 15% DHA) and vitamins A, D and K_1. For these reasons this species is highly prized in the U.S and Europe (Wong 2008; Kucharczyk 2018). According to whole-bodied proximate analyses, there are considerable similarities in the body composition between burbot and cod (Wong 2008; Table 1). Thus, people can get high macro nutrient similar with Atlantic cod by consuming burbot.

Table 1. Nutritional content of cod and burbot muscle tissues. Value per 100 g of edible portion (skinless tissue) (Adapted from Wong 2018; Anonymous 2008a, b).

	Energy [kJ]	Protein [g]	Total lipid [g]	Ash [g]
Atlantic cod, *Gadus morhua*	343	17.81	0.67	1.16
Burbot*, Lota lota*	377	19.31	0.81	1.16

Fish Diet

The most expensive cost item of aquaculture production is feed (Tibbetts et al. 2006). The most expensive nutrient in aquafeeds is protein (Lee et al. 2002; Li et al. 2009; Khan et al. 2019). Also, dietary protein is a crucial factor that affects growth performance of fish (Lee et al. 2002), and a major protein source in fish feed is fishmeal (Jobling et al. 2016; Cardinaletti et al. 2019). Carnivorous species are typically fed with protein-rich diets (Jobling 2016) to enhance growth and feed efficiency ratio, but an excessive amount of protein in diets can make the diets unbalanced, increases feed cost, and causes nitrogen excretion and aquatic pollution (Lee et al. 2002; Khan et al. 2019). On the other hand, if protein needs are unmet in fish diets, it can cause growth reduction, nonregulated feed intake and energy deficiency. Fish should use dietary protein just for tissue synthesis not for energy to perform an economic production. If there is an energy deficiency in diet, fish use protein inefficiently as an energy source instead of tissue synthesis. That increases production cost (Khan et al. 2019). To increase dietary protein utilization for fish growth, protein levels can be partially replaced with lipid to enhance dietary energy levels (Lee et al. 2002; Khan et al. 2019). Therefore, it is possible to compose bio-economically viable and environmental diets (Lee et al. 2002) through proper balancing in dietary ingredients (Khan et al. 2019).

Protein requirement of fish shows variety depending on species and size (Arslan et al. 2013). Carnivorous juvenile fish require high levels of dietary protein for fast growth and economic production (Biswas et al. 2009). For instance, the optimum diet composition for sea bass fingerlings growth is 52% protein and 16% lipid (García-Meilán et al. 2016), but for grow-out stages the optimum protein level is 40% (Amin et al. 2014). In addition, juvenile rockfish fed with six diets containing three levels of digestible protein (37%, 42% and 47%) and two levels of lipid (7% and 14%). Rockfish fed with 42% protein and 14% lipid composition feed showed the best growth performance. The optimum diet composition for growth of juvenile rockfish was 42% protein and 14% lipid (Lee at al. 2002); however, Kim et al. (2004) indicated that the optimum dietary digestible protein level for juvenile Korean rockfish was between 45.1% and 50.9%. According to Arslan et al. (2013), nine diets (protein levels are 40-45-50%, lipid levels are 12-16-20%) were formulated to test the optimum diet formulation for juvenile catfish growth. Fish fed with 45% protein and 16% lipid had significantly higher growth performance compared to fish fed other diets. Also, the optimum protein/lipid level in feed for two-banded seabream fingerlings (*Diplodus vulgaris*) is 35/15% (Bulut et al. 2014), and for Pacific bluefin tuna (*Thunnus orientalis*) is 61.9/17.9% (Biswas et al. 2009).It is recommended that for best growing performance of juvenile shi brum (*Umbrina cirrosa*) digestible protein in diet should be more than 50% and lipid level should be 10% (Kokou et al. 2019). Furthermore, juvenile Manchurian trout (*Brachymystax lenok*) were fed with eight formulated diets (40, 45, 50, 55% crude protein and 8-16% lipid) to clarify optimum diet for fish growth. The diet that contains 45% protein and 16% lipid was determined optimum composition for juvenile Manchurian trout (Xu et al. 2015). According to research of Amin et al. (2014), brook trout (*Salvelinus fontinalis*) performed best growth when fed a 40% protein and 23% lipid diet. In addition, the optimum protein was 41% for juvenile Atlantic cod (Hamre 2006), and the optimum diet composition for fingerling cod was 48% protein and 16% lipid (Morais et al. 2001). Larval burbot fed with a commercial diet containing 50, 57, 60% protein and 10, 14, 15% fat, the larvae fed with 60% protein and 15%lipid had highest growth metrics (weight gain, SGR) (Jensen et al. 2011). However, there is lack of information about optimum diet composition for juvenile and sub-adult burbot rearing.

Soy Protein Source in Aquaculture

Feed for aquaculture production often relies on fishmeal produced from marine resources as a protein source for carnivorous fish (Wacyk et al. 2012; Jobling. 2016). Increased of aquaculture production has increased the demand for feed and fishmeal (Arslan et al. 2013). Overfishing of wild fish for feed production can impact the limited supply of wild fish used for fishmeal production (Wacyk et al. 2012) and raise the price of fishmeal for the market (Burr et al. 2012; Biswas et al. 2019). Fishmeal levels have decreased in feeds

(Naylor et al. 2009) and alternative protein sources are being used routinely in aquaculture feeds (Wacyk et al. 2012; Biswas et al. 2019).

Alternative protein sources are needed for sustainability in aquaculture, but these must contain high protein levels and digestible nutrients to provide growth and health (Gatlin et al. 2007; Cardinaletti et al. 2019). Plant protein is common and can be a good alternative to fishmeal for some species (Burr et al. 2012; Wacyk et al. 2012). Soybean meal (SBM) is one of the most appropriate alternative protein sources to fishmeal in terms of availability, low price, nutrient composition and balanced amino acid profile (Cheng and Hardy 2004; Minijarez-Osorio et al. 2016). The crude protein level of SBM ranges 44-48.5% (Cheng and Hardy 2004), but deficient with methionine and lysine. SBM contains high crude fiber and anti-nutritional factors (Biswas et al. 2019). To gain more crude protein and eliminate the antinutritional factors, bioprocessing technology is used. Sugars and antinutritional factors associated with raw SBM are removed, and soy protein concentrate (SPC) is produced and is more digestible for carnivorous fish and includes more than 60% crude protein (Cheng and Hardy 2004; Refstie et al. 2006; Tibbetts et al. 2006; Walker et al 2010; Minijarez-Osorio et al. 2016). Researchers used fungus to aerobically processed SBM resulting in enhanced protein content, developed digestibility, and decreased anti-nutritional compounds (Senevirathne et al. 2016). Microbially enhanced soy protein (fermented soy protein) has higher protein at level of 58.4% (Sinn et al. 2016) than SBM and higher digestibility than SBM, and can be a good alternative to fishmeal for aquaculture feed (Sindelar 2014; Senevirathne et al. 2016). Fish species (Førde-Skjærviket al. 2006) and size (Refstie et al. 2006) are important for digestion and absorption of soy protein throughout the intestine. For instance, SBM and SPC are useful to replace with fishmeal in red drum

(*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*) feeds when supplemented with amino acids. Fishmeal replacement with SBM and SPC is suitable at ratio of 75% (Minijarez-Osorio et al. 2016). It is stated that the suitable ratio of fishmeal to SBM for sea bream ranges from 20.5 – 39.5% (Martinez-LLorenz et al. 2008). Another study indicates that replacement of 70% fishmeal with SPC in sea bream diet did not change the growth performance of fish (Biswas et al. 2019).

Rainbow trout have high level apparent digestibility coefficient (ADC) for soy ingredient protein and amino acids (Cheng and Hardy 2004). Perera et al. (2019) indicate that total plant-based diets were used for rainbow trout feeding and that such diets can be suitable with slight effects on growth performance and metabolism of rainbow trout. According to Harlioglu (2011), 40% of fishmeal can be replaced in rainbow trout feed with solvent extracted SBM with no statistical differences on nutrient utilization of carcass composition. Replacing 87% of fishmeal with soy protein concentrate (SPC) in rainbow trout fingerlings has also been shown to be possible without affecting growth (Burr et al. 2012). Replacement of fishmeal protein with plant protein in Atlantic cod diet did not do any negative effect (Hansen et al. 2007). Furthermore, 24% of fishmeal replaced with soybean meal in Atlantic cod diet and this did not cause any negative effect on fish (Førde-Skjærvik et al. 2007).

Anti-nutritional Aspect of Soybean Meal

Although soybean meal is a viable alternative protein source for fish feed, it has some antinutrients like trypsin inhibitors, lectins, phytic acid and indigestible soluble oligosaccharides (Kaushik et al. 1995).

Table 2. Antinutritional factors in soybean meal (SBM), soy protein concentrate (SPC) and fermented soy protein (USSEC technical bulletin; Refstie et al. 2005; Francis et al. 2001; Gao et al. 2013; Chen et al. 2013).

Antinutritional Factors	SBM	SPC	Fermented Soy protein
Trypsin (mg/g)	5-8	<4	1.6
Phytic acid (mg/kg)	9.20	13.83	6.2
Lectins (mcg/g)	10-200	<0.1	<0.1
Saponins (%)	0.6	0	-
Oligosaccharides (%)	15	3	10

These can damage the mucosal integrity of the gastrointestinal tract, decreased pancreatic and brush-border enzymes, much nitrogen in the fecal, suppress thyroid hormone, lower mineral absorption, reduce palatability, and suppress of the immune system in fish (Alberksan et al. 2006; Colburn et al. 2012; Fuentes-Quesada et al. 2018). High levels of soybean meal (64%) have been found to negatively affect the immune system of *Totoaba macdonaldi*, via reduced expression levels of IL-8 (interleukin-8) one of the main immune-relevant cytokines (Fuentes-Quesada et al. 2018). Soybean meal has ~20% nonstarch polysaccharides that enhance the viscosity of the digesta and reduce nutrient absorption in fish (Førde-Skjærvik et al. 2007). Antinutritional factors can decrease utilization of proteins and reduce growth rates in salmonids (Olsen et al. 2007). In addition, SBM includes a low level of methionine (Hansen et al. 2007; Salze et al. 2010; Lin and Luo 2011). Vitamin B12 (cobalamin) plays a coenzyme role on methionine synthetase which converts homocysteine to methionine, and lack of vitamin B12 in SBM causes absent of the methionine. B12 should be added in sufficient amount in plant protein resources (Hansen et al. 2007). High levels of antinutritional and antigenic factors, indigestible carbohydrates (Fuentes-Quesada et al. 2018; Kaushik et al. 1995; Colburn et al. 2012; Alberksan et al. 2006), and absence of methionine in soybean meal (SBM) have limited high level replacement of soybean meal with fishmeal in some fish feeds (Colburn et al. 2012; Kaushik et al 1995; Aksnes et al. 2006; Tibbetts et al. 2006; Hansen et al. 2007; Salze et al. 2010; Walker et al. 2010).

Although previous studies have shown that a significant success in partially or totally replacing fishmeal (FM) with SBM in feed of some omnivorous species such as carp (*Cyprinus carpio)*, channel catfish (*Ictalurus punctatus)*, and tilapia (Colburn et al. 2012; Lin and Luo 2011; Kaushik et al 1995), some fish species have variable sensitivity to SBM. Performance effects include growth reduction and morphological changes such as higher HSI, gastrointestinal growth and differences in amino acid and fatty acid compositions (Walker et al 2010; Alberksan et al. 2007). Furthermore, histological changes (intestinal inflammation, damage of intestinal integrity, deleterious changes in the intestines), microbiota changes (damage of mucosal integrity and changes in intestinal microflora), and impacts on the immune responses (reduction of IL-8 gene expression) have been linked to SBM antinutrients (Lin and Luo 2011; Hansen et al. 2007; Fuentes-Quesada et al. 2018).

Previous studies show that soy protein affected the non-specific defense mechanisms in rainbow trout and caused poor growth performance in salmonids (Kaushik et al 1995; Hansen et al. 2007) and Atlantic cod (Alberksan et al. 2007). Dietary SBM (10-30%) feed inclusions for Atlantic salmon diets have been found to reduce growth (Walker et al. 2010), but Atlantic cod had a high tolerance level for dietary soybean meal and could tolerate at least 25% extracted soybean meal in the diet (Førde-Skjærvik et al. 2007; Walker et al. 2010) without affecting growth and body composition (Refstie et al. 2006; Hansen et al. 2007).

For other carnivorous fish feeds, SBM is not favorable for a complete replacement of fishmeal because of low crude protein levels (Salze et al. 2010), high fiber content, amino acid imbalance, poor palatability and the presence of anti-nutritional factors or toxicants (Tibbetts et al. 2006). Although the SPC is more expensive than SBM, it does not have an alcohol soluble fraction and includes higher essential amino acid concentrations and nutrient digestibility for piscivorous marine species compared with SBM (Colburn et al. 2012; Walker et al. 2010). Thus, global aquaculture industries can gain advantage from replacing fishmeal with SPC by keeping sustainability in aquaculture production (Walker et al. 2010). Juvenile cod feeds need to contain 50–60% protein (Alberksan et al. 2007), and these feeds can have 50–60% protein from plants (SPC) with no reduction in growth (Alberksan et al. 2007; Hansen et al. 2007). On the other hand, replacing higher quantities (75-100%) of fishmeal with plant protein in Atlantic cod feed decreased growth, feed efficiency, and protein efficiency in fish (Walker et al. 2010).

References

Aksnes, A., Hope, B., Høstmark Ø., Albrektsen, S., 2006. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. Aquaculture 261:1102–1110.

Albrektsen, S., Mundheim, H., Aksnes, A., 2006. Growth, feed efficiency, digestibility and nutrient distribution in Atlantic cod (*Gadus morhua*) fed two different fishmeal qualities at three dietary levels of vegetable protein sources. Aquaculture 261:626–640.

Amin, M.N., Katersky Barnes, R., Adams, L.R., 2014. Effects of different protein and carbohydrate levels on growth performance and feed utilisation of brook trout, *Salvelinus fontinalis* (Mitchill, 1814), at two temperatures. Journal of Applied Ichthyology 30:340–349.

Anonymous 2008a. Nutritional content of raw burbot (*Lota lota* L.). United States Department of Agriculture (USDA). NDB No. 15006. https://fdc.nal.usda.gov/fdc-app.html#/food-details/171950/nutrients.

Anonymous 2008b. Nutritional content of raw Atlantic cod (*Gadus morhua* L.). United States Department of Agriculture (USDA). NDB No. 15015. https://fdc.nal.usda.gov/fdc-app.html#/food-details/171955/nutrients.

Arslan, M., Dabrowski, K., Ferrer, S., Dietrich, M., Rodriguez, G., 2013. Growth, body chemical composition and trypsin activity of South American catfish, surubim (*Pseudoplatystoma sp*.) juveniles fed different dietary protein and lipid levels. Aquaculture Research 44:760–771.

Barron, J.M., Jensen, N.R., Anders, P.J., Egan, J.P., Ireland, S.C., Cain, K.D., 2012. Effect of temperature on the intensive culture performance larval and juvenile North American burbot (*Lota lota maculosa*). Aquaculture 364-365:67-73.

Barron, J.M., Jensen, N.R., Anders, P.J., Egan, J.P., Ireland, S.C., Cain, K.D., 2013. Effects of Stocking Density on Survival and Yield of North American Burbot Reared under Semi-Intensive Conditions. Transactions of the American Fisheries Society 142(6):1680-1687.

Beard, Z.S., 2017. Survival, Movement, and Distribution of Juvenile Burbot in a Tributary of the Kootenai River. North American Journal of Fisheries Management 37:1274–1288.

Biswas, A., Araki, H., Sakata, T., Nakamori, T., Takii, K., 2019. Optimum fishmeal replacement by soy protein concentrate from soymilk and phytase supplementation in diet of red sea bream, *Pagrus major.* Aquaculture 506:51–59.

Biswas, B.K., Ji, S.C., Biswas, A.K., Seoka, M., Kim, Y.S., Kawasaki, K., Takii, K., 2009. Dietary protein and lipid requirements for the Pacific bluefin tuna *Thunnus orientalis* juvenile. Aquaculture 288:114–119.

Bruce, T.J. and Brown, M.L., 2017. A Review of Immune System Components, Cytokines, and Immuno-stimulants in Cultured Finfish Species. Open Journal of Animal Sciences 7: 267-288. https://doi.org/10.4236/ojas.2017.73021.

Bulut, B., Yigit, M., Ergun, S., Kesbic, O.S., Acar, U., Gultepe, N., Karga, M., Yılmaz, S., Guroy, D., 2014. Evaluation of dietary protein and lipid requirements of two-banded seabream (*Diplodus vulgaris*) cultured in a recirculating aquaculture system. Aquacult Int 22:965–973.

Burr, G.S., Wolters, W.R., Barrows, F.T., Hardy, R.W., 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture 334-337:110–116.

Cardinaletti, G., Randazzo, B., Messina, M., Zarantoniello, M., Giorgini, E., Zimbelli, A., Bruni, L., Parisi, G., Olivotto, I., Tulli, F., 2019. Effects of Graded Dietary Inclusion Level of Full-Fat *Hermetia illucens* Prepupae Meal in Practical Diets for Rainbow Trout (*Oncorhynchus mykiss*). Animals 9:251.

Chen, L., Madl, R.L., Vadlani, P.V., Li, L., Wang, W., 2013. Value - Added Products from Soybean: Removal of Anti-Nutritional Factors via Bioprocessing. Chapter 8 DOI:10.577/52993. http://dx.doi.org/105772/52993.

Cheng, Z.J., Hardy, R.W., 2004. Effects of Microbial Phytase Supplementation and Dosage on Apparent Digestibility Coefficients of Nutrients and Dry Matter in Soybean Product-Based Diets for Rainbow Trout *Oncorhynchus mykiss*. Journal of the World Aquaculture Society Vol. 35, No. 1.

Colburn, H.R., Walker, A.B., Breton, T.S., Stilwell, J.M., Sidor, I.F., Gannam, A.L., Berlinsky, D.L., 2012. Partial Replacement of Fishmeal with Soybean Meal and Soy Protein Concentrate in Diets of Atlantic Cod. North American Journal of Aquaculture 74:330–337.

Cordeiro, C.M., 2019. A corpus-based approach to understanding market access in fisheries and aquaculture international business research: A systematic literature review. Aquaculture and Fisheries (Article in press).

Egan, J.P., Johnson, R.D., Anders, P.J., Cain, K.D., 2015. Initial Characterization of Embryonic Development in North American Burbot. North American Journal of Aquaculture 77(1):37-42.

Foltz, J.R., Jensen, N.R., Polinski, M.P., Ireland, S.C., Cain, K.D., 2012. Characterization of Oocyte Development in Hatchery-Reared Burbot. North American Journal of Aquaculture 74 (3):408-412.

Førde-Skjærvik, O., Refstie, S., Aslaksen, M.A., Skrede, A., 2006. Digestibility of diets containing different soybean meals in Atlantic cod (*Gadus morhua*); comparison of collection methods and mapping of digestibility in different sections of the gastrointestinal tract. Aquaculture 261:241–258.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197–227.

Fuentes-Quesada, J.P., Viana, M.T., Rombenso, A.N., Guerrero-Rentería, Y., Nomura-Solís, M., Gomez-Calle, V., Lazo, J.P., Mata-Sotres, J.A., 2018. Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. Aquaculture 495:78-89.

Gallagher, C.P., Dick, T.A., 2015. Winter feeding ecology and the importance of cannibalism in juvenile and adult burbot (*Lota lota*) from the Mackenzie Delta, Canada. Hydrobiologia 757:73–88.

Gao, Y., Wang, C., Zhu, Q., Qian, G., 2013. Optimization of Solid-State Fermentation with Lactobacillus brevis and Aspergillus oryzae for Trypsin Inhibitor Degradation in Soybean Meal. Journal of Integrative Agriculture 12(5):869-876.

García-Meilán, I., Ordóñez-Grande, B., Machahua, C., Buenestado, S., Fontanillas, R., Gallardo, M.A., 2016. Effects of dietary protein-to-lipid ratio on digestive and absorptive processes in sea bass fingerlings. Aquaculture 463:163-173.

Gatlin III, D.M., Barrows, F.T., Paul Brown, P., Konrad Dabrowski, K., Gaylord, T.G.,Hardy, R.W., Herman, E., Gongshe Hu, G.,Krogdahl, A., Richard Nelson, R., Overturf1, K., l Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., EveWurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38:551-579.

Gonzalez, E.B., Boer, F. 2017. The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. Fish Sci 83:661–670.

Grealisa, E., Hynesa, S., O'Donoghueb, C., Vegaa, A., Oscha, S.V., Twomeyc, C., 2017. The economic impact of aquaculture expansion: An input-output approach. Marine Policy 81:29–36.

Guillen, J., Asche, F., Carvalho, N., Fernandez Polanco, J.M., Llorente, I., Nielsen, M., Villasante, S., 2019. Aquaculture subsidies in the European Union: Evolution, impact and future potential for growth. Marine Policy 104:19–28.

Hamre, K., 2006. Nutrition in cod (*Gadus morhua*) larvae and juveniles. ICES Journal of Marine Science 63:267-274.

Hansen, A.C., Grethe Rosenlund, G., Ørjan Karlsen, Q., Wolfgang Koppe, W., Hemre, G., 2007. Total replacement of fishmeal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I — Effects on growth and protein retention. Aquaculture 272:599–611.

Harlioglu, A 2011. The Influence of Replacing Fishmeal Partially in Diet with Soybean Meal and Full-fat Soya on Growth and Body Composition of Rainbow Trout (*Oncorhynchus mykiss*). Pakistan J. Zool. 43(1):175-182.

Harrison, P. M., Gutovsky, L.F.G., Martins, E.G., Patterson, D.A., Cooke, S.J., Power, M., 2016. Burbot and large hydropower in North America: benefits, threats and research needs for mitigation. Fisheries Management and Ecology 23:335–349.

Jensen, N.R., Anders, P.J., Hoffman, C.A., Porter, L.S., Ireland, S.C., Cain, K.D., 2011. Performance and Macronutrient Composition of Age-0 Burbot Fed Four Diet Treatments. North American Journal of Aquaculture 73(3):360-368.

Jensen, N.R., Ireland, S.C., Siple, J.T., Williams, S.R., Cain, K.D., 2008. Evaluation of burbot egg incubation methods and larval feeding regimes for North American burbot. North American Journal of Aquaculture 70:162–170.

Jensen, N.R., Zuccarelli, M.D., Patton, S.J., Williams, S.R., Ireland, S.C., Cain, K.D., 2008. Cryopreservation and Methanol Effects on Burbot Sperm Motility and Egg Fertilization. North American Journal of Aquaculture 70:38–42.

Jobling, M., 2016. Fish nutrition research: past, present and future. Aquacult Int 24:767–786.

Kaushik, S.J., Cravedi b, J.P., Lalles, J.P., Sumpter, j., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fishmeal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss***.** Aquaculture 133:257-274.

Khan, K.U., Rodrigues, A.T., Menegasso Mansano, C.F., Almeida Queiroz, D.M., Sakomura, N.K., Souza Romaneli, R., Nascimento, T.M.T., Fernandes, J.B.K., 2019. Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices. Latin American Journal of Aquatic Research 47(2):232-239.

Kim, K.W., Wang, X., Han, K., Bai, S.C., 2004. Optimum Dietary Protein Level and Protein-to-Energy Ratio for Growth of Juvenile Korean Rockfish *Sebastes schlegeli*. Journal of the World Aquaculture Society Vol. 35, No. 3.

Kokou, F., Henry, M., Nikoloudaki, C., Kounna, C., Vasilaki, A., Fountoulaki, E., 2019. Optimum protein-to-lipid ratio requirement of the juvenile shi drum (*Umbrina cirrosa*) as estimated by nutritional and histological parameters. Aquaculture Nutrition 25:444–455.

Kucharczyka, D., Nowosada, J., Kujawaa, R., Dietricha, G., Biegaja, M., Sikoraa, M., Luczynskib, M.J., 2018. Comparison of spontaneous and hormone-induced reproduction of burbot (*Lota lota* L.) under hatchery conditions. Aquaculture 485:25–29.

Lee, S.M., Jeon, I.G., Lee, J.Y, 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture 211:227–239.

Li, P., Mai, K., Trushenski, J., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37:43–53.

Lin, S., Luo, L., 2011. Effects of different levels of soybean meal inclusion in replacement for fishmeal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Animal Feed Science and Technology 168:80–87.

Martinez-Llorenza, S., Vidal, A.T., Garcia, I.J., Torres, M.P., Cerda, M.J., 2008. Optimum dietary meal level maximizing growth and nutrient utilization of non-growing gilthead sea bream (*Sparus aurata*). Aquaculture Nutrition 15:320-328.

McCullough, D.E., Roseman, E.F., Keeler, K.M., DeBruyne, R.L., Pritt, J.J., Thompson, P.A., Ireland, S., Ross, J.E., Bowser, D., Hunter, R.D., Castle, D., Fischer, J.L., Provo, S., 2015. Evidence of the St. Clair-Detroit River System as a dispersal corridor and nursery habitat for transient larval burbot. Hydrobiologia 757:21–34.

Minjarez-Osorio, C., Castillo-Alvarado, S., Gatlin III, D.M., González-Félix, M.L., Perez-Velazquez, M., Rossi Jr, W., 2016. Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*): A comparative study. Aquaculture 453:122-129.

Mohantya, B.P., Mahantya, A., Gangulya, S., Mitraa, T., Karunakaran, D., Anandan,R., 2019. Nutritional composition of food fishes and their importance in providing food and nutritional security. Food Chemistry 293:561–570.

Morais, S., Bell, J.G., Robertson, D.A., Roy, W.J., Morris, P.C., 2001. Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth, feed utilization, muscle composition and liver histology. Aquaculture 203:101–119.

Nadarajah, S., Flaaten, O., 2017. Global aquaculture growth and institutional quality. Marine Policy 84:142–151.

National Agricultural Statistics Service (NASS), Agricultural Statistics Board, United States Department of Agriculture (USDA), 2019. Available at https://usda.library.cornell.edu/concern/publications/t722h882h.

Naylora, R.L., Hardy, R.W., Bureau, D.P., Chiua, A., Elliottd, M., Farrelle, I., Gatlin, D.M., Goldburgh, R.J., Huac, K., Nichols, P.D., 2009. Feeding aquaculture in an era of finite resources. National Academy of Sciences of the United States of America 106 (36): 15103-15110.

Olsen, R.E., Hansen, A.C., Rosenlund, G., Hemre, G.I., Mayhew, T.M., Knudsen, D.L., Eroldoğan, O.T., Myklebust, R., Karlsen, Q., 2007. Total replacement of fishmeal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) II — Health aspects. Aquaculture 272:612–624.

Palin´ ska-Zarska, K., Zarski, D., Krejszeff, S., Nowosad, J., Biłas, M., Trejchel, K., Kucharczyk, D., 2014. Dynamics of yolk sac and oil droplet utilization and behavioural aspects of swim bladder inflation in burbot, Lota lota L., larvae during the first days of life, under laboratory conditions. Aquacult Int 22:13–27.

Pereraa, E., Simó-Mirabeta, P., Shinb, H.S., Rosell-Molla, E., Naya-Cataláa, F., Herasa, V., Martos-Sitchaa, J.A., Karalazosc,V., Armerod, E., Arizcune, M., Chavese, E., Berbelf, C., Manchadof, M., Afonsob, J.M., Calduch-Ginera, J., Pérez-Sáncheza, J., 2019. Selection for growth is associated in gilthead sea bream (*Sparus aurata*) with diet flexibility, changes in growth patterns and higher intestine plasticity. Aquaculture 507:349–360.

Refstie, S., Skjærvik, O.F., Rosenlund, G., Rørvik, K.A., 2006. Feed intake, growth, and utilisation of macronutrients and amino acids by 1- and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. Aquaculture 255:279–291.

Refstie, S., T, Stefan Sahlstrom, S., Brathen, E., Baeverfjord, G., Krogedal, P., 2005. Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). Aquaculture 246:331–345.

Salze, G., McLean, E., Battle, P.R., Schwarz, M.H., Craig, S.R., 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fishmeal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. Aquaculture 298:294–299.

Senevirathne, N.D., Anderson, J.L., Rovai, M., 2016. Effect of water treatment on drinking preferences of dairy heifers. Journal of Animal Science 94:166-167.

Senten, J., Engle, C.R., Hartman, K., Johnson, K.K., Gustafson, L.L., 2018. Is there an economic incentive for farmer participation in a uniform health standard for aquaculture farms? An empirical case study. Preventive Veterinary Medicine 15:58-67.

Sindelar, S., 2014. Utilization of Soybean Products as Fish-Meal Protein Replacement in Yellow Perch *Perca flavescens* Feeds. Master`s Thesis. South Dakota University, Vermillion, South Dakota.

Sinn, S.M., Gibbons, W.R., Brown, M.L., DeRouchey, J.M., Levesque, C.L., 2016. Evaluation of microbially enhanced soybean meal as an alternative to fishmeal in weaned pig diets. Animal 11(5):784–793.

Stańczak, K., Król, J., Żarski, D., Hliwa, P., 2017. Marking of Burbot (*Lota Lota* L.) with Fluorescent Dyes Using the *Per Os* Method. Turkish Journal of Fisheries and Aquatic Sciences 17:121-127.

Stapanian, M.A., Myrick, C.A., 2015. Ecology, culture, and management of Burbot: an introduction. Hydrobiologia 757:1–2.

Stapanian, M.A., Paragamian, V.L., Madenjian, C.P., Jackson, J.R., Lappalainen, J., Evenson, M.J., Neufeld, M.D., 2010. Worldwide status of burbot and conservation measures. Fish and Fisheries 11:34–56.

Stoll, J.S., Leslie, H.M., Britsch, M.L., Cleaver, C.M., 2019. Evaluating aquaculture as a diversification strategy for Maine's commercial fishing sector in the face of change. Marine Policy 107:103583.

Tacon, A.G.J., Metian, M., 2008. Global overview on the use of fishmeal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285:146–158.

Terrazas, M. M., Adams, J.R., Sudheesh, P.S., Cain, K.D., 2017. Effects of Diel Temperature Fluctuation on Growth, Stress Response, and Immune Function of Burbot. Transactions of the American Fisheries Society 146(5):996-1007.

Tibbetts, S.M., Milley, J.E., Lall, S.P., 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). Aquaculture 261:1314–1327.

USSEC (U.S. Soybean Export Council) Soy Protein Concentrate Technical Bulletin for Aquaculture Feeds. How Soy Protein Concentrate is Manufactured Types of Soy Protein Concentrate Specifications for Aquaculture Feeds.

Wacyk, J., Powell, M., Rodnick, K., Overturf, K., Hill, R.A., Hardy, R., 2012. Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets. Aquaculture 356–357:223–234.

Walker, A.B., Sidor, I.F., O'Keefe, T., Cremer, M., Berlinsky, D.L., 2010. Partial Replacement of Fishmeal with Soy Protein Concentrate in Diets of Atlantic Cod. North American Journal of Aquaculture 72:343–353.

Wong, A. 2008. Lipid profiles of tissue and liver oil of burbot, *Lota lota* (L.). ActaIchthyol Piscat 38(1):55–61.

Xu, G.F., Wang, Y.Y., Han, Y., Liu, Y., Yang, Y.H., Yu, S.L., Mou, Z.B., 2015. Growth, feed utilization and body composition of juvenile Manchurian trout, *Brachymystax lenox* (Pallas) fed different dietary protein and lipid levels. Aquaculture Nutrition 21:332-340.

Young, N., Brattland, C., Digiovanni, C., Hersoug, B., Johnsen, J.P., Karlsen, K.M., Kvalvik, I., Olofsson, E., Simonsen, K., Solås, A.M., Thorarensen, H., 2019. Limitations to growth: Social-ecological challenges to aquaculture development in five wealthy nations. Marine Policy 104:216–224.

Chapter 2: Evaluation of commercial trout-type and marine-type diets on burbot (*Lota lota maculosa*) growth performance and health.

Abstract

Burbot (*Lota lota*) are the only freshwater member of the cod family (Gadidae). Burbot appear to have water quality requirement similar to trout, have desirable white meat fillets, and can be produced under controlled conditions. Therefore, it is hypothesized that burbot may be a potential commercial aquaculture species and if cultured in trout facilities could offer a valued product that would diversify production. A first step in successful commercial production is to determine basic nutrient requirements and the best feed types for growth of cultured fish. There is limited information about burbot nutrient requirements or feed preference for commercial production. This study was designed to provide a baseline comparison of growth and health indices following feeding of two types of diets. Sub-adult burbot (initial weight 131.7g±7.2) were fed with four different diets (two commercial diets and two reference diets); commercial marine-type diet (Skretting, Europa), commercial trout-type diet (Skretting, Oncor), formulated marine-type diet (Burbot 1), and formulated trout-type diet (Burbot 2) for 90 days to evaluate effects of diets on burbot growth and health. A completely randomized experiment was designed, and each treatment consisted of 3 replicate tanks of 100 fish per tank (1000). Burbot were fed each diet at 1.5% body weight (BW) per day using automated feeders. Results showed no significant differences ($p>0.05$) between dietary treatments for specific growth rate (SGR), relative growth (RG), or feed conversion ratio (FCR). However, there were noted differences between treatments on fillet yield and gastrointestinal indices (GII). Fillet yield ratio of burbot fed the formulated Burbot

2 diet was significantly lower than other diets ($p<0.05$). These Burbot 2 diet group fish did not utilize dietary protein efficiently to develop muscle in fillet. The gastrointestinal index (GII) of fish fed the Oncor commercial trout-type diet was significantly higher than fish fed the Burbot 1 diet ($p<0.05$). The reason for these differences is unclear, but it is clear that sub-adult fish grow equally well on a commercial trout diet compared to a higher protein/higher cost marine/cod diet. Results from this work would be helpful to formulate new diets for burbot.

Introduction

The overall goal of commercial aquaculture is generally to produce a high quality, healthy product for human consumption at the lowest possible cost. Feed for aquaculture is expensive and if formulated with excessive amounts of protein or lipid the diets may be unbalanced, costly, and causes high levels of nitrogen and phosphorus excretion, which can impact fish health and pollute aquatic environment (Lee et al. 2002; Khan et al. 2019). Therefore, it is important to have an understanding of nutritional requirements (protein, lipid) of the cultured fish species of interest (Arslan et al. 2013).

Burbot early life stage rearing techniques such as egg incubation and larval feeding strategies have previously been evaluated (Jensen et al. 2008; Wocher et al. 2013). Larval burbot were fed with nine diets combined 50, 57, 60% protein and 10, 14, 15% lipid, and burbot showed the highest growth when fed a diet containing 60% protein and 15% fat (Jensen et al. 2011). However, growth during the grow-out phase of farmed burbot has not been well defined (Wocher et al. 2011). This lack of information and limited knowledge of feed requirements for this species may slow the progress of burbot aquaculture. It will be important to define general requirements at this point, since there are no burbot-specific feeds on the market. It is stated that there are similarities between burbot and trout, namely that they are both freshwater species thriving and sharing cold water habitats. This has resulted in Trejchal et al (2014) feeding burbot with trout-type feed (Skretting, Norway; 54 % protein, 18 % fat) to rear burbot for their research. On the other hand, due to strong similarities between burbot and Atlantic cod species, burbot have also been produced on cod-type diets (58–62% protein, 13–15% lipid) during the rearing process (Wocher et al 2011). It is not known how general dietary characteristics (i.e. protein, lipid, carbohydrates, etc.) relate to growth performance for this species. It is important to determine burbot growth performance and health by testing different diet formulations in order to provide producers recommendations for commercial production (Trejchel et al. 2014).

In this study, it was hypothesized that burbot *(Lota lota maculosa)* growth would be affected by diet type. To address this, an experiment was conducted to evaluate growth performance and associated health indices (organosomatic indices) of sub-adult burbot (grow out stage) fed select commercial and formulated reference diets. Four diets were tested, two commercial diets (Skretting Europa and Oncor) and two formulated reference diets (Burbot 1 and Burbot 2; provided by Dr. Rick Barrows) and fish were fed each diet for a period of three months.

Materials and Methods

Fish

Sub-adult burbot (initial weight 131.7±7.2g) produced at University of Idaho, Aquaculture Research Institute (UI, ARI) Moscow, ID and reared during the trial at the UI College of

Naturel Resources (CNR) wet laboratory (supplied with flow-through dechlorinated municipal water). The rearing methods followed current practices and published work (Barron et al. 2012; Palińska-Żarska et al. 2014; Trejchel et al. 2014). Fish were combined and graded then transferred randomly to 12 fiberglass tanks (1000L flow-through circular tanks, 152 cm diameter and water depth 91 cm receiving aeration and water flow at rate of 12L/min), each tank had 100 fish and the total number of sub-adult fish used in the trial was 1200 in the CNR Aquatic Animal Laboratory (AAL). Water quality in each tank was maintained daily at standards reported for culture of juvenile burbot (dissolved oxygen > 5 ppm, water temperature at 13.7°C, and negligible ammonia levels; Barron et al. 2012; Trejchel et al. 2014). Water temperature and dissolved oxygen levels were measured daily and photoperiod was timer controlled and set to 12h light: 12h dark. In order to properly assess survival, fish were individually counted at stocking. Each tank housed 100 fish and the total number of sub-adult fish used in the trial was 1200. Two extruded commercial diets (Skretting, Europa and Oncor) that are considered marine-type or trout-type feeds respectively, were selected along with two extruded open control (reference diets) diets (Burbot 1 and Burbot 2) formulated by Dr. Rick Barrows (Table 1).

There were 4 different treatment diets and each tank (3 replicate tanks per treatment diet) represented an experimental unit in a single-factor randomized study. The auto feeders were set at 24h and were loaded once daily. In the pretrial period burbot were fed at a ratio of 2% body weight per day and some feed wasting was observed in tanks, so the feeding ratio was decreased to 1.5 % body weight per day (BW/d) during trial period. Prior to the start of the trial, fish were fed a mixed diet that consisted of (25% Oncor, 25% Europa, 25% Burbot 1, and 25% Burbot 2) for an acclimation period of 7d prior to initiation of the trial.

The tank biomass was recorded at day 0, 45, and 90, and 25 fish were randomly sampled for individual weights and lengths weekly throughout the 90 d study. Fish were starved for 48h and sampled fish were anaesthetized with MS-222 at 100mg/L added into 20 L tank water (buffered to pH 7.0-7.5 with sodium bicarbonate) with aeration. The following metrics were analyzed at 45 and 90 d: feed conversion ratio (FCR), specific growth rate (SGR), relative growth (RG), Fulton's condition factor (K-value), thermal growth coefficient (TGC), and protein efficiency ratio (PER). At day 90, whole-body proximate analysis and the following organosomatic indices were calculated (see formulas below): visceral fat index (VFI), hepatosomatic index (HSI), fillet yield (Skin-on left side fillet weight was multiplied to calculate total fillet weight and main formula was used to calculate fillet yield), gastrointestinal index (GII), and splenosomatic index (SSI).

FCR = the amount of feed consumed/ by the amount of weight gained.

Specific growth rate [SGR; %/d] = $100\times[\ln W2 - \ln W1]/T$

Where W2 is final weight, W1 is initial weight and T is time in number of days.

RG; %= [(W2-W1)/W1]*100

Where W2 is final weight and W1 is initial weight.

Condition factor (K) =$[W/L^3] \times 10^3$

Where W is weight of fish and L is length of fish

TGC= $1000* [\sqrt[3]{Wf} - \sqrt[3]{Wi}]/T*t$

Where Wf is the final weight Wi is the initial weight, t is the number of days between the initial and final measurement and T is the average temperature during the period considered.

VFI= [visceral fat content (g)/ whole fish weight (g wet)]*100.

HSI= [liver weight (g) / whole fish weight (g wet)]*100.

Skin-on left side fillet weight*2= Total fillet weight

Fillet yield= [total fillet weight (g)/ whole fish weight (g wet)]*100.

GII= [weight of gastrointestinal tract (g)/ whole fish weight (g wet)]*100.

SSI= [spleen weight (g)/ whole fish weight (g wet)]*100.

Sampling

At the end of the trial (on the 90th day) 25 fish were sampled randomly from all tanks for total length, wet weights, and tank biomass. Sixteen fish (6 fish for whole-body proximate analysis and 10 fish for organosomatic indices out of 25 fish were euthanized in 250 mg/L MS-222 added to 20 L of rearing water for length and weight measurement. For organosomatic indices, whole fish were weighed then fish were dissected and liver, spleen, heart, gastrointestinal content, fillet and visceral fat were weighed separately (Bruce et al. 2016) to check effects of diets on organosomatic indices of fish. Left side, skin on fillets were removed from body and weighed separately and the weight of one side fillet multiplied with 2 to calculate total fillet yield value of fish.

Growth Evaluation

The sampled burbot were measured to the nearest mm and weighed using an electronic scale (Mettler-Toledo, Capacity 6000g) weekly. Daily feed ration was adjusted after each weekly sampling based on the sampling results and estimated growth. The individual measurement of weight and length was used to calculate fish condition factor (K) according to the formulas provided above. The tank biomass was used for growth calculations at 45 and 90 d.

Proximal Analysis and Morphometrics

At the end of the study (day 90), all fish were counted and 25 fish were randomly sampled from each replicate for total length and wet weight, then 6 fish from each tank were sampled for whole-body proximate analysis and placed at -20^{0}C, as described above. Proximate analysis of fish was completed at the Hagerman Fish Culture Experiment Station, ID, US, and proximate analysis of the diet samples was completed at Midwest Labs in Omaha, NE, US. Briefly, each frozen fish was ground and all six fish from each tank were pooled and grinded to homogenize all fish samples from each tank. Then approximately 1g homogenized sample was used for each protein, lipid, ash and energy analyses. Crude protein was determined by using machine called Elementar Rapid N Exceed, crude lipid was calculated by using the Ankom XT 15 Extraction Suptem, ash was determined by the machine called Isotemp Muffle Furnace, and energy was calculated by the Parr 6300 Calorimeter at the Hagerman Fish Culture Experiment Station, ID, US.

Cost Projection

The feed cost of a fish from 100g to 400g (potential market size for burbot) was calculated for both Europa and Oncor diets. The approximate price of feeds is estimated in general at \$2.69/kg (Skretting Europa) and \$1.65/kg (Skretting Oncor) and FCR values of fish fed with these diets were used to calculate the cost of feed.

FCR= the amount of feed consumed/ by the amount of weight gained.

The cost calculation of a fish fed with Europa diet:

2.06=the amount of feed consumed/300

The amount of feed consumed=618g

The price of 618g Europa diet (\$2.69/kg) was calculated.

The cost calculation of a fish fed with Oncor diet:

1.94= the amount of feed consumed/300

The amount of feed= 582g

The price of 582g Europa diet ($1.65/kg) was calculated.

Statistical Analysis

The experimental design was a completely randomized design and results for dietary treatment effects on burbot growth and organosomatic indices were analyzed via GraphPad Prism software. The data from 90^{th} day was used to calculate growth parameters. Shapiro-Wilk test was conducted to check normal distribution on samples and Bartlett`s test was conducted to check variances equality of samples. One-way ANOVA and Tukey`s Multiple comparison Test were run to analyze the growth data. For statistical analyses of organosomatic indices data Kruskal-Wallis test was conducted instead of ANOVA, because the variance of samples was not equal between groups. Treatment effects were considered significant at $p < 0.05$

Results

Growth and Survival

There were no significant differences in survival and growth between treatments (Table 2.4). Numerically, lowest FCR value was represented by fish fed the Oncor diet and the highest FCR value was represented by fish fed the Burbot 1 diet (Table 2.4). Also, fish fed the Oncor diet had the highest RG and SGR levels respectively. Burbot 1 represented the lowest RG and SGR levels respectively (Table 2.4). In addition, fish fed the Oncor diet showed the highest TGC value and the lowest value was found in fish fed the Burbot 1 diet (Table 3).

Proximate Analysis

The proximate analyses of diets (dry matter basis) indicated that, the Europa diet has the highest protein level and was followed by Burbot 1, Oncor and Burbot 2 diets (Table 2.3). According to statistical analyses of whole-body fish proximate, there were no significant differences between treatments for crude protein, crude lipid, energy and ash (Table 2.5), although there was a slight difference of moisture between treatments ($p=0.042$). The highest protein level was in fish fed with the Europa diet (15.5%), followed by Burbot 1 (15.33%), Oncor (15.31%) and Burbot 2 diets (14.8%) (Table 2.5).

Organosomatic Indices

No significant differences were found for HSI, SSI and VFI between treatments, but skin on fillet yield of fish fed the Burbot 2 diet was significantly lower than fish fed other diets (Table 6). Furthermore, fish fed with the Oncor diet had significantly higher GII than fish fed with the Burbot 1 diet (Table 2.6).

Cost Projection

According to the economical calculations, the cost of Oncor diet for a fish grown from 100g to 400g is $0.96 and the cost of Europa diet is $1.66 (Figure 2.7).

Discussion

This study has shown that there were no significant differences between marine type and trout type diets on sub-adult burbot growth or survival. Organosomatic indices showed no significant differences between treatments for HSI, VFI and SSI (Table 6). Low overall levels of visceral fat were observed in sub-adult burbot. In addition, there were significant differences on fillet yield and GII between treatments. Fish fed with the Burbot 2 diet, which contained the lowest protein level (44.9%), had the lowest fillet yield level when compared with other diets. This indicates that this group put less muscle tissue on when compared with fish in other groups, and it is possible that dietary protein affected this for fish fed the Burbot 2 diet. Fish fed with other diets had higher level than 52% skin-on fillet yield. FAO reported that Atlantic cod had 47% skin-on fillet yield and trout had 69% fillet yield. Thus, burbot had higher fillet yields than Atlantic cod, a comparable species.

Fish fed the Oncor diet demonstrated a significantly higher GII level than the Burbot 1 diet group fish. This indicated that fish fed the Oncor diet showed physiological changes by increasing the gastrointestinal tract growth. Feed proximate analyses (Table 4) indicated that the Oncor diet protein level was lower than the Burbot 1 diet and lipid levels were same. Previous studies state that carnivorous fish fed with high level plant protein showed morphological changes such as gastrointestinal growth (Aksnes et al. 2006; Walker et al 2010) because of high dietary fiber content and low digestible energy concentration (Refstie et al. 2006). Plant sources have high fiber and indigestible carbohydrates that decrease digestibility and nutrient absorption in carnivorous fish (Førde-Skjærvik et al. 2006), to compensate for this problem fish increase feed intake and gastrointestinal growth (Refstie et al. 2006). Results of the present study agree with Refstie et al (2006), and the gastrointestinal growth in fish fed with Oncor diet may be due to high level of plant sources in the diet. In addition, proximate analyses indicated that there were no significant differences in whole body composition of fish. It appears that relative dietary protein and lipid levels correspond to fluctuations in whole body composition of fish fed respective diets.

According to the economic analyses comparing the two commercial diets, feeding sub-adult burbot with the Oncor diet would be more economical than feeding with Europa diet. Burbot producers can save $0.70 per fish by using Oncor diet instead of Europa diet to rear fish from 100g fish to 400g. This suggest a substantial savings and although growth was similar between diets, producers would clearly choose the less expensive diet for burbot production.

According to results of the present study, there were no statistically significant differences on sub-adult stage burbot (*Lota lota maculosa*) growth between diets (Europa, Burbot 1, Oncor and Burbot 2). However, given such growth trends combined with lower costs associated with a commercial trout diet compared to a higher protein marine feed, the Oncor diet (or similar trout diets) would be the diet of choice, among the four evaluated, for burbot culture during the grow out stage for this species. This work provides a general evaluation of these specific diet types and further work to fully define nutritional requirements of burbot at various life stages is needed as the potential of adapting burbot as a commercial aquaculture species expands.

References

Aksnes, A., Hope, B., Høstmark, Ø., Albrektsen, S., 2006. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. Aquaculture 261:1102–1110.

Arslan, M., Dabrowski, K., Ferrer, S., Dietrich, M., Rodriguez, G., 2013. Growth, body chemical composition and trypsin activity of South American catfish, surubim (*Pseudoplatystoma sp*.) juveniles fed different dietary protein and lipid levels. Aquaculture Research 44:760–771.

Barron, J.M., Jensen, N.R., Anders, P.J., Egan, J.P., Ireland, S.C., Cain, K.D., 2012. Effect of temperature on the intensive culture performance larval and juvenile North American burbot (*Lota lota maculosa*). Aquaculture 364-365:67-73.

Bruce, T.J., Sindelar, S.C., Voorhees, J.M., Brown, M.L., Barnes, M.E., 2016. Performance and immunological responses of rainbow trout (*Oncorhynchus mykiss*) fed bioprocessed plant-based proteins. Aquaculture Nutrition 23:1160–1168.

Bulut,B., Yigit, M., Ergun, S., Kesbic, O.S., Acar, U., Gultepe, N., Karga, M., Yılmaz, S., Guroy, D., 2014. Evaluation of dietary protein and lipid requirements of two-banded seabream (*Diplodus vulgaris*) cultured in a recirculating aquaculture system. Aquacult Int 22:965–973.

Food and Agriculture Organization (FAO) of the United Nation 2019.

Førde-Skjærvik, O., Refstie, S., Aslaksen, M.A., Skrede, A., 2006. Digestibility of diets containing different soybean meals in Atlantic cod (*Gadus morhua*); comparison of collection methods and mapping of digestibility in different sections of the gastrointestinal tract. Aquaculture 261:241–258.

García-Meilán, I., Ordóñez-Grande, B., Machahua, C., Buenestado, S., Fontanillas, R., Gallardo, M.A., 2016. Effects of dietary protein-to-lipid ratio on digestive and absorptive processes in sea bass fingerlings. Aquaculture 463:163-173.

Jensen, N.R., Ireland, S.C., Siple, J.T., Williams, S.R., Cain, K.D., 2008. Evaluation of burbot egg incubation methods and larval feeding regimes for North American burbot. North American Journal of Aquaculture 70:162–170.

Jensen, N.R., Anders, P.J., Hoffman, C.A., Porter, L.S., Ireland, S.C., Cain, K.D., 2011. Performance and Macronutrient Composition of Age-0 Burbot Fed Four Diet Treatments. North American Journal of Aquaculture 73(3):360-368.

Jobling, M., 2016. Fish nutrition research: past, present and future. Aquacult Int 24:767–786.

Khan, K.U., Rodrigues, A.T., Menegasso Mansano, C.F., Almeida Queiroz, D.M., Sakomura, N.K., Souza Romaneli, R., Nascimento, T.M.T., Fernandes, J.B.K., 2019. Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices. Latin American Journal of Aquatic Research 47(2):232-239.

Lee, S.M., Jeon, I.G., Lee, J.Y, 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture 211:227–239.

Palin´ ska-Zarska, K., Zarski, D., S. Krejszeff, S., Nowosad, J., Biłas, M., Trejchel, K., D. Kucharczyk, D., 2014. Dynamics of yolk sac and oil droplet utilization and behavioural aspects of swim bladder inflation in burbot, *Lota lota* L., larvae during the first days of life, under laboratory conditions. Aquacult Int 22:13–27.

Rawles, S.D., Gaylord, T.G., Snyder, G.S., Freeman, D.W., 2010. The Influence of Protein and Energy Density in Commercial Diets on Growth, Body Composition, and Nutrient Retention of Sunshine Bass, *Morone chrysops* ♀ × *Morone saxatilis* ♂*,* Reared at Extreme Temperature. Journal of the World Aquaculture Society Vol. 41, No. S2.

Refstie, S., Skjærvik, O.F., Rosenlund, G., Rørvik, K.A., 2006. Feed intake, growth, and utilisation of macronutrients and amino acids by 1- and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. Aquaculture 255:279–291.

Saillant, E., Ma, L., Wang, X., Gatlin III, D.M., Gold, J.R., 2007. Heritability of juvenile growth traits in red drum (*Sciaenops ocellatus* L.). Aquaculture Research 38:781-788.

Terrazas, M. M., Adams, J.R., Sudheesh, P.S., Cain, K.D., 2017. Effects of Diel Temperature Fluctuation on Growth, Stress Response, and Immune Function of Burbot. Transactions of the American Fisheries Society 146(5):996-1007. https://doi.org/10.1080/00028487.2017.1334701.

Trejchel, K., Zarski, D., Palin´ ska-Z_ arska, K., Krejszeff, S., j Dryl, B., Dakowski, K., Kucharczyk, D., 2014. Determination of the optimal feeding rate and light regime conditions in juvenile burbot, *Lota lota* (L.), under intensive aquaculture. Aquacult Int 22:195–203.

Walker, A.B., Sidor, I.F., O'Keefe, T., Cremer, M., Berlinsky, D.L., 2010. Partial Replacement of Fishmeal with Soy Protein Concentrate in Diets of Atlantic Cod. North American Journal of Aquaculture 72:343–353.

Wocher, H., Alexander Harsa´nyi, A., Frieder J Schwarz, F.J., 2013. Larviculture of burbot (*Lota lota* L.): larval rearing using Artemia and weaning onto dry feed. Aquaculture Research 44:106–113.

Wocher, H., Harsányi, A., Schwarz. F.J., 2011. Husbandry conditions in burbot (*Lota lota* L.): Impact of shelter availability and stocking density on growth and behavior. Aquaculture 315:340–347.

Table 2.1. Experimental diet composition (% on as is basis) of the two formulated diets for burbot.

Ingredient	Burbot 1	Burbot 2
Fishmeal [a]	29.20	19.40
Poultry meal [b]	27.31	21.42
Wheat flour [c]	18.0	33.30
Soy protein concentrate [d]	9.85	6.85
Dicalcium phosphate [e]	2.20	3.11
Vitamin premix [f]	1.0	1.0
Mineral premix [g]	0.1	0.1
Stay-C [h]	0.2	0.2
Lysine HCl [i]	-	0.31
DL-Methionine [j]	-	0.2
Choline chloride [k]	0.6	0.6
Fish oil [l]	11.54	13.51
Totals	100.0	100.0

[a]SeaPro 75, BioOregon Protein, Warrenton, OR; [b] American Dehydrated Foods, Lillington, NC; [c] Swan Pastry Flour, ADM, Decatur, IL; [d] Profine VF, DuPont, St. Louis, MO; [e] Wilbur Ellis, Ogden, UT; [f] ARS 702 premix, Nelson and Sons, Murray, UT (as g kg^{-1} in premix; Vitamin A palmitate (500IU mg^{-1}) 1.93, Cholecalciferol 0.0165, Tocopheral acetate 13.20, Menadione sodium bisulfite 0.47, Thiamine mononitrate 0.91, Riboflavin 0.96, Pyridozine HCl 1.37, Pantothenate 10.11, Cyanocobalamine 0.003, Niacin 2.18, Biotin 0.033, Folic Acid 0.25, Wheat flour 968.6); [g] ARS 1440 trace mix, Nelson and Sons, Murray, UT(as g kg^{-1} premix; $CaCO_3$ 349.18, $CuSo_4$-$5H_2O$ 59.00, $FeSO_4$-$7H_2O$ 398.50, $MnSO_4$-H_2O 52.60; KI 7.86; Na_2SeO_3 0.96, $ZnSO_4$-$7H_2O$ 131.90); [h]DSM Nutritional Products, Parsippany, NJ; [i] ADM, Decatur, IL; [j] ADM, Decatur, IL; [k] BalChem Corporation, New Hampton, NY; [l] Whitting Trimming Oil, Pacific Seafood, Clackamas, OR.

Table 2.2. Ranked ingredient listing of commercial diets used to test effects of diets on burbot growth and health (Europa and Oncor).

Europa	**Oncor**
Fishmeal	Whole wheat
Wheat flour	Poultry by-product meal
Wheat gluten	Fishmeal
Fish oil	Feather meal
Lysine	Soybean meal
Mold inhibitor[a]	Blood meal
Vitamin premix[b]	Animal poultry fat
Mineral premix[c]	Fish oil
Water	DL Methionine
Vitamin C	Choline chloride
Choline Chloride	Calcium propionate
Vitamin E	Calcium L-ascorbyl-2- monophosphate
Ethoxyquin (preservative)	A vitamin premix[d]
	A mineral premix[e]
	Ethoxyquin (preservative)

[a]Propionic acid, Sodium Carbonate, Calcium hydroxide, Silicon Dioxide, Lemon oil Terpenes; [b]Calcium Carbonate, Vitamin E, Vitamin C, Inositol, Niacin Pantothenate, Mineral Oil Vitamin B2, vitamin B6, vitamin K3, Biotin, Vitamin B1, Folic Acid, Vitamin D5, Vitamin A, Vitamin B12; [c]Calcium Carbonate, Zinc Sulphate, Manganese Sulphate, Mineral oil, Copper Sulphate, Calcium iodate; [d]Vitamin A Acetate, Vitamin D3 Supplement, Vitamin E Supplement, Inositol, Calcium Pantothenate, Riboflavin, Nicotinic Acid, Thiamine Mononitrate, Pyridoxin Hydrochloride (B6), Vitamin B12 Supplement, D-Biotin, Folic Acid, Ascorbyl Polyphosphate C, Menadione Sodium Bisulfite Complex (Vitamin K), [e]Manganese Sulphate, Zinc Methionine, Calcium Iodate, Copper Sulfate, Ferrous Sulphate, Sodium Selenite.

Table 2.3. Proximate composition of experimental diets (as is) used burbot performance trials. GE=gross energy.

Proximate	Europa	Burbot 1	Oncor	Burbot 2
Crude Protein (%)	62.9	56.9	53.7	44.8
Crude Lipid (%)	16.0	20.0	20.0	19.8
GE (Kcal/g)	5543	5860	6056	5702
Ash (%)	13.62	6.20	7.67	6.11
Moisture (%)	7.12	2.79	7.97	3.02

Table 2.4. Growth indices of sub-adult burbot sampled at day 90. Values shown as mean ± SEM. Treatment effects were considered significant at $p < 0.05$ and N=3.

	Europa	**Oncor**	**Burbot 1**	**Burbot 2**	***P*-value**
Initial weight (g)	130.9±6.5	133.9±9.1	139.9±12.7	133.5±1.4	0.891
Final weight (g)	176.8±12.6	211.6±36.1	197.5±14.2	199.1±5.6	0.698
Initial Biomass (g)	11967±285	12700±551	13067±657	12700±189	0.441
Tank BG (g)	7100±458	9067±2691	7340±1279	7953±247	0.727
RG (%)	59±3	70±19	56±7	63±1	0.814
SGR (%/d)	0.52±0.02	0.58±0.12	0.49±0.05	0.54±0.01	0.814
TGC	3.10±0.13	3.65±0.89	3.01±0.37	3.30±0.08	0.814
Total fed (g)	14564±735	15512±1829	15857±1466	16142±169	0.817
FCR	2.06±0.05	1.94±0.40	2.22±0.17	2.03±0.06	0.789
PER	0.83±0.02	1.13±0.22	0.83±0.07	1.13±0.03	0.192
K	0.68±0.00	0.57±0.13	0.68±0.01	0.69±0.01	0.764
Survival (%)	100±0	100±0	99.7±0.003	96±0.02	0.170

Tank biomass gain (g), relative growth (RG; %), specific growth rate (SGR; %/d), feed conversion ratio (FCR), protein efficiency ratio (PER), Fulton`s condition factor (K), thermal growth coefficient (TGC), initial weight (g), final weight (g), total feed (g), initial biomass (g)

Table 2.5. Proximate composition of whole body fish (wet basis) fed with Europa, Burbot1, Oncor, Burbot2 diets. Values shown as mean ± SEM. Treatment effects were considered significant at $p < 0.05$ and N=3.

Proximate	Europa	Burbot1	Oncor	Burbot2	p- value
Crude Protein (%)	15.52±0.22	15.33±0.2	15.31±0.1	14.84±0.04	0.079
Crude Lipid (%)	5.89±0.29	6.68±0.55	6.13±0.42	7.63±0.51	0.105
Energy (Kcal/g)	5818±81	5953±76	5930±119	5943±198	0.867
Ash (%)	2.42±0.05	2.51±0.05	2.36±0.31	2.36±0.14	0.915
Moisture (%)	75.58±0.44	74.60±0.53	75.65±0.07	73.99±0.34	0.043*

Table 2.6. Organosomatic indices; fillet yield, hepatosomatic index (HSI), spleen somatic index (SSI), visceral fat index (VFI), gastrointestinal index (GII). Values given are mean ± SEM. Samples derived from three replicate tanks of four treatments. The Kruskal - Wallis test was run for the statistical analyses. Treatment effects were considered significant at $p < 0.05$ and N=3.

Treatment	Europa	Oncor	Burbot 1	Burbot 2	*P*-value
Fillet yield (%)	52.13 ± 0.36^{a}	53.12 ± 0.53^{a}	52.24 ± 0.44^{a}	49.98 ± 0.27^{b}	0.004*
HSI (%)	7.87±0.38	8.17±0.53	9.04±0.09	8.75±1.35	0.294
SSI (%)	0.13±0.02	0.10±0.01	0.11±0.01	0.09±0.01	0.233
VFI (%)	0.58±0.14	0.45±0.17	0.36±0.14	0.29±0.20	0.483
GII (%)	3.29 ± 0.05^{ab}	3.43 ± 0.14^{b}	3.04 ± 0.02^{a}	3.19 ± 0.05^{ab}	0.048*

Table 2.7. The calculation of feed cost of burbot from 100g to 400g fed with Europa and Oncor diets.

	Feed Price (1kg)	**Cost Production ($) for 100-400g fish**
Europa	2.69	1.66
Oncor	1.65	0.96

Chapter 3: Digestibility of different types of soy protein ingredients in burbot (*Lota lota maculosa*)

Abstract

Alternative ingredients are needed for fishmeal replacement in aquaculture diets. In the present study, apparent digestibility of various soy ingredients was determined for an emerging aquaculture species (burbot; *Lota lota maculosa*). Fish were fed a reference diet, and diets containing soybean meal (SBM), soy protein concentrate (SPC), and fermented soybean meal MESBM to determine digestibility of these ingredients. Results showed that dry matter digestibility of SPC (67.5%) diet was significantly higher than SBM (64.5%) diet there was no significant difference in dry matter digestibility of SPC and MESBM (66.6%) diets. Also, soy protein ingredient digestibility of SPC (93.5%) was significantly higher than MESBM (88.1%) for adult burbot but there was no significant difference between ingredient digestibility of SPC and SBM (92.5%) diets. Furthermore, there was no significant difference in ingredient lipid digestibility among diets in burbot. These results indicate that burbot can digest high level of soy protein ingredients, and both diet dry matter digestibility and ingredient protein digestibility of SPC were high for burbot.

Introduction

The constantly increasing demand of fishmeal is limiting feed resources for aquaculture (Fuentes-Quesada et al. 2018) and this inherent demand increases the price of fishmeal (Salze et al. 2010). It is important to determine if alternative sources of protein can be used as replacements for fishmeal for new and alternative fish species being considered for aquaculture (Aksnes et al. 2006; Walker et al 2010). Such alternatives should be widely available, competitively priced, and contain the appropriate nutritional characteristics for the target production species (Gatlin et al. 2007). Under most circumstances, a variety of plant sources are considered good alternative protein sources (grains, pulses and oilseeds), capable of partially replacing fishmeal for certain fish species (Salze et al. 2010). Soybean meal (SBM) typically has the highest protein content (40–48% crude protein) among the plant-based ingredients and is one of the more favorable alternative protein sources (Fuentes-Quesada et al. 2018). Soy-derived ingredients have been found to be viable alternative protein sources to fishmeal (Walker et al. 2010) for many species due to their availability, relatively low cost, high protein levels, and favorable amino acid profiles (Refstie et al. 2006; Salze et al. 2010; Lin and Luo 2011; Calburn et al. 2012).

High digestibility is one of the most important factors for a protein source to be viewed favorably as a fishmeal replacement in aquaculture diets (Gatlin et al. 2007). Soybean meal contains high levels of undigestible ingredients such as several heat-stable antinutritional factors (trypsin inhibitors, phytic acid) and high levels of α-galactoside sugars and non-starch polysaccharides. (Refstie et al. 2006; Hansen et al. 2007). These indigestible soy meal ingredients negatively affect growth, immunity, nutrient utilization, intestinal integrity and fish welfare in carnivorous fish (Refstie et al. 2006; Skjaervik et al. 2007; Aksnes et al. 2006; Alberksan et al. 2007; Tibbetts et al. 2006; Olsen et al. 2007; Fuentes-Quesada et al. 2018). Digestion and absorption of soy protein along the intestine varies among fish species (Skjaervik et al. 2007) and fish size (Refstie et al. 2006). For instance, although 10–30% SBM replacement reduced growth and protein digestibility and caused intestinal inflammation in salmonids (Refstie et al 2006; Gatlin et all. 2007), no negative effects were observed in Atlantic cod fed diets containing 24% dietary soybean meal (Forde-Skjaervik et al. 2007). Atlantic cod have elevated tolerance for high levels of soybean meal protein and can digest high levels of energy from these diets (Tibbetts et al. 2006). Further, Atlantic cod can adapt to these soy diets by increasing length and weight of gastrointestinal content, changes to the intestinal microflora and increased feed intake (Walker et al. 2010). However, indigestible carbohydrates in SBM decrease nutrient uptake by augmenting fecal moisture content and increasing the rate of gut evacuation (Walker et al. 2010). In addition, high levels of extracted soy protein in Atlantic cod diets can lead to decreased digestibility of amino acids and lipids, and reduced feed efficiency ratio (FER) and protein retention (Aksnes et al. 2006). Advanced ingredient processing technologies have made a significant contribution to the aquaculture industry. Many current products may be considered sustainable alternative sources of dietary protein, which have crude protein levels similar to fishmeal (Salze et al. 2010).

Bioprocessing, a biotechnological process that removes α-galactoside sugars and antinutritional factors associated with raw soybean meal (Refstie et al. 2006), has a positive effect on digestibility and growth of fish (Tibbetts et al. 2006). Soy protein concentrate (SPC) that has more than 60% crude protein is produced at the end of the process (Cheng and Hardy 2004; Minijarez-Osorio et al. 2016). No differences were observed on Atlantic cod growth when 87% of the fishmeal was replaced with SPC. (Burr et al. 2012). In addition, the entire replacement of SPC with fishmeal in diet did not negatively affect the growth and feed conversion in juvenile Atlantic cod (Walker et al. 2010).

Aerobically processed soybean meal is metabolized via fungus to enhance protein content, develop digestibility, and decrease anti-nutritional compounds (Senevirathne et al. 2016). At the end of the process, microbially enhanced soy protein, a good alternative protein source to fishmeal with higher protein content and digestibility than SBM, is produced (Sindelar 2014; Senevirathne et al. 2016).

Effects of soy protein ingredients on fish growth and health have been studied for several species, but soy protein digestibility of burbot (*Lota lota maculosa*) still needs to be addressed. Based on similarity between burbot and Atlantic cod species, it is hypothesized that burbot will be able to effectively digest soybean protein ingredients. The aim of the study is to evaluate digestibility of soy protein ingredients in burbot to obtain baseline data on soy utilization, which may be important as we test or formulate diets for this emerging aquaculture species

Materials and Methods

Fish

Adult burbot (~310g), which were produced at UI-ARI (Moscow, ID), were reared at UI-ARI (Moscow, ID) wet lab in a recirculating aquaculture system (RAS) that has fluidized biofilter and UV filter. The aim of the experiment was to determine the digestibility of soy protein ingredients in burbot. The rearing methods followed current practices and published work (Barron et al. 2012; Palińska-Żarska et al. 2014; Trejchel et al. 2014). Six fiberglass tanks (1130L recirculating aquaculture system (RAS), receiving aeration and water at rate of 12L/min) were used at the ARI coldwater laboratory. The water temperature and dissolved oxygen levels were measured daily and 1/8th of the tank water was replaced daily via lifting the inner standpipe to clean tanks. Water quality in each tank was held to standards reported for culture of adult burbot and measured everyday (dissolved oxygen > 5 ppm, water temperature at 15°C, ammonia levels negligible; Barron et al. 2012; Trejchel et al. 2014). Photoperiod was timer controlled and set 10h light: 12h dark. Four diets with 3 replicates for each were distributed among tanks, and each tank contained 72 adult burbot fish. The experiment was conducted in two rounds after the tank randomization. Fish were fed with control diet and Soybean meal diet in first round. After the fecal sample collection, tanks were randomized again, and fish were fed with SPC diet and MESBM fermented diet for 10 days, followed by fecal collection.

Feeding

We used a fishmeal reference ingredient, a soybean control (hexane extracted), a soy protein concentrate (SPC), and a fermented soybean meal (MESBM) as dietary treatment groups. 30% of the reference diet was replaced with dietary soy protein ingredient in the experimental diets (soybean meal, concentrated soybean meal, fermented soybean meal diets). The source of the SPC was SoyCoMil-P (ADM; Decatur, IL), while the MESBM was a bioprocessed soybean meal provided from Prairie AquaTech (Brookings, SD). An inert marker (yttrium oxide) was included in dietary formulations of reference diet (Table 1) and experimental diets that have 70% reference diet and 30% soybean protein ingredient (Sindelar 2014). Adult burbot (~310g) were fed to apparent satiation twice daily with FM reference diet (42.9% protein and 19.3% lipid) during the pretrial period (7 days). Satiation was determined by hand-feeding and monitoring feeding activity. When feeding activity slowed and a significant number of pellets were visible at the bottom of the tank, apparent satiation was assumed. After 7 days, fish were fed the experimental diets for 10 additional days and fecal material was collected via stripping as described below.

Sampling

Fecal samples were collected by distal gastrointestinal stripping following the method of (Sindelar 2014). Briefly, fish were anesthetized in MS-222 (100 mg/L; buffered to pH 7.0-7.5) prior to sampling. Fish were sampled in batches and fish were individually removed then abdominal pressure was applied in a peristaltic motion to the distal portion of the intestine (Sindelar 2014) until 50g wet fecal material was obtained from fish in each tank. Fish were placed in recovery baths (rock salts (6g/L) and aerated water, and then just aerated water) prepared using rearing water from the RAS. Fecal samples were pooled in a pre-weighed aluminum pan. Sample pans were weighed and frozen at -20°C for future drying process and determination of moisture content. The empty aluminum pans were preliminarily weighed and recorded, and then to make it sure we had at least 50g wet fecal sample from each tank a total weight of pan and fecal material was recorded after sampling. The fecal samples were placed in a drying oven at 75^{0}C for 24h then ground (mortar and pestle) and submitted for yttrium analysis (~1 g) at the Analytical Sciences Laboratory, UI Moscow campus. The remainder of the ground samples were sent to the Hagerman Fish Culture Experiment Station Nutritional Service Center to determine apparent digestible protein (ADP) and lipid (ADL).

In addition, experimental feeds were ground and provided to the Analytical Sciences Laboratory (UI campus) for yttrium analyses and to the Hagerman Fish Culture Experiment

Station Nutritional Service Center for protein and lipid analyses. Also, ground feed samples were dried at 105°C for four hours in an oven to calculate moisture ratio in trial feed.

Based on the results from the laboratories, apparent digestion coefficient of dry matter and soy ingredient protein were calculated by using the ADC (%) and $ADC_{ingredient}$ formulas (Cheng et al. 2004).

Moisture= Weight (Wet fecal+pan) – Weight (Dry fecal+pan) (AOAC, 1999)

ADCs (%)5 100 *[1-(amount of Y in diets/amount Y in fecal)] was used for dry matter digestibility calculation.

Where Y is yttrium.

ADC (%) = 100 – (1- (amount of Y in diets/amount of Y in fecal)* (amount of nutrient in fecal/ amount of nutrient in diets))

Where Y is yttrium (Cheng et al. 2004).

$ADC_{ingredient}$ (%) = (ADCtest (1-i) * ADCref)*i^{-1}

Where ADCtest and ADCref are the apparent digestibility coefficients of nutrients for the test and reference diets, respectively, and i is the percentage of ingredient included in the experimental diets (Cheng et al. 2004).

Statistical Analyses

The experiment design was completely randomized design and results for dietary treatment effects on burbot digestibility of soy protein ingredient were analyzed via GraphPad Prism software. One-way ANOVA and Tukey`s Multiple Comparison Test were run to analyze data. Treatment effects were considered significant at $p < 0.05$.

Results

According to statistical analyses, dry matter digestibility of SPC (67.5%) diet was significantly higher than SBM (64.5%) diet and there was no significant difference between dry matter digestibility of SPC and MESBM (66.6%) diets (Figure 3.1). In addition, a statistical difference between treatments on soy ingredient protein ADC of burbot was observed ($p<0.05$). The SPC ingredient protein ADC was significantly higher than MESBM digestibility (Figure 3.2), though it was not observed any significant difference on soy protein ingredient digestibility between SPC and MESBM diets. SPC ingredient protein ADC (93.5 %) in fish was the highest level compared to other treatments and is followed by SBM (92.3%) and MESBM (88.1 %) diets (Figure 3.2). Furthermore, the result of the present study stated that there was no significant difference on soy ingredient lipid digestibility in fish between diets ($p>0.05$). The highest level of soy ingredient lipid ADC was represented with SPC diet (87.1 %) and was followed by MESBM (84.2 %) and SBM (73.4 %) diets (Figure 3.3).

Discussion

. Dry matter ADC level of SPC diet was significantly higher than SBM diet, but there was no significant difference between dry matter ADC level of SPC and MESBM diets. Also, soy ingredient protein digestibility of the SPC diet was significantly higher than MESBM, but there was no significant difference on ingredient protein digestibility between SPC and SBM. Based on these results of the study, adult burbot are able to highly digest SPC and SBM protein better than MESBM. The ratio of digestion and absorption of nutrients along the intestine depends on species (Forde-Skjaervik et al. 2006), and fish size and some other factors (Refstie et al. 2006). The macronutrients digestibility level of 1-year old and 2 year-old Atlantic cod fed with FM, SBM and SPC diets were not significantly different, but the replacement of fishmeal with soybean meal decreased protein and lipid digestibility (Forde-Skjaervik et al. 2006). On the other hand, amino acids digestibility level of fish fed FM, SBM, BPSBM variated between 1-year old and 2-year-old Atlantic cod (Refstie et al. 2006). The soy protein ingredient ADC of experimental diets (SBM and SPC) were respectively 91.5% and 94% in Atlantic cod (Tibbets et al. 2006). Also, soy ingredient protein digestibility of SBM and SPC in rainbow trout was respectively 92.1% and 97.9% (Glencross et al.2005), and SBM digestibility for Nile tilapia was 84.7% (Engin and Ozkan 2008). In addition, Atlantic salmon soy ingredient protein ADC of SBM and SPC were 85% and 89% (Refstie et al. 1999). It is seen that ingredient protein ADC of SBM and SPC diets in Atlantic cod and in burbot are strongly similar, and rainbow trout digestibility of SPC ingredient level was higher than burbot SPC ingredient digestibility. Also, burbot can digest higher soy ingredient protein ADC of SBM and SPC than Atlantic salmon. The present study showed that adult burbot can digest high level of SPC, SBM and MESBM ingredients protein.

On the other hand, there was no significant difference on soy ingredient lipid ADC ratio between treatments it may due to low lipid level in soy ingredients.

Non-starch polysaccharides in SBM increase water content in fecal material of fish (Gatlin III et al. 2007). In addition, Walker et al. (2010) reported that replacement of 25% fishmeal with SBM in cod diets enhanced fecal moisture content and caused diarrhea because of the non-starch polysaccharides and α-galactoside oligosaccharides. In the present study, burbot fed with SBM diet were observed to have greater amounts of watery fecal material compared to fish fed other diets.

The result of the study showed that adult burbot are highly capable of digesting soy protein ingredients. The soy protein ingredient ADC of SPC diet was significantly higher than MESBM diet. Lipid content of soy ingredients was so low and there was no significant diet effect on soy ingredient lipid digestibility of burbot.

This study provides an initial evaluation of soy ingredient digestibility for adult burbot. Similar digestibility studies should be conducted on juvenile and grow-out stage burbot.

References

Aksnes, A., Hope, B., Høstmark., Ø., Albrektsen, S., 2006. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. Aquaculture 261:1102–1110.

Albrektsen, S., Mundheim, H., Aksnes, A., 2006. Growth, feed efficiency, digestibility and nutrient distribution in Atlantic cod (*Gadus morhua*) fed two different fishmeal qualities at three dietary levels of vegetable protein sources. Aquaculture 261:626–640.

Barron, J.M., Jensen, N.R., Anders, P.J., Egan, J.P., Ireland, S.C., Cain, K.D., 2012. Effect of temperature on the intensive culture performance larval and juvenile North American burbot (*Lota lota maculosa*). Aquaculture 364-365:67-73.

Burr, G.S., Wolters, W.R., Barrows, F.T., Hardy, R.W., 2012. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (*Oncorhynchus mykiss*), and early or late stage juvenile Atlantic salmon (*Salmo salar*). Aquaculture 334-337:110–116.

Cheng, Z.J., Hardy, R.W., 2004. Effects of Microbial Phytase Supplementation and Dosage on Apparent Digestibility Coefficients of Nutrients and Dry Matter in Soybean Product-Based Diets for Rainbow Trout *Oncorhynchus mykiss*. Journal of the World Aquaculture Society Vol. 35, No. 1.

Cheng, Z.J., Hardy, R.W., Huige, N.J., 2004. Apparent digestibility coefficients of nutrients in brewer's and rendered animal by-products for rainbow trout (*Oncorhynchus mykiss* (Walbaum)). Aquaculture Research 35:1-9.

Colburn, H.R., Walker, A.B., Breton, T.S., Stilwell, J.M., Sidor, I.F., Gannam, A.L., Berlinsky, D.L., 2012. Partial Replacement of Fishmeal with Soybean Meal and Soy Protein Concentrate in Diets of Atlantic Cod. North American Journal of Aquaculture 74:330–337.

Engin, K., Özkan, F., 2008. Apparent Nutrient Digestibility of Balanced Diets with Soybean Extract or Meat and Bone Meal in the Nile Tilapia (*Oreochromis niloticus L*.) Infected with Vibrio anguillarum. Turk. J. Vet. Anim. Sci. 32(3):173-181.

Førde-Skjærvik, O., Refstie, S., Aslaksen, M.A., Skrede, A., 2006. Digestibility of diets containing different soybean meals in Atlantic cod (*Gadus morhua*); comparison of collection methods and mapping of digestibility in different sections of the gastrointestinal tract. Aquaculture 261:241–258.

Fuentes-Quesada, J.P., Viana, M.T., Rombenso, A.N., Guerrero-Rentería, Y., Nomura-Solís, M., Gomez-Calle, V., Lazo, J.P., Mata-Sotres, J.A., 2018. Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. Aquaculture 495:78-89.

Gatlin III, D.M., Barrows, F.T., Paul Brown, P., Konrad Dabrowski, K., Gaylord, T.G.,Hardy, R.W., Herman, E., Gongshe Hu, G.,Krogdahl, A., Richard Nelson, R., Overturf1, K., l Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., EveWurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38:551-579.

Glencrossa, B., Evansa, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R., Sipsas, S., 2005. Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. Aquaculture 245:211–220.

Hansen, A.C., Grethe Rosenlund, G., Ørjan Karlsen, Q., Wolfgang Koppe, W., Hemre, G., 2007. Total replacement of fishmeal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I — Effects on growth and protein retention. Aquaculture 272:599–611.

Lin, S., Luo, L., 2011. Effects of different levels of soybean meal inclusion in replacement for fishmeal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Animal Feed Science and Technology 168:80–87.

Minjarez-Osorio, C., Castillo-Alvarado, S., Gatlin III, D.M., González-Félix, M.L., Perez-Velazquez, M., Rossi Jr, W., 2016. Plant protein sources in the diets of the sciaenids red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*): A comparative study. Aquaculture 453:122-129.

Olsen, R.E., Hansen, A.C., Rosenlund, G., Hemre, G.I., Mayhew, T.M., Knudsen, D.L., Eroldoğan, O.T., Myklebust, R., Karlsen, Q., 2007. Total replacement of fishmeal with plant proteins in diets for Atlantic cod (*Gadus morhua L.*) II — Health aspects. Aquaculture 272:612–624.

Palin´ ska-Zarska, K., Zarski, D., S. Krejszeff, S., Nowosad, J., Biłas, M., Trejchel, K., D. Kucharczyk, D., 2014. Dynamics of yolk sac and oil droplet utilization and behavioral aspects of swim bladder inflation in burbot, *Lota lota* L., larvae during the first days of life, under laboratory conditions. Aquacult Int 22:13–27.

Refstie, S., Skjærvik, O.F., Rosenlund, G., Rørvik, K.A., 2006. Feed intake, growth, and utilisation of macronutrients and amino acids by 1- and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. Aquaculture 255:279–291.

Refstie, S., Svihus, B., Shearer, K.D., Storebakken, T., 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean products. Animal Feed Science and Technology 79:331-345.

Salze, G., McLean, E., Battle, P.R., Schwarz, M.H., Craig, S.R., 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fishmeal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. Aquaculture 298:294–299.

Senevirathne, N.D., Anderson, J.L., Rovai, M., 2016. Effect of water treatment on drinking preferences of dairy heifers. Journal of Animal Science 2:166-167.

Sindelar, S., 2014. Utilization of Soybean Products as Fish-Meal Protein Replacement in Yellow Perch *Perca flavescens* Feeds. Master`s Thesis. South Dakota University, Vermillion, South Dakota.

Tibbetts, S.M., Milley, J.E., Lall, S.P., 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). Aquaculture 261:1314–1327.

Trejchel, K., Zarski, D., Palin´ ska-Z arska, K., Krejszeff, S., j Dryl, B., Dakowski, K., Kucharczyk, D., 2014. Determination of the optimal feeding rate and light regime conditions in juvenile burbot, *Lota lota* (L.), under intensive aquaculture. Aquacult Int 22:195–203.

Walker, A.B., Sidor, I.F., O'Keefe, T., Cremer, M., Berlinsky, D.L., 2010. Partial Replacement of Fishmeal with Soy Protein Concentrate in Diets of Atlantic Cod. North American Journal of Aquaculture 72:343–353.

Table 3.1. Ingredient listing of reference diet and experimental diets used for soy protein ingredient diet digestibility for burbot.

Ingredients	**Reference Diet (%)**	**SBM (%)**	**SPC(%)**	**MESBM**
Ytrium Oxide	0.01	0.01	0.01	0.01
Whole Cleaned Wheat	28.79	20.15	20.15	20.15
Fishmeal	55.00	38.50	38.50	38.50
Vitamin Premix	1.25	0.87	0.87	0.87
Choline Chloride	0.60	0.42	0.42	0.42
Mineral Premix	0.75	0.52	0.52	0.52
Stay C (L-Ascorbat-2-Mono)	0.30	0.21	0.21	0.21
Fish Oil – Menhaden	13.30	9.31	9.31	9.31
Test Ingredient	0.00	30.00	30.00	30.00
Total	100.00	100.00	100.00	100.00.

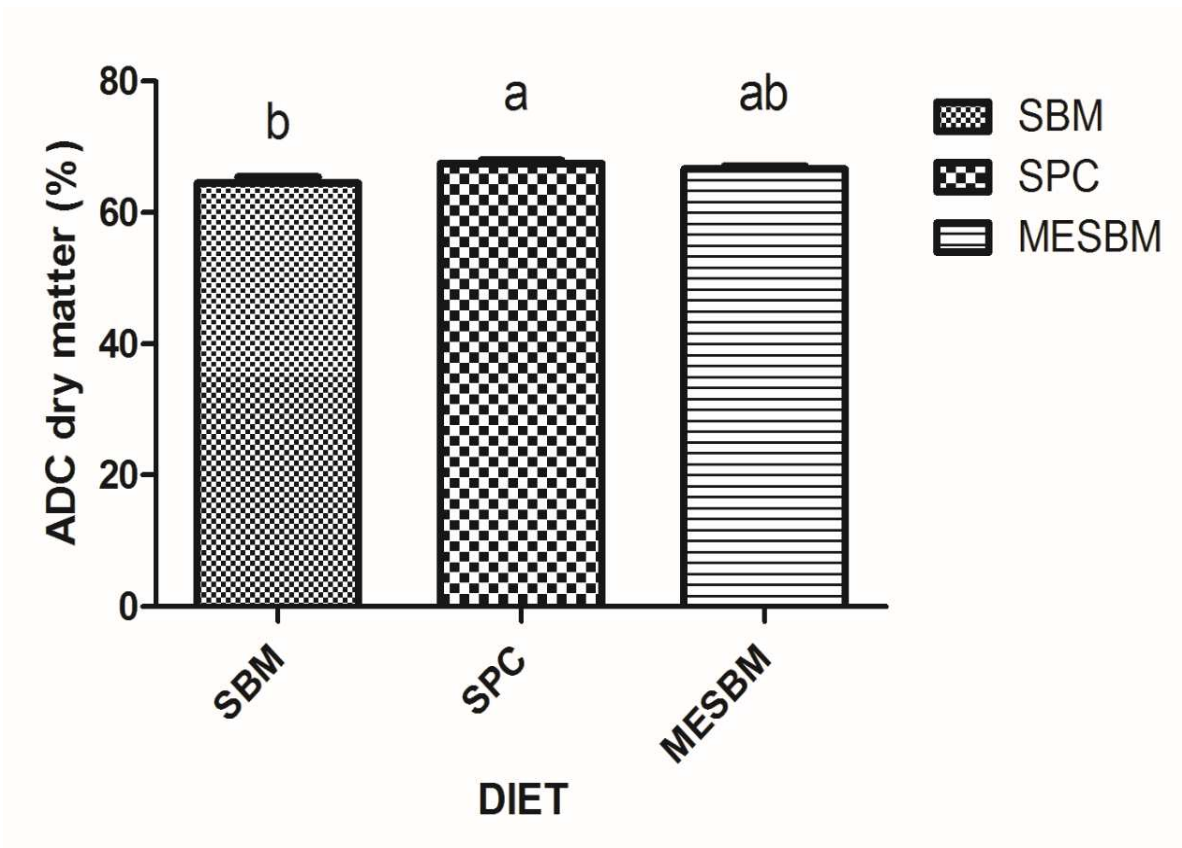

Figure 3.1. ADC $_{dry\ matter}$ (apparent digestibility coefficient of dry matter). Diet dry matter digestibility level of burbot fed with SBM (Soybean meal), SPC (Soy protein concentrate), MESBM (microbial enhanced soybean meal) diets.

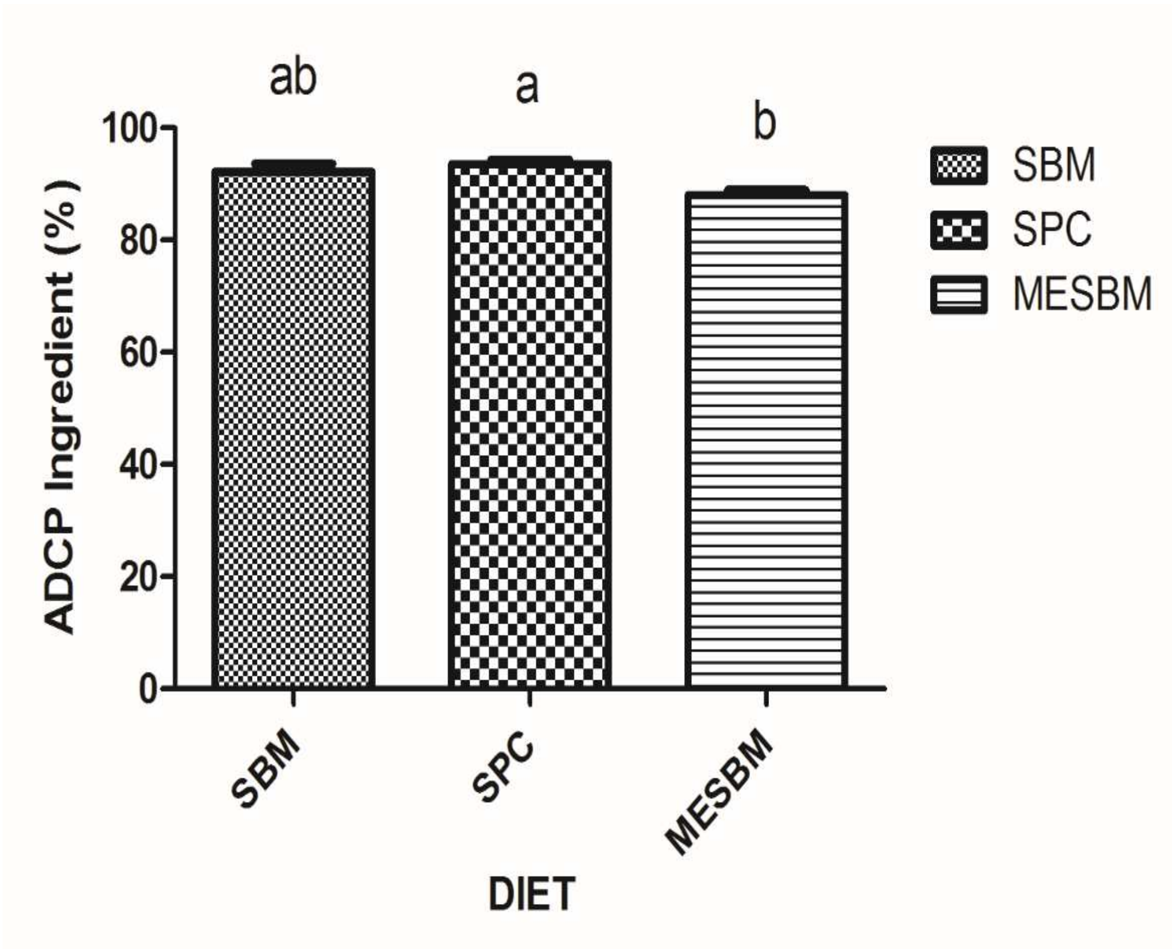

Figure 3.2. $ADCP_{ingredient}$ (apparent digestibility coefficient of ingredient protein). Soy ingredient protein digestibility ratio of burbot fed with SBM (Soybean meal), SPC (Soy protein concentrate), MESBM (microbial enhanced soybean meal) diets.

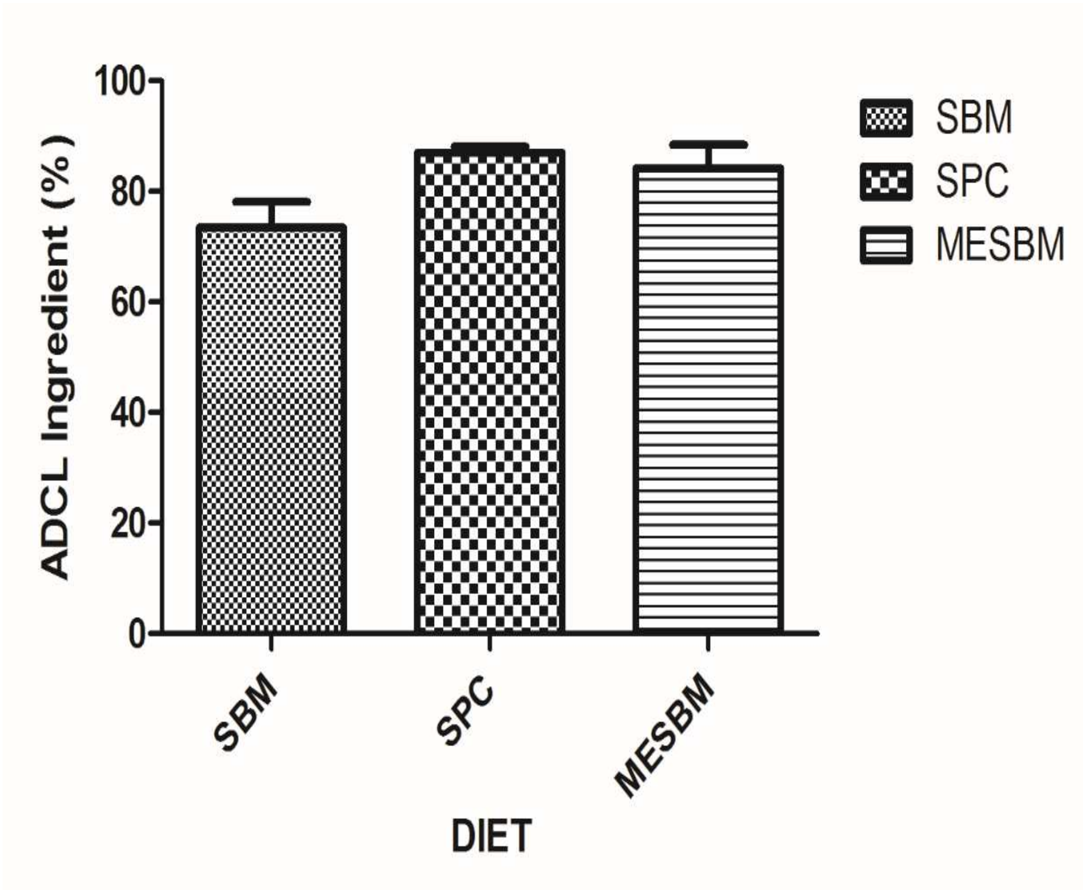

Figure 3.3. ADCL$_{ingredient}$ (apparent digestibility coefficient of ingredient lipid). Soy ingredient lipid digestibility ratio of burbot fed with SBM (Soybean meal), SPC (Soy protein concentrate), MESBM (microbial enhanced soybean meal) diets.

General Discussion

This study identified effects of marine type (Europa, Burbot 1) and trout type (Oncor, Burbot 2) diets on sub-adult burbot growth and health. The marine type and trout type diets had no significant effect on burbot growth. Economic analysis indicated that using Oncor diet for feeding sub-adult burbot is more cost effective than using Europa diet. Although there was no significant diet effect on fish growth, the economic analysis showed that a more economical trout-type commercial diet would be recommended over a more expensive higher protein marine-type diet. In addition, there were no significant differences for HSI, VFI and SSI between diets while there were significant dietary effects on Fillet yield and GII of burbot. Fish fed with Burbot 2 diet had significantly lower Fillet yield level. Although there was no significant difference on growth between diets, fish fed with Burbot 2 diet did not create muscle efficiently probably because of the low protein level. This result agrees with Khan et al. (2019) who reported that fish fed with protein-insufficient diets use protein as an energy source instead of muscle production. Furthermore, fish fed with Oncor diet had significantly higher GII level than fish fed with Burbot 1 diet. Walker et al (2010) and Fuentes-Quesada et al. (2018) reported that using high level of plant protein in carnivorous fish diets caused gastrointestinal growth in fish. This gastrointestinal growth increase becomes because of high fiber contents and low digestible energy level in plant sources (Refstie et al. 2006). Oncor diet had high level plant ingredient, so the high level of GII in fish fed Oncor diet may due to the high plant ingredient in Oncor diet. According to results of the study we can initially recommend the Oncor diet to burbot producers to feed sub-adult burbot for an economical commercial production.

This study also served to test adult burbot digestibility of soy protein ingredients in diets for the future sustainable and commercial burbot production. Dry matter digestibility of SPC and SBM diets were higher than MESBM diet. Also, soy ingredient protein digestibility of fish was significantly higher for SPC diet compared MESBM diet. However, there was no significant difference on soy ingredient protein ADC level for burbot between SPC and SBM diets. According to results of Tibbets et al. (2006), there was similarity on ingredient protein ADC level of SBM and SPC between cod and burbot. Also, there was similarity between ingredient protein ADC of SBM in rainbow trout and burbot, and soy ingredient protein digestibility of SBM and SPC in burbot was higher than Atlantic salmon. Furthermore, there was no significant difference on soy ingredient lipid ADC levels between treatments. Finally, adult burbot have high level soy ingredient protein ADC and SPC diet had the highest ingredient protein ADC ratio. SPC and SBM protein ingredient can be good protein source in the future in terms of digestibility to do a sustainable burbot production. SPC is more expensive than SBM, because processing technology is expensive (Walker et al. 210). Based on the result of the present study there was no significant difference on ingredient and dietary digestibility between SPC ($13.3/kg) and SBM ($0.30/kg) diets. Thus, usage of the SBM ingredient as protein source in burbot diets would be more economical for producers. For further knowledge, juvenile and sub-adult burbot soy ingredient digestibility should be investigated to test if soy protein ingredients can be a good protein source in these age burbot diets regarding digestibility.

References

Fuentes-Quesada, J.P., Viana, M.T., Rombenso, A.N., Guerrero-Rentería, Y., Nomura-Solís, M., Gomez-Calle, V., Lazo, J.P., Mata-Sotres, J.A., 2018. Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. Aquaculture 495:78-89.

Khan, K.U., Rodrigues, A.T., Mansano, C.F.M., Queiroz, D.M.A, Sakomura, N.K., Romaneli, R.S., Nascimento, T.M.T., Fernandes, J.B.K., 2019. Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices. Latin American Journal of Aquatic Research 47(2):232-239.

Refstie, S., Skjærvik, O.F., Rosenlund, G., Rørvik, K.A., 2006. Feed intake, growth, and utilisation of macronutrients and amino acids by 1- and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. Aquaculture 255:279–291.

Tibbetts, S.M., Milley, J.E., Lall, S.P., 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). Aquaculture 261:1314–1327.

Walker, A.B., Sidor, I.F., O'Keefe, T., Cremer, M., Berlinsky, D.L., 2010. Partial replacement of fishmeal with soy protein concentrate in diets of Atlantic cod. North American Journal of Aquaculture 72:343–353.