

7-2013

Processing Approaches to Improve Functionality and Value of Soybean Products

Jordan J. Foley

Iowa State University, jjfoley@iastate.edu

Kurt A. Rosentrater

Iowa State University, karosent@iastate.edu

Buddhi P. Lamsal

Iowa State University, lamsal@iastate.edu

Nitya Poovaiah

Iowa State University, nityap@iastate.edu

Follow this and additional works at: http://lib.dr.iastate.edu/abe_eng_conf



Part of the [Agriculture Commons](#), and the [Bioresource and Agricultural Engineering Commons](#)

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/abe_eng_conf/341. For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

This Conference Proceeding is brought to you for free and open access by the Agricultural and Biosystems Engineering at Iowa State University Digital Repository. It has been accepted for inclusion in Agricultural and Biosystems Engineering Conference Proceedings and Presentations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Processing Approaches to Improve Functionality and Value of Soybean Products

Abstract

Soybeans have high concentrations of proteins, amino acids and unsaturated lipids. Various soybean products, such as soybean meal, soy protein concentrate, and soy protein isolate are available and have been studied, due to their abundant nutrient contents. They are acceptable substitutes for meat for vegetarians and vegans, due to their high nutritional value. Soy products have been extensively utilized in the animal feed industry as a protein source as well. Unfortunately, there are challenges associated with soybeans and soy products. Soybeans cannot be consumed without being processed. The main problem is the presence of Anti Nutritional Factors (ANFs), which are a major cause of poor protein digestibility. With respect to animal feeds, other problems include morphological and pathological changes, such as abnormalities in the digestive systems in non-ruminant animals. Hence, the objective of this study was to review various methods of processing soybeans, which can reduce anti-nutritional factors, increase nutrient availability, and effectively utilize the nutritional value of soybeans.

Keywords

Enzymes, Feed, Fermentation, Fish Meal, Food, Protein, Soybeans

Disciplines

Agriculture | Bioresource and Agricultural Engineering



2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

An ASABE Meeting Presentation

Paper Number: 131592967

PROCESSING APPROACHES TO IMPROVE FUNCTIONALITY AND VALUE OF SOYBEAN PRODUCTS

Jordan J. Foley, Kurt A. Rosentrater

Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, 50011

Buddhi Lamsal, Nitya Poovaiah

Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, 50011

Written for presentation at the

2013 ASABE Annual International Meeting

Sponsored by ASABE

Kansas City, Missouri

July 21 – 24, 2013

Abstract. *Soybeans have high concentrations of proteins, amino acids and unsaturated lipids. Various soybean products, such as soybean meal, soy protein concentrate, and soy protein isolate are available and have been studied, due to their abundant nutrient contents. They are acceptable substitutes for meat for vegetarians and vegans, due to their high nutritional value. Soy products have been extensively utilized in the animal feed industry as a protein source as well. Unfortunately, there are challenges associated with soybeans and soy products. Soybeans cannot be consumed without being processed. The main problem is the presence of Anti Nutritional Factors (ANFs), which are a major cause of poor protein digestibility. With respect to animal feeds, other problems include morphological and pathological changes, such as abnormalities in the digestive systems in non-ruminant animals. Hence, the objective of this study was to review various methods of processing soybeans, which can reduce anti-nutritional factors, increase nutrient availability, and effectively utilize the nutritional value of soybeans.*

Keywords. *Enzymes, Feed, Fermentation, Fish Meal, Food, Protein, Soybeans.*

The authors are solely responsible for the content of this meeting presentation. The presentation does not necessarily reflect the official position of the American Society of Agricultural and Biological Engineers (ASABE), and its printing and distribution does not constitute an endorsement of views which may be expressed. Meeting presentations are not subject to the formal peer review process by ASABE editorial committees; therefore, they are not to be presented as refereed publications. Citation of this work should state that it is from an ASABE meeting paper. EXAMPLE: Author's Last Name, Initials. 2013. Title of Presentation. ASABE Paper No. ---. St. Joseph, Mich.: ASABE. For information about securing permission to reprint or reproduce a meeting presentation, please contact ASABE at rutter@asabe.org or 269-932-7004 (2950 Niles Road, St. Joseph, MI 49085-9659 USA).

INTRODUCTION

Soybeans are one of the main sources of vegetable proteins. A majority of the soybeans produced are converted into feed and food ingredients such as defatted flour, soybean meal, whole soybean flour, soy protein isolate and soy protein concentrate (Liu, 2000). Soybeans are believed to have originated in China. Soybeans were first brought to the United States from China in the 1800s as cost-effective ballast for their ships and were unloaded at the coast. In the year 1829, soybeans were first grown in the United States and were used for miscellaneous purposes. In 1929, numerous varieties of soybeans were shipped into the United States from China for research, which resulted in an exponential growth of soybean production (Cromwell, 2012).

Soybeans account for approximately 55% of the total production of oilseeds globally. Soybean meal is produced by extracting oil from the seeds of the soybeans. The protein concentration in soybean meal ranges from 44% to 49%. It also has a balanced amino acid profile and is rich in lysine, tryptophan, threonine, isoleucine, and valine (Cromwell, 2012). Due to the high protein content of soybeans, they are a good substitute for meat for vegetarians. Soy products are predominantly used in place of animal proteins and in processed foods (Liu, 2000). The consumption of soybean meal in China was approximately 56 million tons in 2010 (Teng et al, 2011).

In the United States, approximately 97.5% of the total soybeans produced are used in livestock and poultry feed as a protein source (ASA, 2008). The demand for fish meal is increasing with the growing global aquaculture industry, which has in turn resulted in higher prices (Tacon and Metian, 2008). In fact, soybean products can be incorporated into fish feed in order to reduce the use of fish meal, which is expensive and accounts

for a majority of expenditures in aquaculture. Several studies have been conducted incorporating soybean products in aquaculture and other animal feeds. Major problems can be the development of physiological abnormalities, such as a decrease in the bile acid content, presence of large vacuoles in the mucosal folds of the distal intestine, growth retardation, and other changes in the digestive system (Iwashita et al., 2008). If soybeans are not processed in any way, and are directly fed to young pigs, there will be a reduction in growth due to the trypsin inhibitors, whereas older pigs may be able to tolerate these more (Cromwell, 2012).

Problems associated with soybeans are mainly due to the presence of anti-nutritional factors (ANFs). The inhibitors present that prevent protein digestion are mainly trypsin and chymotrypsin inhibitors (Jiao et al., 1992). Trypsin is an enzyme required for the digestion of proteins. Soybeans contain certain factors that inhibit this activity. Deactivation of trypsin inhibitors is key to improving the digestibility of soy proteins. Another anti-nutritional factor, phytic acid, is present, which is a form of phosphorus that is difficult to digest, as humans and non-ruminant animals do not have the digestive enzymes to degrade phytic acid (Cromwell, 1999). Some of the inhibitors that are present in soybeans are not resistant to heat and can be destroyed if treated with heat. Some trypsin inhibitors, glycinin antigens, oligosaccharides, and saponins are resistant to heat and can remain in the soybeans even after heat treatment (Liener, 1994).

The ill-effects caused due to anti-nutritional factors can be reduced through heat treatment or fermentation, as fermentation leads to bio-modification of the substrate. Modification can also be done by treating the soybean products directly with enzymes. Some studies have been conducted in these areas, and it has been shown that

fermentation can improve the nutritional quality of soybean meal (Hong et al, 2004). Another method that is being studied for the reduction of anti-nutritional factors is chemical treatment. A trait seen in most plant inhibitor proteins are the presence of intramolecular disulfides (Garicia et al, 1987). Hence, if these disulfides can be reduced, the effects of the anti-nutritional factors can be controlled.

Thus the aim of the study was to review key methods of processing soybeans, such as fermentation, enzyme treatment, and chemical treatment, which can reduce the problems associated with soybeans.

FERMENTATION AND ENZYMES

Fermentation is a widely used method of processing food and biochemicals. Fermentation breaks down the substrate into a simpler form, which makes it easy for digestion, thus increasing protein availability. Table 1 summarizing several studies related to fermentation and enzymes.

Fermentation can be conducted by several methods, including submerged fermentation and solid state fermentation. Submerged fermentation involves the dissolution of the nutrients in a high quantity of water (Chahal, 1991). Solid state fermentation is a type of fermentation conducted without any free water present in the substrate which contains microorganisms (Lonsane et al, 1985). Solid state fermentation has a few advantages over submerged fermentation, including low cost, better product recovery, no foaming problems, and reduction of the amount of waste water generated through the process. The disadvantages of solid state fermentation, however, are contamination by bacteria, control of moisture levels, and increase in heat buildup (Lonsane et al , 1985, Hasseltine, 1972).

Teng et al (2011) studied the fermentation of soybean meal using *Bacillus subtilis* and *Aspergillus oryzae* (table 1). The medium for fermentation was soybean meal and wheat bran in a ratio of 3:1, with a moisture content of 67%. After 72 h of fermentation they found that the soluble protein had increased by 63.11% and 19.4% for the *Bacillus subtilis* and the *Aspergillus oryzae*, respectively. The amino acid content, namely arginine, serine, threonine, aspartic acid, alanine, and glycine contents, increased by 50.67%, 45.6%, 34.55%, 22.25%, 21.23%, and 18.12%, respectively. Clearly, *Bacillus subtilis* was a good choice for the fermentation process, as it shows a much higher soluble protein and amino acid content post-fermentation.

Solid state fermentation was used by Lio and Wang (2012) to break down the fiber that is present in this product, in order to increase its digestibility and potentially use it for non-ruminant animal feed. They observed that the highest activity of xylanase was 757.4 IU/g when *Trichoderma reesei* and *Phanerochaete chrysosporium* were inoculated and incubated for a period of 36 h, followed by *Aspergillus oryzae* for an additional 108 h (Lio and Wang, 2012).

An increase in the solubilization of soybean proteins through fermentation can improve the digestibility of the soybean product. Kiers et al. (2000) studied the effect of fermentation on digestibility (table 1). Fermentation resulted in the degradation of soybean macromolecules to a large extent, which resulted in an increase in the amount of water soluble components. It also lowered the molecular weight of the compounds due to the biochemical changes that took place. They found that the in-vitro digestibility increased from 29% to 33-43% after 48 h of fermentation (Kiers et al, 2000).

Enzyme modification is done by the addition of enzymes to the substrate directly, or by

inoculating the medium with micro-organisms, which in turn ferments the medium and produces enzymes (Cowieson et al, 2006). Enzymes such as β -glucosidase, α -galactosidase, phytases, and proteases are used to reduce the effects of inhibitors, anti-nutritional factors, and oligosaccharides (Bedford, 2000). Soy isoflavones are found in the form of isoflavone glycosides (Izumi et al, 2000). Isoflavones are known to benefit human health, and they are being studied for their role in health and wellness with respect to cardiovascular disease, osteoporosis, and beneficiary effects on menopausal symptoms (Delmonte et al, 2006). Furthermore, Iowa State University developed a method of enzyme-assisted aqueous extraction processing (EAEP), which is a method for the extraction of soybean oil. A byproduct of this process is soybean cotyledon fiber (de Moura et al, 2011).

Yang et al (2009) studied the extent to which β -glucosidase (*P. thermophile* β -glucosidases and commercial almond β -glucosidase) could be used for deglycosylation of isoflavone glycosides, in order to be able to utilize the benefits of the isoflavones (table 1). Five types of soybean (daidzin, genistin, daidzein, genistein, and glycitein) were used for the study. They also studied the thermostability of β -glucosidase. In their results, they reported that the isoflavone glycosides conversions in soybean flour by *P. thermophile* β -glucosidase to their aglycones were 98%, 95.8% and 99.3% of hydrolysis of daidzin, glycitein and genistin, respectively in 4 h. It was also found that the deglycosylation of isoflavone was higher in *P. thermophile* β -glucosidase than the commercial almond β -glucosidase. Additionally, the thermal stability of the enzyme at 50°C was high, and retained 95% of its initial activity, even after 8 h (Yang et al., 2009). Leomar et al studied the effect of fermentation using *Aspergillus oryzae* on the

conversion of isoflavones from glycosides to aglycones and found that fermented autoclaved whole soy flour contained 75.51% isoflavone aglycones after 48 h of fermentation. More details regarding the effects of feeding fermented or enzymatically-treated soy products can be found in Table 1.

CHEMICAL TREATMENT OF SOYBEANS

Protein digestibility can be improved through chemical treatment also. The major form in which protein is stored in soybeans is glycinin, which contains disulphide bonds. A trait seen in most plant inhibitor proteins is the presence of intramolecular disulfides (Garicia-Olmedo et al, 1987). The primary inhibitors, which prevent protein digestion and are most commonly seen in soybeans, are the Kunitz trypsin inhibitor (trypsin inhibitor) and Bowman-Birk inhibitor (chymotrypsin inhibitor) (Jiao et al, 1992). The number of disulfide bonds present in the Bowman-Birk inhibitor and the Kunitz trypsin inhibitor are 7 and 2, respectively (Birk 1976, Wilson, 1988). Treating soy proteins to high temperature causes denaturation of a part of the anti-nutritional factors but is often not enough to completely inactivate them. For example, the Kunitz trypsin inhibitor was inactivated only by 80% by heating it for half an hour at a temperature of 120°C (Friedman et al, 1991). During chemical modification, there may be a change in the three dimensional structure of the protein due to reduction that makes the proteins more available for digestion (Herkelman et al, 1991).

Wang et al (2009) studied the use of mild temperatures along with sulfite and metasulfite chemical treatments, in order to increase the digestibility of soy protein (table 2). Soy white flour produced through hexane extraction was used for the study. Results showed that the samples, which had not been subjected to heat, had lower

sulfhydryl content after treatment with the reducing agents, which was attributed to the re-oxidation of sulfhydryl compounds. Due to the inactivation of trypsin inhibitors by sodium metabisulfite and sodium sulfite, the in-vitro digestibility was higher for the samples that were treated when compared to the untreated samples; this was true even at a mild temperature of 55°C. An increase in either of the two chemicals resulted in higher digestibility values, but the digestibility when sodium metabisulfite was used was higher. Maximum digestibility was recorded at a temperature of 100°C. Digestibility at 100°C was three times greater than the digestibility at 80°C (Wang et al, 2009).

Reduction of disulfide bonds have also been reported by treating soy protein to NADP-thioredoxin (NTS) (Jiao et al, 1992). They found that treating the proteins with NTS at low temperature resulted in inactivation of the inhibitors through reduction (i.e., NADPH reduced). In another study, Faris et al (2008) used thioredoxin to reduce the disulfide bonds in soy white flour. They found that sulfhydryl content was much higher in the samples that were treated with NTS (35.44 µmol/g), when compared to the untreated soy white flour (7.69 µmol/g) or the control (8.66 µmol/g). It was determined that the soy white flour that was treated with NTS showed an increase in digestibility (10.06%), when compared to the untreated white soy (7.78%) and the control samples (6.38%) (Faris, 2008). More details regarding the effects of feeding soy products treated by chemical or physical methods can be found in Table 2.

SOY PRODUCTS AS SUBSTITUTES FOR FISH MEAL

For decades, the primary protein source for fish feeds has been fish meal. Fish meal is mainly made of whole wild-caught fish or meat remains from the fish processing industries. Though fish meal can most readily provide fish with the nutrients that they

require, there are growing long-term problems associated with it. The demand for fish meal is increasing by the day, due to the growing global aquaculture industry, and wild fish production and harvest is not sustainable enough to keep up with this increasing demand. Another problem is the high cost of fish meal (generally between 1500 and 2000 \$/ton), which has been due to increased demand and limited natural supplies. The maximum expenditure for aquaculture production is the fish food. Thus, the dependence of the industry on fish meal is not favorable in the long run, and effective alternatives have to be developed (Ayadi et al, 2012). Soybean products have high nutritional values, and are reasonably priced vis-à-vis fish meal. The primary drawback, however, is the presence of anti-nutritional factors.

Over the years, many studies have investigated the use of soybean meal (SBM) in fish diets (von der Decken and Lied, 1993). For example, Goda et al. (2007) found that SBM and full-fat soybeans supplemented with various amino acids could effectively be used in a 28% crude protein diet fed to Nile tilapia fingerlings; the fish showed higher growth rate, weight gain and feed intake when fed the extruded SBM diet compared to the fish meal control diet, which was attributable to inactivation of heat-labile antinutritional factors in the SBM due to extrusion processing. Other studies also concluded that dehulled solvent-extracted and expeller-pressed SBM at 64% and 68% inclusion levels, could completely replace fish meal in commercial diets containing 32% protein for juvenile tilapia, and did not negatively affect weight gain, survival rate, or palatability (Nguyen et al., 2009). In studies of southern catfish, 39% of the fish meal could be replaced with SBM without adversely affecting growth rate in a 48% crude protein diet (Ai and Xie, 2005).

Soy protein concentrate (SPC) has been investigated in a few studies (Cremer et al., 2008; table 1). It has been used to replace fish meal in feed for salmonids (Carter and Hauler, 2000). Studies at 58% SPC dietary inclusion level in a 46% crude protein diet showed that yellowtail (*Seriola quinqueradiata*) juveniles had comparable growth to fish fed a fish meal-based control diet (Takagi et al., 2008). SPC at 49% inclusion level (substituted for 75% of the fish meal) in 46% crude protein diets for juvenile coho (*Rachycentron canadum*) and did not negatively affect weight gain or growth rate (Salze et al., 2010). It has also been shown that it is possible to totally replace fish meal for juvenile rainbow trout with SPC at 62% inclusion in a 46% crude protein diet without reducing growth rates (Kaushik et al., 1995).

In recent years, soy protein isolate (SPI) has been investigated in aquafeeds as well. For example, in 45% crude protein juvenile coho diets, SPI at 23% dietary inclusion (substituted for 41% of the fish meal) resulted in higher growth rate and weight gain compared to the fish meal-based control diet (Lunger et al., 2007). Conversely, when SPI was fed to post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) at 15% and 30% dietary inclusion levels, growth performance could not be evaluated due to poor palatability (Hajen et al., 1993).

Summaries of additional studies using soy products in aquafeeds are provided in Table 3.

CONCLUSIONS

For years, commercial aquafeeds have been primarily based on fish meal, due to its many advantages, including high protein content, high digestibility, amino acid profile, fatty acid profile, palatability, and market availability. These characteristics make it very

challenging to find less expensive alternatives to fish meal without adversely affecting fish performance and harvest quality. Unfortunately, the long-term sustainability of using fish meal in aquatic diets is not promising, and alternative protein sources must be found. Promising plant protein sources include soybean meal, soy protein concentrate, and soy protein isolate. More research focused on commercial-scale feeding studies, examining feed acceptability, digestibility, fillet quality, and impacts on entire production costs, should be conducted to more completely quantify soy's potential in aquafeeds.

REFERENCES

- Ai, Q. and X. Xie. 2005. Effects of replacement of fish meal by soybean meal and supplementation of methionine in fish meal/soybean meal-based diets on growth performance of the Southern Catfish *Silurus meridionalis*. *Journal of the World Aquaculture Society* 36 (4): 498-507.
- ASA. 2008. Welcome to Soy Stats 2008. The American Soybean Association, St. Louis, MO. Available at: <http://www.soystats.com/2008/Default-frames.htm>. Accessed on 25 June 2013.
- Ayadi, F. Y, K. A. Rosentrater, and K. Muthukumarappan. 2012. Alternative protein sources for aquaculture feeds. *Journal of Aquaculture Feed Science and Nutrition*. 4(1): 1-26.
- Bedford, M.R. 2000. Exogenous enzymes in monogastric nutrition - their current value and future benefits. *Animal Feed Science and Technology* 86(1-2): 1-13.
- Bedford, M.R., and H. Shulze 1998. Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews* 11(1): 91-114.
- Birk, Y. 1976. Proteinase inhibitors from plant sources. *Methods Enzymol.* 45: 695-707.
- Campbell, K. A., C. E. Glatz, L. A. Johnson, S. Jung, J.M.N. de Moura, V. Kapachie, and P. Murphy. 2011. Advances in aqueous extraction processing of soybeans. *Journal of the American Oil Chemists' Society* 88(4): 449-465.
- Carter, C.G. and R.C. Hauler. 2000. Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture* 185 (3-4): 299-311.
- Chahal, D. S. 1991. Chapter 9: Production of *Trichoderma reesei* cellulase system with high hydrolytic potential by solid-state fermentation. In *Enzymes in Biomass Conversion*, 111-122. American Chemical Society. Washington DC.
- Cowieson A.J., M. Hurby, and E. E. Pierson. 2006. Evolving enzyme technology: impact on commercial poultry nutrition. *Nutritional Research Reviews*. 19(1): 90-103.
- Cremer, M. C., Z. Enhua, and Z. Jian. 2008. Soy protein concentrate as a substitute for

- fishmeal in the feed for black carp. ASA-IM/China Aquaculture Program. Available at: <http://www.soyaqua.org/reports/soy-protein-concentrate-substitute-fishmeal-feed-black-carp>. Accessed 25 June 2013.
- Cromwell, G. L. 1999. Soybean Meal – The “Gold Standard.” *The Farmer’s Pride, KPPA News* 11(20).
- Cromwell, G. L. 2012. Soybean Meal - An Exceptional Protein Source. Animal and Food Sciences Department - University of Kentucky. Available at: <http://www.soymeal.org/ReviewPapers/SBMExceptionalProteinSource.pdf>. Accessed June 25, 2013.
- Delmonte, P., J. Perry, and J. I. Rader. 2006. Determination of isoflavones in dietary supplements containing soy, Red Clover and kudzu: extraction followed by basic or acid hydrolysis. *Journal of Chromatography A*. 1107(1-2): 59-69.
- de Moura, J. M. L. N., D. Maurer, S. Jung, and L. A. Johnson. 2011. Pilot plant proof-of-concept for integrated, countercurrent, two-stage, enzyme-assisted aqueous extraction of soybeans. *Journal of the American Oil Chemists’ Society* 88(10): 1649-1658.
- Faris, R. J., H. Wang, and T. Wang. 2008. Improving digestibility of soy flour by reducing disulfide bonds with thioredoxin. *Journal of Agricultural and Food Chemistry* 56(16): 7146-7150.
- Friedman M., D. L. Brandon, A. H. Bates, and T. Hymowitz. 1991. Comparison of a commercial soybean cultivar and isolate lacking the Kunitz trypsin inhibitor: composition, nutritional value, and effects of heating. *Journal of Agricultural and Food Chemistry* 39(2): 327–335.
- Garcia-Olmedo, F., G. Salcedo, R. Sanchez-Monge, L. Gomez, J. Royo, and P. Carbonero. 1987. Plant proteinaceous inhibitors of proteinases and α -amylases in B Miflin (ed). *Oxford Surveys Plant Molecular and Cell Biology* 4. Oxford University Press-ISPMB New York, pp. 275-334.
- Goda, A.M.A.-S., M.E. Wafa, E.R. El-Haroun, and M.A.K. Chowdhury. 2007. Growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) and tilapia galilae *Sarotherodon galilaeus* (Linnaeus, 1758) fingerlings fed plant protein-based diets. *Aquaculture Research* 38 (8): 827-837.
- Hajen, W.E., D.A. Higgs, R.M. Beames, and B.S. Dosanjh. 1993. Digestibility of various feedstuffs by post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 2. Measurement of digestibility. *Aquaculture* 112 (4): 333-348.
- Hasseltine, C. W. 1972. Biotechnology report: Solid state fermentations. *Biotechnology and Bioengineering* 14(4): 517-532.
- Herkelman K. L., G. L. Cromwell, and T. S. Stahly. 1991. Effects of heating time and sodium metabisulfite on the nutritional value of full-fat soybeans for chicks. *Journal of Animal Science* 69(11): 4477–4486.

- Hong, K.J., C. H. Lee, and S. W. Kim. 2004. Aspergillus oryzae GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *Journal of Medicinal Food* 7(4): 430-435.
- Iwashita, Y., N. Suzuki, T. Yamamoto, J. Shibata, K. Isokawa, A. H. Soon, Y. Ikehata, H. Furuita, T. Sugita, and T. Goto. 2008. Supplemental effect of cholytaurine and soybean lecithin to a soybean meal-based fish meal-free diet on hepatic and intestinal morphology of rainbow trout *Oncorhynchus mykiss*. *Fisheries Science* 74(5): 1083-1095.
- Izumi, T., M. K. Piskula, S. Osawa, A. Obata, K. Tobe, M. Saito, S. Kataoka, Y. Kubota, and M. Kikuchi. 2000. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *Journal of Nutrition*. 130(7): 1695-1699.
- Jiao, J. A., B. C. Yee, K. Kobrehel, and B. B. Buchanan. 1992. Effect of thioredoxin-linked reduction on the activity and stability of the Kunitz and Bowman-Birk trypsin inhibitor proteins. *Journal of Agricultural and Food Chemistry* 40(12): 2333-2336.
- Kaushik, S.J., J.P. Cravedi, J.P. Lalles, J. Sumpter, B. Faunconneau, and M. Laroche. 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 133 (3-4): 257-274.
- Kiers, J.L., A. E. Van Laeken, F. M. Rombouts, and M. J. Nout. 2000. In vitro digestibility of bacillus fermented soya bean. *International Journal of Food Microbiology* 60(2-3): 163-169.
- Liener, I.E. 1994. Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science and Nutrition*. 34(1): 31-67.
- Lio, J. Y., and T. Wang. 2012. Solid-state fermentation of soybean and corn processing coproducts for potential feed improvement. *Journal of Agricultural and Food Chemistry*. 60(31): 7702-7709.
- Liu, K. 2000. Expanding soybean food utilization. *Food Technology*. 54(7): 46-58.
- Lonsane, B. K., N. P. Ghildyal, S. Butiatman, and S. V. Ramakrishma. 1985. Engineering aspects of solid state fermentation. *Enzyme and Microbial Technology* 7(6): 258-265.
- Lunger, A.N., E. McLean, and S.R. Craig. 2007. The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters of juvenile cobia (*Rachycentron canadum*). *Aquaculture* 264 (1-4): 342-352.
- Nguyen, T.N., D.A. Davis, and I.P. Saoud. 2009. Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia, *Oreochromis* spp. *Journal of the World Aquaculture Society* 40 (1): 113-121.
- Rosentrater, K. A. 2012. Influence of multiple amino acid supplementations in yellow perch fed plant protein combinations as fishmeal replacers. (Unpublished).
- Salze, G., E. McLean, P. Rush Battle, M.H. Schwarz, and S.R. Craig. 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. *Aquaculture* 298 (3-4):

294-299.

- Tacon, A. G. J., and M. Metian. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285(1-4): 146-158.
- Takagi, S., H. Murata, T. Goto, M. Endo, H. Yamashita, and M. Ukawa. 2008. Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. *Aquaculture* 280 (1-4): 198-205.
- Teng, D., M. Gao, Y. Yang, B. Liu, Z. Tian, and J. Wang. 2012. Bio-modification of soybean meal with *Bacillus subtilis* or *Aspergillus oryzae*. *Biocatalysis and Agricultural Biotechnology* 1(1): 32-38.
- von der Decken, A., and E. Lied. 1993. Metabolic effects on growth and muscles of soya-bean protein feeding in cod (*Gadus morhua*). *The British Journal of Nutrition* 69(3): 689-697.
- Wang, H., R. J. Faris, T. Wang, M. E. Spurlock, and N. Gabler. 2009. Increased in vitro and in vivo digestibility of soy proteins by chemical modification of disulfide bonds. *Journal of the American Oil Chemists' Society* 86(11): 1093-1099.
- Wilson, K. A. 1988. The proteolysis of trypsin inhibitors in legume seeds. *Critical Reviews in Biotechnology* 8: 197-216.
- Yang, S., L. Wang, Q. Yan, Z. Jiang, and L. Li. 2009. Hydrolysis of soybean isoflavone glycosides by a thermostable β -glucosidase from *Paecilomyces thermophila*. *Food Chemistry* 115(2009): 1247-1252.
- Yamamoto, T., Y. Iwashita, H. Matsunari, T. Sugita, H. Furuita, A. Akimoto, K. Okamoto, and N. Suzuki. 2010. Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout *Oncorhynchus mykiss*. *Aquaculture*. 309 (1-4): 173-180.

Table 1. Studies that have examined fermentation and enzyme treatments of soy.

Objective	Procedure	Results	Author
<p>1) To study the effect of bacterial fermentation on the solubilization and degradation of soya beans that may result in an increase in digestibility</p>	<p>5 strains of bacteria, bacillus species, were selected for the study.</p> <p>100 g of soy beans were soaked overnight. They were autoclaved at 121°C for 30 min, then cooled and inoculated.</p> <p>The beans were fermented at 37°C for 24 h and 48 h. The <i>bacillus subtilis</i> species were inoculated for the following time intervals: 6, 12, 18, 24, 36 and 48 h.</p> <p>10g of the samples were then homogenized using 90 mL of water. Then 1 mL was poured with molten nutrient agar, which was left to solidify. Then a layer of nutrient agar was added and incubated at 37°C for 24 h.</p> <p>The samples were freeze dried and ground. Then they were defatted by extraction with petroleum ether. Then the solubilization, absorbability, and digestibility were determined.</p>	<p>The quantity of soluble and dialyzable matter increased from 22% and 6% up to 65% and 40% respectively.</p> <p>The first 18 h resulted in a release of high levels of peptides and oligosaccharides.</p> <p>In vitro digestibility increased from 29% to 33-43% after 48 h.</p> <p>There was degradation of soy bean macromolecules resulting in water soluble, low molecular weight compounds.</p> <p>Showed the fermentation of soya beans using <i>Bacillus</i> resulted in a large number of biochemical changes, resulting in increased solubility and dialyzable material.</p>	<p>Kiers,2000</p>
<p>2) To analyze the addition of PSG (PepSoyGen) into juvenile rainbow trout</p>	<p><i>Oncorhynchus mykiss</i> was used in the 70 d feeding trial, involving an additional 40 d of experimentation.</p> <p>The temperature of the water was maintained at 11°C and pH at 7.6. The flow rate of water to the tank was maintained at 40 L /min.</p> <p>There were 6 diets formulated, in which the percentages of PSG (PSG) were kept at 0, 10, 20, 30, 40 and 50%. The PSG replaced the FM.</p>	<p>The tanks containing rainbow trout, receiving diets containing 0, 10, 20, and 30% PSG diets were seen to have no difference in the total tank ending weights or the weight gain.</p> <p>Weight gain was seen to be less in the fish receiving diets containing 40% PSG and lesser with the fish receiving 50% PSG diet.</p> <p>No significant difference in protein</p>	<p>Barnes, 2012 (unpublished)</p>

<p>3) To evaluate the effects of partial and total substitution of fish meal with PSG on fish performance and responses and economic potential of replacing fish meal with sustainable soybean feedstuff in yellow perch feed.</p>	<p>24 tanks were taken and the different diet formulations were assigned randomly. There were a total of 200 fish used for the study. Feed amount was recorded every day. The number of fish mortalities was also noted.</p> <p>Once the trial was over the tank weight was recorded and weight gain was calculated. The feed conversion ratio was also calculated.</p> <p>From each of the tanks, 5 fish were euthanized for the determination of the carcass composition.</p> <p>For the secondary trial, 4 diets were used containing 0, 10, 20, and 30% PSG. It was conducted over a period of 40 d. The fish health data was then obtained.</p> <p>5 feeds were made containing increasing amounts of PSG (0, 25, 50, 75, 100%) and decreasing amounts of FM.</p> <p>Fish were grown in a 340 L flow through tank, from which 14 fish were randomly selected.</p> <p>These fish were fed with a control diet for a period of 2 weeks at acclimation period. The fish were then fed the 5 different feeds that were formulated twice a day for a period of 105 d. The water temperature was maintained at 23°C throughout.</p> <p>The fish were then euthanized to determine the results of the trials.</p>	<p>content was seen in the fish receiving diets containing 0-40% PSG. The fish fed diets with 50% PSG had a lesser protein content, when compared to fish fed the 10% PSG diet.</p> <p>For the secondary trial, there were no significant differences in the feed conversion, mortality or gain.</p> <p>There were no mortalities through the course of the study; hence, the diet changes had no effect on the mortality.</p> <p>The diet with PSG met all EAA requirements except lysine.</p> <p>Diets that contained 75% or more PSG failed to produce a 100% weight gain.</p> <p>There was a reduction in mean weight gain as the PSG amounts increased.</p> <p>Fish fed with 100% PSG were seen to have consumed the least.</p> <p>Thus, this study indicates the potential for PSG as an alternative protein source, but unsupplemented diets with more than 25% PSG could not show a performance that was comparable to that of a FM diet.</p>	<p>Rosentrater, 2012 (unpublished)</p>
--	---	---	--

<p>4) To determine the effect of fermenting soybean meal with <i>Bacillus subtilis</i> and <i>Aspergillus oryzae</i>, on the nutrient content, and determine the in vitro digestibility, anti-oxide activity, and content of trypsin inhibitor present.</p>	<p>A medium consisting of soybean meal and wheat bran in the ratio 3:1 was used, and moisture content of 67% was autoclaved at a temperature of 121°C for a period of 20 min.</p> <p>This medium was inoculated at a 10% ratio for both of the strains. The inoculated medium was incubated for 72 h at 28°C and 37°C for <i>Bacillus subtilis</i> and <i>Aspergillus oryzae</i>, respectively.</p> <p>After the 72 h period, the incubated, fermented soybean meal was ground and passed through a 60 mesh.</p> <p>The crude fat and protein contents were determined by Soxhlet extraction and Kjeldahl methods, respectively.</p> <p>Proteins were extracted by adding a phosphate buffer solution to 0.25 g of the ground samples. These samples were centrifuged at a speed of 12,000 rpm at 4°C for 10 min. The soluble protein content was determined by taking samples of the supernatants using the Bradford method.</p> <p>SDS-PAGE analysis was done. The sample supernatants were first denatured using a buffer for 10 min, while the temperature was maintained at 100°C. The samples were then centrifuged at a speed of 12,000 rpm at room temperature for 10 min. A 15 µL aliquot sample of the supernatant was subjected to a 12% gel for SDS-PAGE; this was done at 80 V and 180 V. The protein distribution was represented as a percent of the total.</p> <p>The degree of hydrolysis was determined after a measurement</p>	<p>After the 72 h fermentation period with <i>Bacillus subtilis</i> and <i>Aspergillus oryzae</i>, relative protein crude content was seen to increase by 8.61% and 8.42%, respectively.</p> <p>After 72 h, content of soluble protein found in the fermented soybean, fermented by <i>B. subtilis</i> and <i>A. oryzae</i>, was 32.98% and 8.42%, which was an increase of 63.11% and 19.4%, respectively.</p> <p>Amino acid content increased. The increase in arginine, serine, threonine, aspartic acid, alanine and glycine in PSG with <i>B. subtilis</i> was 50.67%, 45.6%, 34.55%, 22.25%, 21.23%, and 18.12%, respectively.</p> <p>For the soybean meal fermented by <i>A. oryzae</i>, the content of arginine, serine, and threonine increased by 25.22%, 15.09%, 12.77%, and 11.29%, respectively.</p> <p>The samples showed a lot of variance in the anti-oxidative activity. The PSG with <i>B. subtilis</i> and <i>A. oryzae</i> was 32.2 and 26.8, respectively. The control was initially determined to be 8.2%. Hence, a large difference was observed.</p>	<p>Teng, 2011</p>
---	---	--	-------------------

<p>5) To study the thermal stability of β-glucosidase and the extent to which it could be used for deglycosylation of isoflavone glycosides in soy products in order to exploit the health benefits of isoflavones.</p>	<p>of the neutral proteinase activity was done. Anti-oxidative activity assay was also done in order to determine the anti-oxide activity.</p> <p>For the study, five types of soybeans were used: daidzin, genistin, daidzein, genistein, and glycitein.</p> <p>B-glucosidase from almonds was used. It was purified and the activity at 50°C and 37°C was determined. The concentrations of proteins present were determined using the Lowry method by using bovine serum albumen (BSA) as a standard. The thermo-stability was determined at different temperatures.</p> <p>High performance liquid chromatography (HPLC) was used to determine the composition of isoflavones.</p> <p>A coffee grinder was used to grind the beans into fine powders. With the help of a Soxhlet extractor, the ground beans were defatted and extracted using 20 mL of 80% methanol, by stirring overnight at room temperature after they were centrifuged at 11,000 g for 15 min. The supernatants were then filtered in order to quantify the isoflavones using HPLC.</p> <p>In order to separate the soy bean embryo from Zhongdou-27 soybeans, 50 g of the soybeans were soaked in water for 8 h, and then the embryos could be separated. These were dried, crushed, extracted with 80% methanol, and centrifuged at 11,000 g, after the supernatant was filtered.</p> <p>The soybean flour and embryo extracts were hydrolyzed using a 0.01 U/ml β-glucosidase. The β-</p>	<p>The thermal stability of the enzyme at 50°C was high and retained 95% of its initial activity, even after 8 h. At a temperature of 60°C, more than 70% of the initial activity was retained after 8 h.</p> <p>The range of isoflavone in the five varieties studied was between 190 to 446 mg/100 g. The isoflavone that was seen to be predominant was mainly glycosides, which were in a range of 101-309 mg/100 g.</p> <p>The isoflavone glycosides conversion in soybean flour by <i>P. thermophile</i> B glycosidase to their aglycones were 98%, 95.8% and 99.3% of hydrolysis of diadzin, glycitin and genistin in 4 h, respectively.</p> <p>For the soy flour extract and soybean embryo extract, hydrolyzed by <i>P. thermophile</i> β-glucosidase concentration of isoflavones after 4 h were seen to increase by 38.3 and 30.6 times the original concentration.</p> <p>Deglycosylation of isoflavone was higher in <i>P. thermophile</i> β-glucosidase than the commercial almond β-glucosidase.</p> <p>In control reactions without enzymes, almost no hydrolysis was seen.</p>	<p>Yang, 2009</p>
--	---	--	-------------------

	<p>glucosidase was in a 450 µL of 50 mM MES buffer with a pH 6.2. The samples were then incubated at either 50°C or 37°C, depending on whether it was for the thermophile β-glucosidase or for the almond β-glucosidase for 10 m and 4 h, respectively. Mixtures of acetic acid-acetonitrile were used to dilute the samples. The final determination of isoflavones was done by HPLC.</p>		
<p>6) To study the effect of fermentation of soybean meal and the fermentation conditions for the non-fish meal based diet of rainbow trout.</p>	<p>The feed consisted of defatted and heat treated SBM, which was fermented using bacteria (<i>bacillus</i> species).</p> <p>There were 2 different conditions used for the fermentation:</p> <p>1) PSG1- SBM was fermented for 7 h, containing a water percentage of 30% until the temperature reached 80°C.</p> <p>2) PSG2- SBM was fermented for a period of 10 h, having a water percentage of 45% until temperature reached 80°C.</p> <p>4 diets were prepared for the fish, i.e., FM, SBM, PSG1 and PSG2. For the non-fish meal diets corn gluten was used at a 21% level.</p> <p>Essential amino acids were supplemented for the non-fish meal diets.</p>	<p>Lower digestibility values of carbohydrates and lipids were observed in fish that consumed the SBM diet. The diet was improved by fermentation of the SBM.</p> <p>Food consumption rate for PSG2 was seen to be lesser than the FM diet.</p> <p>Protein digestibility was found to be lowest on fish who consumed the diets containing fish meal. Protein digestibility increased with fermentation of SBM (SBM<PSG1<PSG2). The fish fed with the PSG2 diet showed protein digestibility that was significantly higher than the SBM diet.</p> <p>Body weight of fish fed with SBM was lesser than when fish were fed with PSG.</p>	<p>Yamamoto, 2010</p>
<p>7) To study the effect of using PSG along with Methionine and additional amino acids in the diet of Juvenile Rainbow Trout</p>	<p>The temperature of the water in which the fish were grown was maintained at 11°C, and the pH was maintained at 7.6. The tanks were loaded with 40 fish, and the total tank weight was recorded. The fish feeding started, and it continued for a period of 36 d. The</p>	<p>The overall weight gain, percent weight gain, and feed conversion ratio were greater in the control diet than in the diets containing PSG, although feed conversion ratios were below one for all of the diets.</p>	<p>Barns, 2012 (unpublished)</p>

	<p>fish were fed once a day by hand.</p> <p>The 15 tanks were randomly assigned one of the 5 different diets formulated.</p> <p>There were 4 diets formulated, other than the control diet. Out of the 4 diets, 2 contained 10% FM and 40% PSG, and the other 2 contained 50% PSG.</p> <p>Methionine was added to all the diets, while lysine, isoleucine, and histidine were added to one of the 40% and one of the 50% PSG diets.</p> <p>At the end of the trial, total weight of the tanks were taken in order to find the weight gain.</p> <p>The feed conversion ratio for each tank was calculated.</p> <p>5 fish from each tank were randomly selected and weighed. The length was measured, health profile was completed, and the protein digestibility was noted.</p> <p>6 fish were euthanized to detect the carcass composition.</p>	<p>A single mortality was observed during the trial.</p> <p>The protein digestibility was least in the controlled diet and increased with the increase in the PSG concentration.</p> <p>The addition of Amino Acids had no significant effect.</p> <p>There was no major difference in the length, weight, and health parameters of the fish.</p>	
--	---	---	--

Table 2. Studies that have examined chemical and physical treatments of soy.

Objective	Procedure	Results	Author
<p>1) To use mild temperatures and sulfite and metabisulfite chemical treatments to increase the digestibility of soy proteins.</p> <p>To find a feasible method to treat the native protein fractions from aqueous-extraction processes (AEP), and overcome the technical problems in order to improve the quality to use it in animal feeds.</p>	<p>Soy white flour from hexane extraction was used for the study with a dispersibility index greater than 85. The sulfhydryl quantification of the soy flour was determined. The crude protein content was determined by the micro Kjeldahl method for both the soy white flour as well, as the sodium metabisulfite (SMBS)-treated soy flour.</p> <p>An in vitro digestibility of the samples was done using trypsin, in terms of protein hydrolysis.</p> <p>2 g of the soy white flour sample was added to 20 mL of 10 mM phosphate buffered 0.85% saline and was incubated at 55°C for an hour and 25°C for an hour, respectively. The reducing agents that were initially selected were SMBS and sodium sulfite. These were added at 2 different conditions, 0.2 mmol/2g of white flour and 1.0 mmol/2g of white flour. The samples were incubated at 55°C. An additional treatment without 55°C incubation was also done. The samples were then dialyzed against water for a time period of 3 d and lyophilized, after incubation and reaction.</p> <p>Effects of the temperature, time of heating, and reducing agent SMBS on in vitro digestibility of soy proteins by Trypsin were determined. In order to conduct these tests, 1g of soy white flour sample was dispersed in 20 mL of 0.1 M phosphate buffer at a pH of 7. The samples contained either 0 or 5% SMBS. These were either heated at 80°C or 100°C for 15 min or not heated. Each of the treatments mentioned were repeated 3 times.</p> <p>The in vivo feeding test was prepared. Two types of soy proteins were prepared. The first type, which was treated with 0.9 mol SMBS after 1.8 kg white soy flour was dispersed in 9 L of 10 mM phosphate buffered 0.85%</p>	<p>Samples, which had not been subjected to heat, had a lower sulfhydryl after the treatment with the reducing agents; this was pointed out to be attributed to the re-oxidation of sulfhydryl compounds.</p> <p>Digestibility tests, which were done in vitro with trypsin, showed a higher digestibility for all the treated samples. Even a mild heat of 55°C showed an increase, which was attributed to the inactivation of trypsin inhibitors.</p> <p>Increased sodium sulfite or SMBS showed higher digestibility values.</p> <p>SMBS was better in increasing the digestibility, when compared to sodium sulfite.</p> <p>From the data obtained, it suggests that the chemically reduced soy protein digestibility may not correspond directly to the sulfhydryl content that is detectable. This was due to new sulfide bonds formed by the generated sulfhydryl groups.</p> <p>With respect to heat treatment, at 100°C in vitro, digestibility was the highest.</p> <p>Heating time and addition of SMBS had no significant effect on the value of digestibility.</p> <p>The heat treatment done at 100°C was more than 3 times the value of</p>	<p>Wang et al, 2009</p>

<p>2) To discuss the aqueous extraction processes and highlight their advantages and disadvantages.</p>	<p>saline at a pH of 7.5, and a control, which was not treated with SMBS at the same conditions. The samples were incubated at a temperature of 55°C for 1 h. This is how the preparation of the in vivo feeding test was done, and then the feeding test was conducted on chicks. 16 pens were used for the test consisting of 4 pens for each treatment, and each pen contained 3 birds. The treatments consisted of a high protein control containing 23% crude protein, a low protein control in which 17.25% crude protein was present, a low protein soy control containing 17.25% in untreated soy flour, and a low protein SMBS treated soy flour containing 17.25% crude protein</p> <p>Aqueous extraction processing includes grinding the feed, extracting the oil out of the feed, centrifugation, followed by de-emulsification. The technologies used for the separation of oil in the cream dictates the cost of this process.</p> <p>Physical methods used on soybeans include mainly, flaking, grinding, and extruding.</p> <p>Both grinding and flaking result in the release of the intracellular material from the inside of the cells, but few remain intact far away from the surface of the particles.</p> <p>Flaking enables hexane to pass through a larger surface of the soybeans and thus improves the efficiency of hexane extraction.</p> <p>The yield of protein extraction is the amount of protein that is removed from the residual fraction.</p> <p>Most aqueous extraction processes on flaked and ground soybeans have been successful.</p> <p>Extraction from flaked soybeans, soy</p>	<p>digestibility at 80°C, which in turn suggested that a large amount of heat is needed to inactivate the trypsin inhibitors.</p> <p>In the feeding trial, chicks that were fed the SMBS diet had a higher gain. The feed ratio increase was 27% and PER increase was 57%, when compared to those that were fed the control (LPSC).</p> <p>Commercialization is still a big problem, although the performance of aqueous extraction processes and enzyme assisted aqueous extraction processes have improved considerably with very high yields.</p> <p>Although the cells are disrupted after extrusion is carried out, the absorption of oils by insoluble proteins make it difficult for the recovery of oils.</p> <p>The droplets of oil should have a size such that it is able to pass through the disrupted cell matrix.</p> <p>With respect to the cost-effectiveness of aqueous extraction processes and enzyme assisted aqueous extraction processes, utilization of the skim fraction is an important factor. An easy method to do this is by precipitation.</p>	<p>Campbell et al, 2011</p>
---	--	---	-----------------------------

	<p>flours, and extraction of intact oil bodies were studied. In addition to this, the demulsification of aqueous extraction processes cream of flour and flakes were studied.</p>		
--	---	--	--

Table 3. Studies that have examined use of soy products as substitutes for fish meal.

Objective	Procedure	Results	Author
<p>1) To review all the alternative sources for aquaculture, including plant protein sources: SBM, SPC, DDGS.</p>	<p>Fish meal was made from surface dwelling fish (anchovy, herring, mackerel, sardine, tuna, etc.) or from wastes obtained from sea food processing industries. It is light brown in color and is made by cooking, pressing, drying, and milling raw fish.</p> <p>Animal protein sources included aquatic food, such as meat and bone meal. Since there is a high amount of meat production in the US, most organic waste is seen from the food processing sector. This can be used as a protein supplement (prohibited to use in ruminant feeds in many countries because of the Bovine Spongiform Encephalopathy).</p> <p>Blood meal is made of blood from animal processing plants and is dried after blood is centrifuged, in order to eliminate any foreign material. Bone meal has also been avoided in aquaculture due to Bovine Spongiform Encephalopathy.</p> <p>Soy bean meal is obtained by removing oil from whole soy beans, toasting the flakes, and grinding it into meal. SBM is a commonly used plant protein because of its nutritional value, availability, consistent composition, and reasonable cost. The limitations are the presence of ANFs.</p> <p>Soy protein concentrate is one of the major soy proteins used in aquaculture feed. It is derived from dehulled solvent extracted with ethanol or other acids so that soluble carbohydrates and the various ANFs are removed.</p> <p>Soy protein isolate is the most pure form of soy protein and has the highest protein content of all soy products. It is made from further processing SPC to remove the insoluble fiber, using alkaline extraction.</p> <p>DDGS is the main coproduct of ethanol production from corn grain in the US. As a result of fermentation, high amounts of most nutrients are present compared to corn, including proteins, fats, minerals and fiber. One limitation is the low levels of essential amino acids.</p>	<p>Traditionally, fish meal has a high protein content.</p> <p>Plant proteins lack essential amino acids, unlike fish meal.</p> <p>Potential plant protein sources include SBM, SBC, SPI, and DDGS.</p> <p>DDGS seems more advantageous due to its lower pricing.</p> <p>Absence of ANFs is another advantage of DDGS over soy proteins.</p>	<p>Ayadi et. al., 2012</p>

<p>2) To use DDGS along with high quality SPC as a replacement to fish meal for fish feed.</p> <p>To examine the utilization of nutrients and the performance of yellow perch, which were fed these mixed plant-based alternative proteins with and without supplements of amino acids.</p>	<p>There were four experimental feeds that were initially made containing FM, DDGS, and SPC. The DDGS levels were made for testing with and without AA supplements. The compositions of the FM, DDGS, and SPC are given as: FM (70% crude protein and 9.7% lipid), SPC (73% crude protein and 0% lipid), and 20% or 40% DDGS (29% crude protein and 11.9% lipids).</p> <p>A 340 L flow through tank was used for the analysis. Yellow perch were taken in the tank, and they were made to be accustomed to a pelletized diet for a period of 60 d. A set of 25 fish were randomly selected for the study.</p> <p>The temperature of the water was kept at 23°C, and the pH was kept between 8-8.6 during the course of the trial.</p> <p>The fish were hand fed every day for 63 d, and the consumption was monitored. Total tank weight was also measured in 21 d.</p> <p>Upon completion, total tank weight was measured along with the length of the fish and their individual weights.</p> <p>The body, viscera, liver, and fillet weights were necropsied in order to examine the condition, muscle ratio, and muscle tissues. They were collected for the analysis of crude protein, crude fat, crude fiber, moisture, ash and amino acid profiles as well.</p>	<p>It was seen that the fish accepted the feed actively and readily.</p> <p>There were no mortalities through the trial of the experiment.</p> <p>Weight gain was highest in the fish that were supplemented with amino acids.</p> <p>Fish performance was better when DDGS was incorporated at 40%.</p> <p>Diet composition was seen to have no effect on muscle ratio.</p> <p>The analysis of the diets also showed that only diets with AA supplements completely met or exceeded the suggested AA required for yellow perch.</p>	<p>Rosentrater et. al., 2012 (Unpublished)</p>
<p>3) To study the effect that soybean meal protein has on the growth and muscle metabolism in fish.</p>	<p>Cod averaging 40 g was taken in a sheltered aquarium with sea water running at a rate of 4-5 L/min. The water was maintained at a temperature of 7 to 8°C and 3.5% salinity.</p> <p>The photoperiod was automatically regulated at 12 h of light and 12 h of dark.</p> <p>The fish were acclimated for a period of 4 weeks to the experimental conditions.</p>	<p>The replacement of 200 g/kg high quality fish meal by soya bean protein allowed a normal growth of the fish, but at a replacement of 300 g/kg, the growth rate declined.</p> <p>Food intake level in</p>	<p>von der Decken and Lied, 1993</p>

<p>4) To demonstrate to fish farmers and feed millers that SPC can be used as an all protein fish feed in place of FM that would allow better sustainability in the industry.</p>	<p>The study involved 8 groups in which each group consisted of fifteen fish, 2 groups of each of the 4 treatments (i.e. 0, 10, 20, and 30% soybean protein, and the rest consisting of fish meal).</p> <p>The trial lasted for a period of 43 days. The fish were fed twice daily and were fed until they were satiated.</p> <p>After the 43 d period, the fish were starved for 3 d and then euthanized, weighed, and examined.</p> <p>Black carp were taken in 6 ponds, 2.0 mu (0.13 ha) with a density of 600 fish per mu (9000/ha), along with 100 silver carp per mu (1500/ha).</p> <p>The fish were fed twice per day to satiation.</p> <p>The fish in three of the ponds were fed with fish feed containing 20% FM, while the black carp in the other three ponds were fed with feed in which the fish meal was replaced completely with SPC. This feed was also supplemented with methionine.</p> <p>All the feed was fed in an extruded, floating pellet form.</p> <p>This was conducted for a period of five months. The six trial ponds were then harvested.</p> <p>All the fish from each of the ponds were weighed to obtain data on the net production and feed conversion efficiencies.</p> <p>Sub samples of fish were obtained from each pond to determine the fish survival and the average weights for black and silver carp.</p>	<p>the group fed with 30% soya bean protein had a food intake level that was lower than the other groups.</p> <p>There was a reduced growth rate of fish associated with a high plant protein diet.</p> <p>With an increase in the soya bean protein, the deposition of glycogen in the muscle fell.</p> <p>The target market size of 750 g was exceeded for all the black carp that was grown.</p> <p>The black carp was noticed to grow larger and resulted in a higher harvest biomass when fed with the fish feed containing SPC, when compared to the FM.</p> <p>The silver carp grew from a weight of 280 g to 1833 g in the SPC ponds and from an initial weight of 270 g to a weight of 1650 g in the FM ponds.</p> <p>The average survival rates for the black carp was 65.2% and for the silver carp was 98% in the SPC tank, and 71.5% and 99%,</p>	<p>Cremer et. al., 2008</p>
---	---	--	-----------------------------

		<p>respectively in the FM tanks.</p> <p>This indicates that black carp do not have a dietary requirement for FM, and if the SPC is formulated well, it can be used to replace the FM.</p>	
--	--	---	--