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# SOYBEAN BY-PRODUCT: AS AN ALTERNATIVE TO FISH MEAL AS PROTEIN SOURCE FOR AQUACULTURE INDUSTRY

ZULHISYAM ABDUL KARI[1,2]*, KHAIRIYAH MAT[1,2], MUHAMMAD ANAMUL KABIR[2,3], W.A. AMIR ZAL[4], MOHAMMAD BODRUL MUNIR[5,6], LEE SEONG WEI[1,2] AND GUILLERMO TÉLLEZ-ISAÍAS[7]

*[1]Department of Agricultural Sciences, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia. [2]Advanced Livestock and Aquaculture Research Group, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia. [3]Department of Aquaculture, Sylhet Agricultural University, Sylhet-3100, Bangladesh. [4]Institute for Poverty Research and Management (InsPEK), Universiti Malaysia Kelantan, 16300 Bachok, Kelantan, Malaysia. [5]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia. [6]Faculty of Agriculture, Universiti Islam Sultan Sharif Ali, Bandar Seri Begawan, BE1310, Brunei Darussalam. [7]Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA.*

**Corresponding author: zulhisyam.a@umk.edu.my*
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**Abstract**: Proteins in commercial quality animal feed are one of its most important components and one of the costliest. Due to high costs and fluctuating production, some protein sources cannot satiate the increasing demand from fish feed manufacturers. Fish nutrition has been accorded particular focus with less costly feedstuff in aqua feed, given extra weightage. There has been increasing attention in recent years on finding methods to recycle the by-products of feed manufacturing. Due to its vital amino acid content and high protein composition, soybean by-product (SBP), a renewable resource, is expected to be a viable raw material option for replacing a fish meal or as a supplement. This review intends to cover the information regarding SBP associated with fermentation and probiotics used in the aquaculture industry potentially as an alternative to replacing FM.

Keywords: Soybean by-product (SBP), plant protein sources, Fish Meal (FM), protein replacement.

## Introduction

There has been tremendous growth in the aquaculture feed industry in the past few years. This growth has accompanied various in-depth research into the science and technology of fish species cultivation. The types and quantities of cultured and farmed species have increased steadily. Worldwide, aquaculture has grown at a median rate of 8.9% per year since 1970 (Huang & Nitin, 2019). The consistent supply and the cost of acquiring feed are the main issues in aquaculture. Feed also contributes the most to the cost of sustainable fish farming for all species. The feed cost is crucial because it normally makes up 30-70% of the whole operational costs and influences profitability in aquaculture investments (Muzinic *et al*., 2006; Daniel, 2018; Kari *et al*., 2022a; Maulu *et al*., 2022).

This makes the research into other protein sources vital as they could replace Fish Meal (FM) which is gradually decreasing production. Higher FM prices have affected the sustainable production of fish products. Overfishing is a current major concern globally, negatively affecting the production of FM, which is projected to be unable to meet demand worldwide by 2050 as aquaculture continues to expand (Sharawy *et al*., 2016; Jiang *et al*., 2018). The partial or full replacement of FM with other protein sources has been accelerated by the rise in FM cost, reduced availability, irregular supply, and poor quality (Ramachandran & Ray, 2007; Sharawy *et al*., 2016; Hazreen-Nita *et al*., 2022).

Plant proteins' wide availability and reasonable cost have made them the most viable choice as an alternative. As such, the identification and development of plant ingredients as an alternative to FM and its high cost has received sustained interest in the flourishing world aquaculture sector (Peres & Oliva-Teles, 2005; Cheng *et al*., 2013; Azarm & Lee, 2014; Anis Mohamad Sukri *et al*., 2022; Dawood, El Basuini, *et al*., 2022; Hamid *et al*., 2022; Nandi *et al*., 2023). This will eventually decrease the use of FM. Due to its stable supply, price and nutritional value, soybean by-product (SBP) is deemed to be a potential alternative protein source from the various plant-based feed being researched as an FM substitute (Rahman *et al*., 2014).

Soybean (*Glycine max*) is a leguminous oilseed and one of the world's largest and most efficient plant protein sources. There were several types of SBP used in aquaculture feed, namely Soybean Meal (SBM), Fermented Soybean Meal (FSBM), Fermented Soy Pulp (FSP), Soy Pulp (SP), Soybean Curd Residue (SCR) and Soybean Protein Concentrates (SPC). SBP potentially contains various biologically active compounds from soybean since it is a by-product of bean curd manufacturing. Soybean makes tofu, milk, oil, sauce, powder, dried tofu, and juice. The waste product is also called soybean curd residue, soybean curd lees, tofu cake, okara, or ampas tahu. The manufacturing of soybean products results in the primary residue, which is usually deemed as waste; normally it is thrown out or burned, or given to ruminants as feed as it has high nutritional value and superior functional characteristics (Rahman *et al*., 2014; Harthan & Cherney, 2017).

The long-term use of low-cost soy waste may interest farmers due to its nutritional composition. SBP is primarily made up of protein, lipids, fibre, and minerals. Some researchers have demonstrated SBP as having multiple functional characteristics of antioxidant capability, cardiovascular disease prevention, calorie intake reduction, and the maintenance of health and hepatic lipid profile (Jiménez-Escrig *et al*., 2008; Kim *et al*., 2016). SBP can also be dried and used in feed for terrestrial animals and fish as a protein source as it has a high protein content and is comparatively low in price (Yang, 2005). Several other research works have evaluated SBP to utilise it as a source of protein for ruminant farming (Rahman *et al*., 2014; Harthan & Cherney, 2017), swine husbandry (Pope *et al*., 2023), chicken farming, (Sinha *et al*., 2013) and fish (Jiang *et al*., 2018; Zulhisyam *et al*., 2020; Abdul Kari *et al*., 2021; Kari *et al*., 2022b; Zakaria *et al*., 2022).

Limitations related to plant protein used in fish feed are well known. Anti-nutritional factors (ANFs) present in the plant-based feed are the primary constraint, an example being phytate, which is phosphate (P) in its main storage form. Trypsin inhibitors and lectins are ANFs that interfere with the activity of digestive enzymes (Gemede & Ratta, 2014). As a solution, research into Solid State Fermentation (SSF) has focused on developing bio-processes for bio-remediation and biological detoxification of agro-industrial wastes to eliminate or reduce these wastes through fermentation. Reducing ANFs and improving the nutritional benefits of plant-based protein sources such as SBM may be achieved through the fermentation approach (Azarm & Lee, 2014; Shiu *et al*., 2015; Jiang *et al*., 2018; Zulhisyam *et al*., 2020). Several recent research has looked into FSBM and FSP usage in animal feed (Rahman *et al*., 2014) and aquaculture (Yamamoto *et al*., 2010; Azarm & Lee, 2014; Kari *et al*., 2022b) to replace FM as SBP is viewed as a novel protein source that possesses a lower amount of ANFs such as trypsin inhibitor while containing superior nutritional elements of high protein content, small sized peptide and concentrations of free amino acids.

The animal feed industry, livestock producers and the aquaculture sector are paying higher attention to utilising fermented products as animal feed components to improve yields. Recently, various modification methods have been used to improve plant-based protein sources or feed processing by-product quality. These methods are chemical, biological, and physical-based. The porosity, nutritional value, and low cost of SBP make it a suitable supporter and carrier (Shi *et al*., 2012). SBP quality has traditionally been improved through microorganism-based fermentation using *Lactobacillus spp*., *Bacillus spp*., *Aspergillus* *spp*., and yeast (Rashad *et al*., 2011; Shi *et al*., 2013). Some studies have also shown that fermentation can improve properties like the nutritional value, flavour, and storage time of SBP (Rashad *et al*., 2011; Vong & Liu, 2016; Zulhisyam *et al*., 2020). ANFs are reduced and animal protein usage can be improved by processing SBP through fermentation. For example, the use of FSBM with *Aspergillus oryzae* in improving the digestibility of protein and carbohydrates in diets has been researched for farmed yellowtail fish (*Seriola quinqueradiata*) (Shiu *et al*., 2015).

Refstie *et al*. (2005) utilised FSBM using *Lactobacillus brevis* in Atlantic salmon (*Salmo salar*) to enhance the digestibility of lipids. Barnes *et al*. (2012) employed the commercial FSBM (which used *Aspergillus spp.* and *Bacillus spp.*) to replace FM in the feed of juvenile rainbow trout (*Oncorhyncus mykiss*). SBP replaces FM as a source of protein and energy towards improved fish health and growth. SBP like FSP has additional benefits such as improving the organic compounds or substrates, delivering probiotics to the fish gut, improving health performance, and increasing the profits of an aquaculture business (Zulhisyam *et al*., 2020). SBP is usually fermented using a single fungus or bacteria. However, fermentation of SBP using a combination of organisms such as *Lactobacillus spp*., *Bacillus spp*., *Aspergillus spp*. and yeast has also been researched by a few studies. These combinations may produce unintended but promising results. At the moment, SBP usage still has some knowledge gaps that need to be researched (Li *et al*., 2012).

**The Usage of Soybean by-product (SBP) in Animal and Aquaculture Industry**

The application of SBP in animal feed is capturing more attention in the aquaculture industry because it has unique features compared with other agricultural by-products. Yang (2005) suggested that its high protein composition and low cost make SBP a suitable feed source for aquatic and land animals. For example, the production of tofu results in SP as a by-product which contains insoluble parts of soybean. It is one of the most popular ingredients for traditional food in East Asia (Nishibori *et al*., 2017). According to Monsoor (2005), 1.2 kg of SP is produced from 1 kg of soybean processed for food and the production is increasing worldwide. Statistically, the annual production of SP from the tofu manufacturing industry in Japan is around 800,000 tonnes (Ohno *et al*., 1993), 310,000 tonnes in Korea (Ahn *et al*., 2010), and about 2,800,000 tonnes in China. In European countries (EU), the same pattern of soybean consumption was reported by Mateos-Aparicio *et al*. (2010).

Spain, for example, imported 2.5 million tonnes of soybean in 2004 and the amount is increasing annually. Liu *et al*. (2007) stated that the consumption of SP by humans is still confusing and not convincing enough because of the high fibre content and because humans are not consuming it in mass quantities. Currently, SP is used as animal feed because it gives an advantage for growth performance, but this depends on the dosage that farmers use for feed manufacture (Wlcek & Zollitsch, 2004; Sinha *et al*., 2013). Various research has been carried out recently to find a use for this by-product for aquaculture freshwater production.

Generally, three methods are used in utilising SBP as an animal feed; raw soy waste, dry soy waste, and ensiled soy waste with other feeds. Many current studies have attempted to evaluate SBP as a protein supplement for ruminants (Rahman *et al*., 2014; Harthan & Cherney, 2017), pigs (Pope *et al*., 2023), and broilers (Sinha *et al*., 2013). In addition, SBP usage in animal feed usually depends on the drying process cost and Anti-nutritional Factors (ANFs) (Li *et al*., 2012). For example, SP's high protein and non-fibre carbohydrate content enables it to be easily digested by goats and dairy cattle (Yang, 2005). It is found that manufacturing feed from SP involves different processes and is much cheaper than using SBM, which makes it highly recommended as feed for cattle, pigs, goats, fish, and chicken as a supplement for existing feed.

An animal with high protein requirements imposes difficulty to feed producers in producing quality feed. Normally, an organic feed product has a higher price than a conventional product and the stock available is also limited. Several researchers have discovered that feed that has been mixed with SBP as a plant-based protein source produces no adverse effects on animal growth performance and health status (Merrifield *et al*., 2010; Sheikhzadeh *et al*., 2012; Kpundeh *et al*., 2015; Guo *et al*., 2016; Dawood, Habotta, *et al*., 2022; Habotta *et al*., 2022; Pope *et al*., 2023).

The majority of agricultural by-products are high in moisture by nature. Farmers usually store them for a certain duration to dry them naturally and save on drying costs. In Japan, most farmers use the mixing method to solve the problem by adding wet by-products to dry feed (Wang & Nishino, 2008; Kari *et al*., 2023). For instance, combining SBM with peanut hulls will result in a mixture that dries out properly and has sufficient fermentable carbohydrates for ideal silage fermentation. Silage of 78:22 SP to peanut hull ratio can also minimise the fibre content and improve silage fermentation efficiency after ensiling for eight weeks (Yang, 2005) and is recommended as an animal feed. In addition, the recent study by Rani *et al*. (2021) revealed that FSP alone could not be used as a supplement feed for animals.

## The Application of Fermentation in Soybean by-product (SBP)

Fermentation is a safe and environmentally friendly process for consumers, consumes less energy and has been proven effective in reducing waste. It is also one of the biodiversity activities and can be applied to many types of agricultural waste, making it a very efficient method to produce quality feed for fish. It has many advantages such as improved nutritive value, digestibility in body systems, and most importantly, it can eliminate or remove ANFs in plant-based ingredients (Kari *et al*., 2022b). Fermentation has been introduced to the feed industry to produce a new feed based on plant protein at a much lower price than FM. When the cost is reduced, it automatically increases the profitability of aquaculture ventures. In recent years, the application of fermented products has received much attention, especially from the feed manufacturers and animal producers focused on improving animal performance and health (Li *et al*., 2014; Li *et al*., 2019). The study showed that SBP's composition and quality could also be enhanced by using microbes like *Lactobacillus spp*., *Bacillus spp*., *Aspergillus spp*. and yeast to ferment it (Rashad *et al*., 2011; Shi *et al*., 2013; Zulhisyam *et al*., 2020).

The fermentation process can degrade the fibre content of SBP into low molecular weight carbohydrates to synthesise protein. The study by Imran *et al*. (2016) reported that fermentation using a mixed structure could increase the protein content by about two times compared with the original sample. The study used SBP and wheat bran with a ratio of 8:2 and A. niger, Trichoderma viride, Saccharomyces cerevisiae, and Candida utilis with ratios of 1:1:1:3 as the mixed culture. In addition, the fermentation technique by different microorganisms is crucial for converting isoflavones and product acceptance (Bao *et al*., 2012; Kuo *et al*., 2006).

Generally, to get the optimum result during the fermentation process, many factors need to be considered such as the variety of raw material, the type of probiotics used, surrounding conditions, the environment and the technique used during the process (Zulhisyam *et al*., 2020). Yamamoto *et al*. (2010) suggested that using *Bacillus spp*. to FSBM for 10 hours enhances the SBM's nutritional value for aquaculture feed. Shiu *et al*. (2015) subsequently showed that a soybean meal fermented for 72 hours has great potential for use as the protein source in the feed for groupers.

Other researchers that used the SBP as a fermented feed and their fermentation process duration are Azarm and Lee (2014) at 15 hours, Zulhisyam *et al*. (2020) at 3 weeks, Sharawy *et al*. (2016) and Feng *et al*. (2007) at 48 hours for both studies. The literature review indicates that the time range used for fermentation varies and depends on the abovementioned factors. The literature shows that the lowest time used in fermentation is 10 hours and the longest is around 3 weeks depending on the properties and feed preparation.

**The Application of Probiotics Associated with Soybean by-product (SBP) in Aquaculture**

The aquaculture industry is currently the world's most rapidly growing food production sector, though bacterial infections and other types of infections are still major obstacles to the industry's development (EL-Haroun *et al*., 2006; Pieters *et al*., 2008). The infection by microorganisms in aquaculture results is the major problem faced by the farmers and is a big challenge for the industry to sustain production. Because of this problem, vaccines and antibiotics have been used to prevent diseases for decades. The extended use of antibiotics and vaccines in aquaculture has raised complications for consumers in tissue accumulation of the substances and immune suppression (EL-Haroun *et al*., 2006).

Coastal areas have also been environmentally damaged by fish and shellfish farming (Gildberg *et al*., 1997). As a result, there is increased concern about the ill effects of using chemical compounds in aquaculture. Interestingly, this concern is related to aquaculture and other fields like human medicine and agriculture, which are looking for alternative methods to replace chemical compounds (Rollo *et al*., 2006). Antibiotics are also facing a crisis of confidence among the people because of these factors and they have been banned in the European Union (De Angelis *et al*., 2006).

According to Panigrahi *et al*. (2010), to avoid the usage of antibiotics and vaccines in the aquaculture industry, probiotics have been introduced as one of the alternatives to replace them. A recent study shows that SBP is a suitable vehicle for sending the probiotics to the fish gut and is a suitable substrate for bacteria propagation (Abdul Kari *et al*., 2021). Besides that, fish growth is promoted by lactic acid bacteria strains with FSBM as an aquaculture feed additive (Li *et al*., 2014). The addition of probiotics to starter diets appeared to improve SBM utilisation by first feeding rainbow trout, but probiotic use had only limited benefits when they were not continuously provided in the diet (Sealey *et al*., 2009).

The most famous and frequently used probiotics in aquaculture are the *Bacillus, Lactobacillus,* and *Bifidobacterium* genera. In addition, there are many species of *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* that have been reported in the aquaculture industry as probiotics namely *L. acidophilus*, *L. casei*, *L. fermentum*, *L. gasseri*, *L. plantarum*, *L. salivarius*, *L. rhamnosus*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, *L. helveticus*, *L. bugaricus*, *B. bifidum*, *B. breve*, *B. lactis*, *B. longum*, *S. boulardii*, *S. thermophilesand,* and *S. cremoris*. Several reports have indicated too that various aquatic probiotics improve growth performance and enhance the immune system against bacterial pathogens (Barth *et al*., 2009; Chauhan & Singh, 2019).

**Regulation and Safety Assessment of Soybean by-product (SBP) Used for Animal Nutrition Globally**

The consumption of SBP has been increasing for many years because of its health and growth performance benefits. In soy product manufacturing, the residue left is produced more than the products. For example, the soymilk industry has serious disposal problems due to the quantity of SP produced. Its high moisture content and short shelf life make it difficult to handle in large-scale production. Malaysia and various other countries have yet to build a comprehensive food waste management framework or Standard Operating Procedure (SOP), although some steps have been taken in the development phases (Thi *et al*., 2015).

As an alternative, Malaysia can refer to good SOP regarding the waste handling issue from established countries such as Japan, Taiwan, and South Korea. According to Kim *et al*. (2011), South Korea and Taiwan use around 81% of their food waste for animal feed. Compared with these countries, the application of food waste in animal feed is not recommended in developing countries because an improper SOP leads to adverse environmental effects. Hence, good food waste management requires the vital establishment of a good SOP to avoid problems, including environmental pollutants, disease spread and excess food waste. The Feed Act 2009, Laws of Malaysia, 2009 states that not all food waste is suitable for use as livestock animal feed except for the sources that have been commonly used previously.

Normally, a lot of common food waste is used in animal feed such as bone meal, eggshells, and seashells because of their mineral content like calcium and phosphorus (Wong *et al*., 2016). In fact, the use of food waste materials for fish feed has been practised previously by many farmers. The practice of feeding fish with food waste material may differ from other countries where the local farmers prefer to use unprocessed raw food waste as feed, especially for the freshwater species. According to Chiu *et al*. (2013), most farmers in China tend to use pellet feed rather than raw food waste in aquaculture.

Currently, the swilling method especially for pigs, is the most popular food waste management method in many countries. Menikpura *et al*. (2013) reported that the use of food waste by swilling has been practised by many Asian countries, including Korea, Japan, and Taiwan and until now, there have been no safety issues related to the use of food waste for feeding pigs. In addition, Taiwan started recycling food waste in 2,001 and about 2,000 tons of food waste are recycled daily to be used as pig swill. Animal feed is one of the methods through which hazards can enter the human body. As a precaution, feed safety is ensured before it is consumed by the animal and in the aquaculture industry. This is because humans consume animals, so it is crucial to consider safety assessments on both sides, i.e. human and animal, to avoid diseases and infections.

The safety of the farmers and workers should also be assessed thoroughly. A proper feed safety assessment should be established by providing a guideline or SOP as a source of instruction to avoid any problems. Superior safety assessments are generally conducted on a case-by-case basis because of the particular properties and components of a complete feed formula. A good guideline can reduce the risks to both animal and human safety.

**Inclusion of Soybean by-product (SBP) in Aquaculture Feed**

The target species and SBP preparation method influence the inclusion dosage when added as a feed supplement or replacement to the fish feed. The research focus highlights its application in promoting growth and enhancing performance. Many studies involving SBP with various inclusion levels were directed towards utilising SBP as a replacement for FM. The study by Kari *et al*. (2022b) showed that increasing the FSP level up to 75% and 100% in the feed can negatively affect the growth and survival rate of fish and the inclusion of 50% of FSP was recommended for *Clarias gariepinus* production. Sharawy *et al*. (2016) found that Indian prawn shrimp fed with 75% and 100% FSBM had a decreased growth performance due to its various components, particularly non-digestible oligosaccharides, lack of certain amino acids, ANFs compounds in the FSBM, and proteins that could not be digested. These results are supported by Tan *et al*. (2005) and He *et al*. (2020).

An increase in body lipids was seen along with the increased level of FSP, which was caused by the higher carbohydrate content compared with FM (Makkar *et al*., 2007; Sharawy *et al*., 2016). The study conducted by Rahimnejad *et al*. (2019) showed that FSBM can replace up to 40% of FM and still achieve optimum growth, but increasing the level to 80% resulted in inferior growth performance and led to production losses. Besides that, the study by Wangsoontorn *et al*. (2018) indicated that FSBM could replace up to 50% of FM in shrimp diets without any negative effects on shrimp health. So, from the previous research, it is very clear that increasing the level or a high quantity of SBP is not recommended for the growth and health performance of aquatic species and the best inclusion level of SBP, as summarised in Table 1.

Table 1: The inclusion level of SBP in different aquatic species

| Soybean by-product (SBP) | Inclusion Level | Duration | Species | Effects | References |
|---|---|---|---|---|---|
| Fermented soybean meal (FSBM) | 50% | 90 days | Indian white shrimps | Growth performance that was similar and cost-effective when compared with the control diet. | Sharawy *et al*. (2016) |
| Fermented soybean meal (FSBM) | 40% | 12 weeks | Largemouth bass (*Micropterus salmoides*) | Resulted in the highest growth performances. | Jiang *et al*. (2018) |
| Fermented soybean meal (FSBM) | 40% | 8 weeks | Black sea bream (*Acanthopagrus schlegeli*) | Increased survival and specific growth rate compared with the control group. | Azarm and Lee (2014) |
| Fermented soybean meal (FSBM) | 25% | 8 weeks | Asian river prawn (*Macrobrachium nipponense*) | Best growth performances. | Ding *et al*. (2015) |
| Fermented soy pulp (FSP) | 50% | 70 days | African catfish (*Clarias gariepinus*) | Improved growth performances, relative protein digestibility, amino acid profile and immune-related gene expression. | Kari *et al*. (2022b) |
| Fermented soy pulp (FSP) | 50% | 70 days | African catfish (*Clarias gariepinus*) | Improved growth performances and intestinal morphology of fish. | Abdul Kari *et al*. (2021) |
| Fermented soybean meal (FSBM) | 40% | 8 weeks | Japanese seabass (*Lateolabrax japonicus*) | Best growth performances feed utilisation, antioxidant capacity, innate immunity and gut health in juvenile Japanese seabass. | Rahimnejad *et al*. (2019) |
| Fermented soybean meal (FSBM) | 50% | 60 days | Pacific white shrimp (*Litopenaeus vannamei*) | Indicated that FSBM can replace up to 50% of FM in shrimp diets without any negative effects on shrimp health. | Wangsoontorn *et al*. (2018) |

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|---|---|---|---|---|---|
| Soybean meal (SBM) | Less than 75% | 8 weeks | Tilapia (*Oreochromis niloticus*) | The higher substitution levels of SBM induced negative influences on the growth, feed utilisation and serum lysozyme of tilapia. | Lin and Luo (2011) |
| Soybean meal (SBM) | 33% | 8 weeks | Juvenile whiteleg shrimp (*Litopenaeus vannamei*) | Higher weight gain and specific growth rate compared with other treatment. | Yun *et al*. (2017) |
| Soybean meal (SBM) | 15-45% | 109 days | White snook (*Centropomus viridis*) | Improved growth, digestibility, or digestive capacity. | Arriaga-Hernández *et al*. (2021) |
| Soy protein concentrate | Less than 30% | 6 weeks | Juvenile pearl gentian grouper | Average body weight gain (BWG), specific growth ratio (SGR), and weight gain ratio (WGR) had the highest values in fish fed with a diet of 15% soy protein concentrate. | Chen *et al*. (2019) |
| Soybean meal (SBM) | 40% | 8 weeks | Seabass (*Lates calcarifer*) | Improved the growth performances. | Yanti *et al*. (2019) |
| Soybean meal (SBM) | 50% | 12 weeks | Nile Tilapia (*Oreochromis niloticus*) | Improved the growth performances. | Ajani *et al*. (2016) |

### Probiotic Viability and Survival in Soybean by-product (SBP)

In developing probiotic feeds, selecting a suitable vehicle to deliver the probiotics to the Gastrointestinal Tract (GIT) under an optimum condition is crucial (Martinez *et al*., 2011). Researchers have employed multiple strategies to achieve a high probiotic survival rate in the GIT, such as through pre-administration with prebiotics and using a food matrix along with the probiotics (Su *et al*., 2007). The shelf life of SBP associated with probiotic viability is a vital parameter and farmers require the information because they need to manage the feedstock in farms especially in the aquaculture industry. Bedani *et al*. (2013) demonstrated that *L. acidophilus La-5* and *B. animalis Bb-12* in the 4 tested FSP formulations were still viable when stored for up to 28 days in a refrigerator. Microorganism survival in FSP products is around 8 to 9 cfu/g making SP very suitable for use in feed as it makes an excellent medium for bacterial growth. Bedani *et al*. (2013) also showed that probiotic survival in FSP is enhanced and FSP has a crucial function in protecting probiotics from gastrointestinal juices.

The work of Sanders and Marco (2010) suggested that diet components are among the factors that affect probiotic survival rates as they move down to the GIT. The selection of diet components is crucial because if the diet is not suitable, the microorganisms will not be exposed and function well in the gut. They will be prevented from using their toxicity on the probiotics present. A recent study by Zulhisyam *et al*. (2020) indicated that the lactic acid bacteria survival rate in FSP for up to 3 months is around 12 CFU $g^{-1}$ of feed storage in refrigerator conditions. The study used FSP as an edible coating material with commercial feed to observe the growth and health condition of *Clarias gariepinus* production.

**Effect of Growth and Immunity Improvement of Fish Fed with Fermented Soybean By-product**

Several methods have been developed using current technology to remove compounds such as tannins, phenols, and phytates from plant-based feed, i.e. soaking, cooking, and fermentation (Rahman & Osman, 2011). Fermentation has emerged in recent years to become a useful technique for producing health-promoting biological substances in feed. Fermentation has also been demonstrated as a superior method to remove ANFs such as tannins and phytates from plant protein-based feed, as discussed in the previous section. Emmambux and Taylor (2003) showed that tannin reduction results in the microbial degradation of compounds under fermentation, which improves the feed's nutritional value and fish immunity.

The previous studies showed that fermented feed used in experimental diets positively affects the growth and performance of tested animals, as discussed in the previous subsection. The only differences are the way the feed was prepared, the percentage of inclusion, and the types of plant or animal-based protein used as an alternative feed. Basically, the plant ingredients quality used in aqua-feeds is evaluated by indicators like growth performance, biochemical composition, and the effects on disease and histopathology (Poleksic *et al*., 2010; Rašković *et al*., 2011).

The study by Vielma *et al*. (2000) reported that in a feeding trial with trout from 0.25 kg to an average body weight of 2.02 kg, fish fed a diet containing 69% of the protein from soy ingredients (55.6% from SPC and 13.8% from SBM) grew significantly faster than fish fed FM based diet. Kissil *et al*. (2000) reported that 30% of dietary protein from FM replaced by an SPC did not significantly influence feed intake in seabream. However, 60% and 100% replacement reduced feed intake significantly. Regarding the use of soy protein in fish feed, different results were obtained in different studies, which may be related to the quality of soy product, nutritional balance in the diets, the amount of ANFs in the diets and the way of the feed preparation. Thus, a details study was needed to confirm the statement above.

**Effect of Fermented Soybean by-product (SBP) on Intestinal and Liver Morphology of Fish**

The aquaculture industry has prioritised the search for new protein sources that are both sustainable and possess superior properties in its quest to reduce FM use. Past research has reported that SBM generally cause morphological changes in the intestine and liver (Heikkinen *et al*., 2006; Lilleeng *et al*., 2007; Yamamoto *et al*., 2010; Shiu *et al*., 2015). Shiu *et al*. (2015) found these changes in the intestine and liver and digestive enzyme activity at low level in the fish gut, including pepsin in the stomach and trypsin, amylase, lipase, and chymotrypsin in the intestine. These changes occurred when SBM was used to replace FM in the fish diet and the parameters dramatically improved. The findings above indicate the potential of SBP as a protein source in feed manufacturing.

Lilleeng *et al*. (2007) in their work on Atlantic salmon, found that when the fish were fed non-fermented SBM, there were intestinal pathomorphological changes that affected the intestinal folds, caused cells to lose their supranuclear vacuolisation, changes in lamina propria and increased the amount of connective tissue. Refstie *et al*. (2005) found similar pathomorphological changes in the intestines of Atlantic salmon, but pathological changes in the intestine caused by SBM were not as significant in fish given FSBM and this is supported by other studies (Kiers *et al*., 2003; Shiu *et al*., 2015).

Biologically active compounds in the form of ANFs, like glycinin protein and β-conglycinin, could be among the main causes of abnormal intestinal morphology and the reduced capacity for digesting and absorbing animal nutrients (Feng *et al*., 2007).

Yamamoto *et al*. (2010) studied farmed rainbow trout with a diet with FSBM and found improvements in the histopathology of the fish intestines and livers compared with those given a non-fermented feed diet. According to Shiu *et al*. (2015), some pathomorphological changes are believed to have been caused by ANFs in the SBM, which cannot be removed completely by fermentation and depends on the fermentation method. As a solution, further studies are needed on optimising fermentation methods to remove the ANFs or reduce them to an appropriate amount for better fish growth and health performance.

**Effect of Fermented Soybean by-product (SBP) on Blood Biochemical Parameters in Fish**

Generally, one of the indicators of the physiological condition of fish is measured by changes in the blood indices. There are many studies on the effects of nutrition on animals which used haematological, biochemical, and physiological markers as indicators of health and growth performance (Dawood *et al*., 2016; Burgos-Aceves *et al*., 2019; Mat *et al*., 2022; El-Sharkawy *et al*., 2023; Suma *et al*., 2023). Haematological variations have been detected in fish-fed SBP as a major protein source. Uczay *et al*. (2019) suggested that adding protein from vegetables or plant meals in the feed damages the blood parameters. Yang *et al*. (2009) found that the haematological characteristics of hematocrit blood test and haemoglobin values were lowered significantly in silver perch fed with a diet with high FSBM content.

Lim *et al*. (2011) also found decreased hematocrit and haemoglobin levels in the blood parameters of *Takifugu rubripes* fed with a diet of 30% SBM. Anaemia from lowered red blood cell count and haematocrit was discovered in *S. hasta* juveniles with a diet of 34% SBM (Yaghoubi *et al*., 2016). Red sea bream (*Pagrus major*) and cobia (*Rachycentron canadum*) showed similar results when fed with SPC and defatted SBM, respectively, as the alternative dietary protein instead of FM (Takagi *et al*., 2001; Zhou *et al*., 2005).

Takagi *et al*. (2001) suggested that a decline in plasma protein level and a decrease in the plasma transferrin caused by an amino acid imbalance from a poor diet that used SPC as the primary protein source resulted in lowered iron transport and iron supply to reticulocytes. Yang (2005) in their research found that there were higher levels of hematocrit and haemoglobin in the group fed with FSBM and supplements when set against the group fed a diet of only FSBM, which had lower lysine and methionine content. These results demonstrated that hematocrit and haemoglobin levels were enhanced by supplementing the diet of silver perch with essential amino acids and a higher fermented feed level. They suggested that the level of FSBM had a significant effect on plasma Triacylglycerol (TAG) and total cholesterol (CHL) levels observed in the results.

Another study by Hassaan *et al*. (2015) stated that the physiological parameters of haemoglobin, haematocrit, white blood cell, and red blood cell levels are enhanced by substituting with FSBM. In contrast, the study by Abdul Kader *et al*. (2012) showed that hematocrit value was not significantly affected by substituting FM with 48% FSP; increasing the FM substitution level tended to raise the total serum protein level. So, from the information above, it is proven that fermented SBP can improve blood biochemical parameters, and the percentage of inclusion needs to be identified for better growth and health performances in fish.

**Effect of Fermented Soybean by-product (SBP) on Amino Acid Profile and Body Carcass Composition in Fish**

The action of enzymes produced by microorganisms increases feed nutrient levels through the biological method of fermentation

(Khan & Ghosh, 2013). Fermentation has also been reported to be viable as a technique to reduce undesirable components and enhance the nutritional quality of a by-product to get another product (Shi *et al*., 2015). Sharawy *et al*. (2016) found an increase in all essential amino acids, i.e., arginine, histidine, lysine, methionine, leucine, isoleucine, threonine, phenylalanine, and valine in FSBM compared with non-fermented SBM. These increases could be caused by the elevated production of protein cell mass which is essential to body carcass development. Hong *et al*. (2004) also found that fermentation with *A. oryzae* GB-107 can increase SBP's amino acid level.

One of the essential amino acids for fish is methionine, which can be found in SBM and increases with fermentation. A similar trend was found by Kumar and Gomes (2005) suggested that selecting a microorganism that can produce methionine in fermented SBP is necessary for the future of the aqua-feed industry. Body carcass composition is very important in feed nutrient studies and is also one indicator to test the efficiency of the proposed new feed. Li *et al*. (2014) indicated that FSBM could replace FM because it can improve growth and carcass development and has no negative effects on animals tested. Besides that, the same pattern was also observed by Sun *et al*. (2007) reported that fermentation of plant protein could improve animal growth and carcass development.

### Effect of Fermented Soybean by-product (SBP) on Nutrient Digestibility and Antioxidant Enzymatic Enhancement

Crude fibre components in fish feed cannot be digested easily by the fish. When the fibre cannot be digested, other nutrients' digestibility and absorption will also be affected. This will result in protein in the feed being sub-optimally used in body tissue building and negatively affect animal growth and health performance (Farizaldi & Jafrinur, 2017). Fermentation solves the issues by breaking down the hard-to-digest components into simple carbohydrates using enzymes secreted by the microbes (Farizaldi & Jafrinur, 2017). Nutrient digestibility and value are enhanced by SBP fermentation while providing vital elements like calcium and vitamins A and B to the feed (Abdul Kari *et al*., 2021). Hong *et al*. (2004) found an improvement in FSBM nutrient levels from fermentation with mould caused by a decrease in trypsin inhibitor level and a rise in small-sized peptide content. Some other studies have been carried out over the years into SBP application in the diet of young animals like chicken (Hirabayashi *et al*., 1998), piglets (Kiers *et al*., 2003), and broilers (Feng *et al*., 2007).

The other factor that can affect the digestive systems of fish and other animals is the presence of ANFs in most plant-sourced proteins. As discussed in the previous section, this problem can be resolved by applying a fermentation process to the feed before giving it to the animal. The ANFs reduce digestive enzyme activities and subsequently decrease feedstock digestibility; therefore, fermentation is required to break down the plant-based protein in the feed and indicated that a high level of plant-based protein (unfermented protein) results in decreasing protease, lipase, and amylase activities in the intestines of the Chinese sucker (Yong-Hong *et al*., 2005). Shiu *et al*. (2015) found similar results and suggested that trypsin, chymotrypsin, amylase, and lipase activities in the distal intestine deteriorated in fish given a diet of SBM. However, fermentation resulted in trypsin, chymotrypsin, amylase, and lipase, improving their digestive functions in the distal intestine.

According to Ding *et al*. (2015), antioxidant enzymes are important indicators of animals' physical health and reaction in response to external stimuli. As a fundamental, superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) is of primary importance in combating excessive superoxide radicals in fish and has been frequently applied in various fish species by several authors for assessing the impact of dietary protein source on antioxidant enzyme activities in fish (Zheng *et al*., 2012). The study by Lee *et al*. (2016) indicated that plasma antioxidant enzyme activities, in terms of SOD and GPx, were significantly elevated with incremental replacement of FM with FSM in diets for grower rockfish, whereas activities of hepatic antioxidant defence enzymes were not affected by dietary treatment in the current study. It has been suggested that enhanced levels of antioxidant enzymes may reflect a compensatory effect or preceding cellular oxidative stress (Wu *et al*., 2013).

Interestingly, it has been reported that microbial fermentation of SBM, with *A. oryzae*, could enhance the bioavailability of potential antioxidant compounds present in untreated SBM, including flavone and isoflavones, leading to improved antioxidant defence system in parrot fish, *Oplegnathus fasciatus*, tilapia, and flounder (Kim *et al*., 2010). These results agree with the findings of (Abdul Kader *et al*., 2012) reported that oxidative stress was significantly reduced in juvenile red seabream and flounder fed diets containing FSM compared to those fed the FM-based control diet.

**Effect of Fermented Soybean by-product (SBP) on Gene Expression and Activation at the Cellular Level of Aquatic Animals**

Developments in science and technology have opened the opportunity for researchers to explore deeper into genomic studies. Genomics is crucial in the aquaculture industry because it sets a new path for better understanding the link between nutrition, growth, and the immune system at the cellular level. Like fish and other aquatic organisms, lower vertebrates generally employ their immune systems for survival starting from the embryonic stage (Rombout *et al*., 2005). As such, Zhu *et al*. (2013) suggested that fish are likely the most appropriate subjects for studies into immunology and the evolution of immune systems. Transforming Growth Factor Beta 1 (TGF-β1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines (Ghadami *et al*., 2000; Vaughn *et al*., 2000). It has many functions in body systems, including cell growth, differentiation, apoptosis, and proliferation. It is also the strongest and main element in the immune system (Marie *et al*., 2006).

The study of gene expression in fish for TGF-β1 has been reported previously (Laing *et al*., 2000). The study led by Li *et al*. (2019) revealed that the expression level of TGF-β1 remained stable when fish were fed with FSBM compared with non-fermented ones. In their study, the SBM was fermented by *Shewanella sp*. MR-7 and turbot (*Scophthalmus maximus L.*) were used as the model animal.

Heat shock proteins (HSPs) is a stress protein with a molecular weight of around 16–100 kDa and is produced and exists in all organisms when exposed to stress conditions (Roberts *et al*., 2010). To date, the research on HSPs in aquatic species is getting interesting and gaining more attention among researchers, although the information available is rarely systematic (Iwama *et al*., 2004; Sagstad *et al*., 2007; Roberts *et al*., 2010). HSPs comprise several protein families environmental stressors produce (Zhang *et al*., 2011; Qian *et al*., 2012). In the aquaculture industry, the HSP gene is a very important component in developing fish health, food toxins, the relationship between host and environmental pollutants, and finally to bacterial and viral infections in the animal.

Nowadays, various methods are used to enhance the HSP levels in fish to reduce stresses to the animal, especially in husbandry care such as transportation, feeding management, and vaccination (Roberts *et al*., 2010). In fact, any changes that occur in animals related to stressors are called General Adaptation Syndrome (GAS). Normally, the changes occurring in fish involve 3 main phases: Alarm reaction, resistance, and exhaustion. GAS mechanisms will vary, and it is impossible to define them using a specific meaning due to those reactions mentioned above. HSPs are an important issue in feed technology.

Ding *et al*. (2015) showed that HSPs expression increased due to a high level of plant-based protein in feed which induced stress response. They also mentioned that the transcription levels of HSPs in the feed were upregulated at an FSBM replacement level of 100%. Their study used *M. nipponense* as the model animal. Contrastingly, Hansen *et al*. (2006) found unchanged expression levels of HSPs in Atlantic cod after they were given high levels of plant protein in their diet. Roberts *et al*. (2010) suggested that the disparity in results was caused by species-specific differences and/or suboptimal diet variances. Hence, a detailed study is needed regarding the inclusion of plant-based protein in the feed of fish towards growth performance and health related to the HSP gene.

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