

Effects of Different Soybean Meal Sources on Feed Utilization, Growth Performance, and Survival of Channel Catfish (*Ictalurus punctatus*)

by

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Abstract

The economic impact generated by the channel catfish (*Ictalurus punctatus*) industry has become relevant in the U.S. because of the profits produced in the southern states of Alabama, Arkansas, and Mississippi, becoming the most significant aquaculture segment in the nation. However, the hegemony of the catfish industry has not been exempted from setbacks throughout its history. One of the most common complications for the industry is the farmers' demand for low-cost feeds, which may result in the use of low-cost, low-quality ingredients in the diets of channel catfish (*Ictalurus punctatus*). This can negatively impact the production of this asset due to the delay in the days to market and prolonged exposure to pathogens in the environment. The aquaculture industry, including channel catfish production, has traditionally used diets with animal-based proteins. These products' have increasing costs and limited availability which have opened the opportunity for less expensive and more consistent plant-based proteins like soybean meals. Nevertheless, several studies show that the total or partial replacement of animal-based proteins with soybean could impact growth performance, protein intake, and feed utilization in channel catfish. Consequently, this study was initiated to determine if replacing solvent extracted soybean meal in a practical catfish feed could improve the performance. The basal diet (32% protein, 6% lipid) contained 56.4% SBM, which was then partially replaced by including 10% of a corn fermented protein from the ethanol industry (Basal-CFP) , or completely replaced with fermented soybean meal (43% diet) produced by Hamlet Inc (Findlay, OH, U.S.) or 45% of a low oligosaccharide soybean meal (Basal LO) produced by a genetically engineered technology and produced by Benson Hill (St Louis, MO, U.S.A.). Two 12-week growth trials were performed. One in a recirculating aquaculture system using a series of aquaria and juvenile catfish (mean initial weight 2.14 ± 0.03 g). This trial included the before mentioned diets as well

as a 32% protein commercial feed as a reference. Interestingly, enough final weights for fish reared on the test diets were all significantly greater than those reared on the commercial reference. Demonstrating that improvements in feeds can be made. Within the test diet fish were significantly larger and FCR were lower for fish maintained on the diets containing low oligosaccharide soybean meal and fermented corn protein. Under IPRS conditions in ponds, juvenile fish (32.56 ± 0.72 g) were reared on the test diets to a final size of 134.4g to 113.4 for fish offered the fermented soy-based diets to those offered the basal diet. Overall, results indicated that new varieties of soy can help improve the growth of channel catfish and work should be continued to evaluate results under production conditions.

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Hay hombres que luchan un día

Y son buenos

Hay otros que luchan un año

Y son mejores

Hay quienes luchan muchos años

Y son muy buenos

Pero hay los que luchan toda la vida

Esos son los imprescindibles

There are men who fight a day

And they are good

There are men who fight a year

And they are better

There are men who fight many years

And they are better still

But there are those who fight all their lives

These are the indispensable ones

Bertolt Brecht

Table of Contents

Abstract	2
Acknowledgments	4
List of Tables	7
List of Figures	8
List of Abbreviations	9
1. Introduction	10
2. Materials and Methods	18
2.1 Indoor Recirculating Aquaculture System (RAS)	18
2.2 Outdoor Inpond Raceway System (IPRS)	19
2.3 Water Quality	20
2.4 Diets Formulation	20
2.5 Feed Management	21
2.6 Harvest and Sampling	21
2.7 Proximate Analysis	22
2.8 Serum Biochemistry Analysis	22
2.9 Microbiome Analysis	23
2.10 Statistical Analysis	23
3. Results	23
4. Discussion	24
5. Summary	28
6. Conclusion	29
7. References	31

List of Tables

Table 1. (Composition (g/100g as is) of practical diets formulated to contain 32% protein and 6% lipids. Optimal Aquafeed Inc. commercially produced diets)	37
Table 2. (Proximate composition and amino acid profile (g/100 dry Weight) of the four experimental diets used during a 12-week trial to feed channel catfish (<i>Ictalurus punctatus</i>) fingerlings conducted in a RAS system and IPRS system)	38
Table 3. (Response of channel catfish (<i>Ictalurus punctatus</i>) fingerlings offered one of five practical diets over a 12-week growth trial conducted in RAS.)	39
Table 4 (Response of channel catfish (<i>Ictalurus punctatus</i>) fingerlings offered one of four practical diets over a 12-week growth trial conducted in an IPRS.).....	40
Table 5 (Water quality parameters recorded over a 12-week trial for channel catfish (<i>Ictalurus Punctatus</i>) fingerlings fed with experimental diets containing.)	41
Table 6 (Compiled results of statistical analysis for bloodwork values obtained using a portable chemistry analyzer VetScan 2 for channel catfish (<i>Ictalurus punctatus</i>) fingerlings offered one of four practical diets over a 12-week growth trial).....	42
Table 7 (Compiled results of statistical analysis for bloodwork values obtained using a portable chemistry analyzer VetScan 2 for channel catfish (<i>Ictalurus punctatus</i>) fingerlings offered one of four practical diets over a 12-week growth trial in a RAS system)	43

List of Figures

Figure 1 (Proximate Analysis for the experimental diets tested on the trial at the end of a 12-week period for (<i>Ictalurus punctatus</i>), fed with diets containing different soybean products replacing or comixing with solvent-extracted soybean meal and cultured in RAS and IPRS system)	44
Figure 2 (Final Weight obtained from experimental diets testes on a 12-week trial period for (<i>Ictalurus punctatus</i>), fed with diets containing different soybean products replacing or comixing with solvent-extracted soybean meal and cultured in RAS and IPRS system).....	45
Figure 3 (Feeding Conversion Ratio obtained from experimental diets tested on a 12-week trial period for (<i>Ictalurus punctatus</i>), fed with diets containing different soybean products replacing or comixing with solvent-extracted soybean meal and cultured in RAS and IPRS systems.)....	46

List of Abbreviations

ANOVA	Analysis of Variance
Basal	Basal Diet
Basal-CFP	Basal Diet – Corn Fermented Protein
Basal-F	Basal Diet – Fermented Soybean Meal
Basal-LO	Basal Diet – Low Oligosaccharide Soybean Meal
DNA	Deoxyribonucleic Acid
DHA	Docosahexaenoic Acid
EDA	Eicosatetraenoic Acid
FCR	Feed Conversion Ratio
G.M.	Genetic Modified
GMO	Genetic Modified Organism
IPRS	In-Pond Raceway System
ME	Metabolizable Energy
MS222	Tricaine Methasulfonate
NE	Net Energy
RCF	Relative Centrifugal Force
SBM	Soybean Meal
TAN	Total Ammonia Nitrogen
TCI	The Catfish Institute
RAS	Recirculating Aquaculture System
USDA	United States Department of Agriculture

1. Introduction

The catfish industry is the largest segment of aquaculture in the United States and a significant contributor to the local economies in the Southern states of Alabama, Arkansas, and Mississippi, where 95% of U.S. catfish production and processing operations occur. The magnitude of the catfish industry is significant because of the 150,000 MT produced annually (Engle et al., 2022), generating an output of \$1.10 billion in the tristate region, which increases to a total income of \$1.91 billion because of the direct and indirect creation of jobs. (Hegde et al., 2022). Catfish farmers have faced several challenges throughout history, converting it into a resilience example for the other aquaculture segments in the U.S. and worldwide. However, the hegemony of the catfish industry has been threatened by those same challenges that have impacted the industry's production, marketing, and technological aspects since its inception in the 50s. Thus, it is critical to understand the catfish industry's growth, contraction, and recovery, which have driven its dynamics in the different decades. The U.S. catfish production has its origins back in 1910 at the Kansas State fish hatchery when the term aquaculture was used concerning private farms, but the initial efforts by hatcheries supported with public funds established the basis for modern industry (Shell, E.W, 1993). During the 50s, the initial stages of the catfish industry were settled in multiple U.S. states, including Alabama, Arkansas, and Kansas, where private farms were involved in the production of fingerlings; the establishment of fingerling farms was a precursor to the development of the catfish industry (Nelson, B. 1956, Shell, E.W., 1993, Perez, K.R., 2006) by providing a constant supply of fingerlings for catfish production for human consumption. Years later, different economic conditions helped the catfish industry rise. The most remarkable one was the restriction of rice and cotton crops in Arkansas and Mississippi, forcing farmers to find alternative uses for their lands. In this way, farmers tried

first to raise buffalo fish (*Ictiobus spp.*) as a replacement for their previous crops, but due to the lack of knowledge, the production was not suitable. Then, catfish production gained more popularity among farmers due to the shortest production times and a higher yield obtained by feeding the catfish; this new feed consisted of bagged sinking feed produced by Kansas State University (Engle et al., 2022), promoting better growth in the catfish compared to buffalo fish. At that time, the growing catfish industry was designing and constructing specialized equipment, including seines, rollers, haulers, loading baskets, and paddlewheel aerators. In the 60s, catfish farming became a profitable industry, experiencing an expansion around different states of the U.S. as producers developed and installed new processing plants that increased the product's added value. However, this expansion in the following decade presented a few setbacks related to a non-stable supply for producers because the availability of catfish was subjected to the channel catfish growing season from May to November (Meyer et al., 1967). John Waldrop established a comprehensive enterprise budget for better management of the production of catfish at Mississippi State University in early 1978 (Waldrop & Smith, 1980), identifying the different steps involved in the production of catfish and their prices. Furthermore, implementing new advances in nutrition, such as new nutritionally complete balanced diets, increased fish growth (Robinson & Li, 2020) while catfish prices dropped in the 70s from oversupply from foreign places like Brazil and Mexico. Despite the economic difficulties experienced by producers, novel production methods were developed, continuous innovation helped increase catfish production year-round, and harvesting began to produce enough catfish to support a more extensive scale production. Between the 80s and 2000s, catfish emerged as a major farming enterprise, reaching the USDA's attention, where the USDA department began to collect data on catfish farming to improve the sector. Consequently, the catfish industry production developed a

size sufficient to support the feed manufacturing sector. With the addition of the nutritional segment in the catfish industry, the construction of new feed mills was necessary to fulfill their nutritional requirements. It is essential to mention that the development of the nutritional industry for catfish created the foundation for developing a more elaborate production system. After the creation and addition of new feed mills, the industry experienced rapid growth, which promoted the creation of new segments, such as the catfish breeding research programs developed by Auburn University, Mississippi State, USDA research program, and Goldkist, (Dunham et al., 1983,1990., Abass et al., 2022). Several strains were developed in these breeding programs, but some performed better than others. The strain that presented better growth and performance was the hybrid catfish (*Ictalurus punctatus* ♀ x *Ictalurus furcatus* ♂), developed at Auburn University and later adopted nationwide by local farmers. Also, the need to increase the yield production impulse is the addition of floating electric paddlewheel aerators (Boyd et al., 2018) that perform better than aerators powered by tractors. The growth in catfish sales and the expansion in nontraditional consumers increased catfish consumption because the white-fleshed fish were available all year with constant volumes. Also, the creation of The Catfish Institute (TCI) increased the industry's marketing and public relations. In the following decade several obstacles like the entry of new foreign competitors producing catfish at lower prices like Vietnam, a shrinking local economy, and the accelerated growth of other aquaculture sectors, produced a decline in the shares of the catfish industry from 46% in 1998 to 24% in 2018. Even so, the catfish farmers have demonstrated flexibility and capability in adapting to the difficulties by evolving to the demands of the industry and converting into one of the few aquaculture sectors that developed a comprehensive vertical integration system, which assembles four critical actors: catfish fingerlings farms, food size catfish farms, feed mills producing catfish feed, and

processing plants (Engle et al., 2022), and contributing about 75% of the finfish aquaculture volume with a 35% value share (FAO, 2020), and over 55% of total live weight fish in the U.S. (USDA, 2022). Certain inconveniences from the past remain present these days, and the most significant one is that nutritional requirements for channel catfish. Feeds are manufactured based solely on maximizing growth, but this could also impact the economic performance. The ingredients supplying protein in diets tend to have a higher price than other ingredients, which later will alter per unit feed costs (Hatch et al., 1998). All these factors have pushed the catfish industry and aquaculture sector to find new and reliable alternatives for animal-based proteins.

In the past years, global aquaculture has increased its production, expanding seafood production while there is a decline in wild stocks. The aquaculture industry has been profiled as a strong seafood supplier for global markets. However, the continued and expanding need for feed ingredients to support production is presented as one of the most significant challenges. Aquaculture production requires diets with a proper nutrient profile to increase fish production and maintain the good health of the animals. Thus, as in other terrestrial farming-rearing activities, the industry depends on providing nutrients. The increasing demand for feed in the aquaculture segment led to the production and development of novel feed ingredients. In the past, feed manufacturing sectors depend on fish meals as a protein source in their diet due to the high content of proteins and nutrients. However, fish meal is a limited resource depending on the marine fisheries to obtain the raw material for production. The high availability and lower production cost have prompted shifts to plant-based ingredients in different industries, including aquaculture. To complicate the situation, competitors like the poultry and swine industries still use fish meal as a critical ingredient in some diets.

The development and sustainability of the aquaculture sector will depend on identifying new suitable and cost effective plant-based ingredients that could replace animal-based proteins without affecting the performance of the animals (Gatlin et al., 2007). Consequently, many plant-based ingredients, including grains and oilseeds, have become popular among manufacturing producers; wheat, corn, soybean, rapeseed, and peas are among the options. So, plant-based protein has become popular with the most suitable substitutes for animal proteins in feed production; in this plant-based category, soybean has become the most used ingredient because of its properties like high protein content, amino acid composition, reliable production, and low-cost (Gatlin et al., 2007). Plant-based ingredients have not been exempted from problems. Several studies have demonstrated that the complete or partial replacement of animal-based meals could produce an imbalance in nutrients, negatively impacting their growth performance and health. (Hayward & Hafner, 1941). Compared to other plant-based sources, soybean meal has consistently been shown to contain a lower nutrient variability and lower concentrations of anti-nutritional factors, higher amino acid digestibility, and metabolized energy (N. Ruiz, C.M. Parsons, H. H. Stein, C.N. Coon, J.E. van Eys, and R.D. Miles, 2020). According to Hayward, three areas in soybean meal production are essential to understand, which include amino acid digestibility, anti-nutritional factors (ANF), and metabolizable (M.E.) and net energy (N.E.) to obtain a proper utilization of soybean meal when it is used for feeding animals (Hayward & Hafner, 1941).

Nevertheless, the reliance on elevated levels of soybean meal could generate adverse effects in fish production due to anti-nutritional factors and indigestible oligosaccharides in this plant-based protein, reducing channel catfish's growth and feed efficiency. (Lim et al., 1998). At the same time, a higher concentration of animal-based proteins in catfish feed could impact

commercial production. The 60% of total production costs in aquaculture comes from the feed used for producing fish. Hence, soybean meal is a valuable alternative protein source in many feed formulations. Its use and optimization have yet to be developed through the creation of new soybean products that will fulfill the nutritional requirements of multiple aquatic animals.

The use of plant-based ingredients could be limited because of the anti-nutritional components, imbalanced amino acid profile, and lower protein content, affecting all the feed production segments, including the catfish. Anti-nutrient factors are substances that, by themselves, experience changes in the metabolic processes of animals. These anti-nutritional factors are classified into heat-labile and heat-stable compounds. Depending on each group of the anti-nutritional factors, they can be removed with heat treatments or enzyme-inhibiting products, because of these methods, the digestion of carbohydrates, proteins, and lipids can be impacted affecting their food utilization and health in fish. Another problem with most plant-based proteins is that they have an essential amino acid and fatty acid limitation in their formulations. A clear example is soybean meal, which has a high lysine content but a low level of methionine and cysteine. This deficiency of amino acids can be addressed with a combination of other plant-based ingredients; the limitations for fatty acids in plant-based products present as the lack of HUFA (Highly Unsaturated Fatty Acids) like DHA (Docosahexaenoic acid) and EPA (Eicosatetraenoic acid), which are provided by the addition of fish oil. The opportunities for using plant-based ingredients are extensive, but all these products may require different processing techniques to improve the availability of essential amino acids and the other major nutrients in proteins from plants that will help in the growth of fish.

Critical factors in soybean meal production are soybean variety, growing conditions, and processing conditions that may impact the composition of the resultant soybean meal. For this

reason, new processing methods have been developed and applied over the last few years. For several years, solvent-extracted soybean meal, including catfish, has been a significant protein ingredient for omnivorous fish (Naylor et al., 2021), including catfish. The soybean meal production in the U.S. uses solvent extraction as one of the most common methods for separating oil from the remainder of the soybean. This processing method has an extraction efficiency of 99% and can handle large volumes of soybeans. (Grieshop et al., 2003). Significant technological advances in plant-based products have benefited and improved their use as a protein source in feed for aquatic animals without affecting their performance. Those new processing techniques include the addition of missing essential amino acids (Goda et al., 2007), the application of exogenous enzymes to break down proteins into peptides (Liti et al., 2006), the use of additives in the feed (Overland et al., 2000) and engineering seed crops to address the challenges of increasing the proportion of plant-based protein in fish feed (Herman & Schmidt, 2016).

The use of fermented plant-based proteins in the aquaculture industry has received greater attention than previous studies demonstrated that the fermentation process produced by microorganisms helps to reduce the presence of anti-nutritional factors, crude fiber, and toxic substances but also increase the crude protein contents in plant-based proteins (Imorou-Toko et al., 2008 & Jakobsen et al., 2015). Mugwanya explains in his research that the most recommended way to manage the challenges produced by antinutrients factors in plant-based proteins is using the fermentation process because this method potentially improves the feed intake and feed utilization in several aquaculture species. The different fermentation methods, fermentation requirements, and the microbial species used in the fermentation process are essential for understanding and obtaining better results (Mugwanya et al., 2023).

According to Yamamoto et al., 2024, the technological improvements used to refine the co-products from the distiller's industry and manufacture ingredients with superior nutritional quality and consistency are essential for the feed manufacturing industry in aquaculture. The advantage is that the U.S. is the world's largest fuel ethanol producer, providing approximately 55% of the total world production. The primary substrate of ethanol production is corn kernel starch (D. Kumar & Singh, 2019). After fermentation, the corn stillage is produced and can be predestined to feed livestock. This substrate can be processed through a mechanical separation (Williams, 2022) and converted into corn-fermented protein. Fiber and oil are fractionated during this process, helping concentrate the grains' residual proteins. The corn fermented protein (CFP) has a high protein concentration of 48% crude protein, 3-5% fat, and <8% crude fiber, as fed, comparable to the same values of solvent-extracted dehulled soybean meal with limited concentrations of lysine and arginine (Galkanda-Arachchige et al., 2021). The use of CFP has found a big market in the U.S., providing protein ingredients to the catfish industry.

The oligosaccharide content in soybean meal is part of the heat-stable components (Liener, 1981), which meal processing cannot eliminate (Grieshop et al., 2003). The presence of these oligosaccharides in soybean meal increases intestinal content viscosity. It reduces the digestive transit time in animals (Bedford, 1995), producing poor digestion and absorption of nutrients (Pangeni et al., 2017). Thus, genetic engineering and plant transformation have played a critical role in crop improvement by introducing helpful foreign genes or silencing the expression of endogenous genes in crops. These genetically modified crops have valuable traits during their production, including insect resistance, herbicide tolerance, abiotic stress tolerance, disease resistance, and nutritional improvements (K. Kumar et al., 2020). In this way, transgenic soybean has become one of the most widely produced genetically modified (G.M.) crops,

producing 91.9 million hectares around the globe, which represents 48.27% of the world's total genetically modified crops planted (Xiang et al., 2023), and playing an essential role as a potential substitute for other soybean products in the feed market for animals.

The objective of the present study is to evaluate the digestibility factors of Basal-F, Basal-LO, and Basal-CFP for channel catfish (*Ictalurus punctatus*) and evaluate the growth performance, feed utilization, survival rate, physiological responses, intestinal microbiota for catfish fingerlings offered diets containing different levels of Basal-F, Basal-LO, and Basal-CFP, which replaces the solvent-extracted soybean meal on a protein basis.

2 Materials and Methods

2.1 Indoor Recirculating Aquaculture System (RAS)

This study was conducted at the E.W. Shell Fisheries Center in The School of Fisheries, Aquaculture, and Aquatic Sciences at Auburn University. The indoor trial was performed in a RAS (Recirculatory Aquaculture System). The airflow was provided by an airpipe connected to a 1.5 hp Pentair Sweet Water Regenerative Blower (Golden Valley, MN, U.S.). The temperature in the system was maintained at 25°C using a CIHC QDPTY 6-2 immersion heater (Homestead, FL, USA) placed into a recirculatory 800L sump. A low salinity of 2.00 ppt was maintained constantly by adding sodium chloride salt from Champion's Choice (Hurst, TX, USA) into the sump. The indoor trial consists of twenty aquaria with a capacity of 87.5L, each housing twenty channel catfish (*Ictalurus punctatus*) fingerlings from the Delta Pure strain. The catfish fingerlings in the aquaria were hand fed twice daily, and the amount of feed was increased according to the biweekly sampling for growth performance and survival. Throughout the trial, the quantity and pellet size of the feed were adjusted to ensure proper feed intake. The water quality parameters in the indoor system remained constant and did not present problems during

the trial, including dissolved oxygen, temperature, salinity, TAN, and nitrite. The fish were reared during a 12-week period using four experimental diets and a commercial reference; for better results, randomization was implemented by assigning each treatment to a pre-determined number of aquariums and raceways, reducing potential bias. Also, the diets were renamed using alphanumerical and color codes for better distribution and management. After completing the trials, several experimental procedures were performed on the fish, including group weighing and tissue sampling, which were performed under tricaine metal-donate anesthesia. Three fish were pooled from each aquarium for tissue collection and sampling, and the remaining fish from the aquarium and raceways were stocked back.

2.2 Outdoor In-pond Raceway System (IPRS)

The outdoor trial was conducted along with the indoor trial at the E.W. Shell Fisheries Center in The School of Fisheries, Aquaculture, and Aquatic Sciences at Auburn University. It consisted of channel catfish (*Ictalurus punctatus*) juveniles stocked (32.56 ± 0.72 g) in each raceway, set up in a 0.4648 ha pond. The 12-in pond raceways used for this trial were costume-made (Auburn, AL, U.S.) with the dimensions of 4.9 m x 1.3 m x 1.2 m and a capacity of 5300 L; the air provided to the raceways was generated by three S-45 Sweet Water Regenerative Blowers 1.5 HP (Apopka, FL, U.S.) with singular a port manifold and a grid diffuser by Swan Hoses (Sandy Springs, GA, U.S.) which provided a constant and even air supply to each raceway. The water quality parameters in the system remained steady and did not present problems during the duration of the trials, including dissolved oxygen, temperature, salinity, total-ammonia-nitrogen (TAN), and nitrite. The fish in the IPRS was fed during a 12-week period using four experimental diets and a commercial reference; for better results, randomization was implemented by assigning each treatment to a pre-determined number of

aquariums and raceways, reducing potential bias. Also, the diets were renamed using alphanumerical and color codes for better distribution and management.

2.3 Water Quality

For both trials, D.O., temperature, and salinity were evaluated daily using a YSI PRO 2030 probe (Yellow Springs, OH, USA); other parameters like TAN and nitrite were recorded twice per week by using a YSI 9500 Photometer (Yellow Springs, OH, USA) and pH was measured twice per week using as well with a Hanna pHep H198107 meter (Smithfield, RI, USA).

2.4 Diets Formulation

Four practical diets were used for both trials over a 12-week growth experiment conducted in an RAS and IPRS. The basal diet utilize solvent-extracted soybean meal at 56.4% as the primary protein source. The solvent-extracted soybean meal (Basal) was produced by Bunge Inc (St. Louis, MO, USA). The other three diets consisted of soybean products that have replaced SBM on an isoprotititic basis with fermented soybean, genetically modified soybean or a partial replacement using corn-fermented protein. The different experimental treatments were tested to evaluate their impact on enhancing channel catfish fingerlings' nutritional value and performance. The (Basal-F) treatment consisted of the complete replacement of SBM by the addition of fermented soybean meal (43 g/100g diet) produced by Hamlet Inc (Findlay, OH, U.S.). The (Basal-LO) treatment consisted of SBM replaced by the inclusion of 45 g/100g diet of low oligosaccharide soybean meal produced by a genetically engineered technology and produced by Benson Hill (St Louis, MO, U.S.). The (Basal-CFP) treatment consisted of a basal diet modified to contain 44.5 g SBM/100g diet in combination with 10g/100g diet of fermented corn protein, a byproduct of ethanol production (Table 1). A commercial diet with 32% protein

produced by Alabama Catfish Feed Mill (Uniontown, AL, USA) was included as a reference. Once the diets were received, they were stored until needed.

2.5 Feed Management

During the 12-week trial, the fish in each aquarium at the RAS system were fed twice daily by hand, and the fish in each raceway at the IPRS system were fed ad libitum once daily to optimize feed consumption. The time gap for feeding the fish was arranged from 8:00 a.m. to 5:00 p.m. Depending on the protocol for each trial, the fish were group-weighted and enumerated to obtain weight gain, feed utilization, and feed conversion ratio (FCR) every two weeks or once per month. This information and visual feeding observations were used to adjust feed inputs.

2.6 Harvest and Sampling

After both trials, the fish from each system were harvested, grouped, weighed, and sampled to obtain their final weight and total number. The collected data was used to measure growth parameters using the following formulas:

$$\mathbf{Final\ Mean\ Weight} = \frac{\mathbf{Total\ Biomass\ (g)}}{\mathbf{Total\ Number\ of\ Fish\ (g)}}$$

$$\mathbf{Percent\ Weight\ Gain} = \frac{\mathbf{Final\ Weight\ (g) - Initial\ Weight(g)}}{\mathbf{Initial\ Weight(g)}} \times 100$$

$$\mathbf{Survival\ Rate} = \frac{\mathbf{1 - Total\ Number\ of\ Mortalities}}{\mathbf{Initial\ Number\ of\ Fish}} \times 100$$

For the indoor trial, the collected samples included blood, feces, gut, and liver obtained from three pooled fish per aquarium. The fish were anesthetized using tricaine methanesulfonate (MS-222) to obtain a blood sample, which was extracted from the caudal vein using a 0.5 mL syringe. After bleeding out the fish, they were sacrificed to collect fecal matter, gut, and liver samples. The collected tissues were stored under appropriate conditions. Fecal matter was transferred into a 2 mL threaded cryogenic storage vial by Fisherbrand (Waltham, MA, U.S.) and

freeze-dried using liquid nitrogen. After the procedure, the feces samples were stored at -80°C . Liver and gut samples were transferred into a 5 mL tube by Eppendorf (Enfield, CT, U.S.) with an ethanol solution and stored at 10°C for further analyses.

2.7 Proximate Analysis

Collected fish from each aquarium and raceway were pooled by the treatment they received and processed into a homogenous paste using a Globe Planetary Mixer SP20 20 qt (Dayton, OH, U.S.). After completing the previous step, the homogenized paste was transferred into individual bags and stored at -20°C . The samples were weighed out and re-bagged for shipping to Midwest Laboratories (Omaha, NE, U.S.), where proximate analysis was performed.

2.8 Serum Biochemistry Analysis

The blood samples were transferred into a 5mL Eppendorf PCR tube (Enfield, CT, U.S.) without an anticoagulant agent and let sit overnight at 10°C . The following day, samples were centrifuged using a Marathon 16KM by Thermo Fisher Scientific centrifuge (Waltham, MA, U.S.) at 10,000 RCF (Relative Centrifugal Force) for 10 minutes to extract serum from them. The serum samples from the three fish from each aquarium were pooled to analyze the biochemistry of the serum. These factors included Albumin, Alkaline Phosphatase, Alanine Aminotransferase, Amylase, Total Bilirubin, Blood Urea Nitrogen, Calcium, Phosphorous, Creatinine, Glucose, Sodium, Potassium, Total Protein, and Globulin. The biochemistry analysis was performed using a Comprehensive Diagnostic Profile Disc by Abaxis. The analysis was conducted in the VetScan Vs2 and from Abaxis (Union City, CA, U.S.).

2.9 Microbiome Analysis

Feces samples collected during the trial were stored at -80°C . However, the analysis was not completed because, during the shipping process to the laboratory for microbiome analysis,

the samples experienced improper handling and arrived at the lab in inappropriate conditions to perform the requested analysis.

2.10 Statical Analysis

This study employed statistical analyses using SAS software (Cary, NC, U.S.), including an ANOVA to assess differences produced for each treatment in fish growth and feed utilization and Tukey's analyses for post hoc comparisons.

3. Results

Both trials, the indoor trial and outdoor trial, were conducted with no inconvenience related to disease outbreaks and water quality parameters. During the indoor trial, one replicate corresponding to the Basal-LO treatment experienced a higher mortality due to a salinity spike in the system, impacting the total number of fish for that treatment and getting removed from the final statistical analysis. The water quality parameters were maintained in the suitable ranges for the channel catfish production (Table 5). For the indoor trial, the results indicate the presence of significant differences ($P < 0.05$) in final weight, feed conversion ratio (FCR), weight gain (%), and weight gain (g) of channel catfish (*Ictalurus punctatus*) fed with diets containing Basal-CFP and Basal-LO as a substitute of solvent extracted soybean meal (Basal). Mean final weights arranged between 47.91 g to 64.94 g, with the B-LO treatment having the most significant final weight and the commercial diet having the lowest one, FCR values range between 1.04 and 1.25, weight gain (g) values arranged between 45.8 g to 62.8 g, and weight gain (%) arranges between 2199.7% to 2998.91% (Table 3) In contrast, the Biomass and Survival Rate did not present significant differences ($P > 0.05$).

In the outdoor trial, the water quality had the recommended parameters for channel catfish production (Table 5). One replicate from the raceways corresponding to the Basal diet

was excluded from the final statistical analysis because of irregularities in the number and size of the stocked fish. Foam formation was observed during specific periods of time in the raceways due to the accumulation of organic matter at the bottom of the pond. For the outdoor trial, the results do not indicate the presence of significant differences ($P > 0.05$) in final weight, feeding conversion ratio, weight gain (%), and weight gain (g) of channel catfish (*Ictalurus punctatus*) fed with the experimental diets as a substitute of solvent extracted soybean meal (Basal). Mean final weights arranged between 113.4g to 130.4g, with the Basal-F treatment having the most significant final Weight and the commercial diet having the lowest one, FCR values range between 2.2 and 1.6, weight gain (g) values arranged between 81.83 g to 98.54 g, and weight gain (%) arranges between 266% to 309%. Biomass values range between 48.93g and 63.06g, and Survival Rate values range between 70.5% and 81.3%. The bloodwork test performed on both systems included the evaluation of the following parameters Albumin, Alkaline Phosphatase, Alanine Aminotransferase, Amylase, Blood Urea Nitrogen, Calcium, Creatinine, Phosphorous, Glucose, Sodium, Potassium, Total Protein, Globulin, and Total Bilirubin. The different treatments did not present considerable variabilities between all the experimental diets evaluated in the trials. Additionally, the HSI and IPF evaluated after the sampling collection, did not present considerable variabilities either.

4. Discussion

Over the past decades, soybeans have become a widely used ingredient in the feed manufacturing industry. The nutritional benefits of plant-based ingredients used as a reliable replacement for animal-based proteins have created many opportunities for the different seeds and grains available now. Soybean has received considerable attention among plant-based products because of the nutritional quality factors present in this grain. Nevertheless, soybeans

have not been exempted from antinutrient factors and complications from amino acid imbalance. For this reason, several studies have been conducted to demonstrate that the inclusion of new strains of soybeans as well as processing methods for soybean meal can help improve diet formulations when used as ingredients in the feed for fish (Hayward & Hafner, 1941). The Basal-CFP and Basal-LO experimental diets produced the best growth, FCR, and survival in the present study. Corn-fermented protein is a promising protein source obtained from bioethanol production, and it has been previously evaluated in channel catfish (Yamamoto et al., 2024) and Atlantic salmon (*Salmo salar*) (Williams, 2022). Also, the use of low oligosaccharide soybean meal in diets compared with conventional solvent-extracted soybean meal was shown to have a greater concentration of digestible amino acids (Pangeni et al., 2017). The number of studies presenting the benefits of new sources of soybean meal demonstrated that corn-fermented protein and low oligosaccharide (Baker et al., 2011) could be utilized without producing adverse effects on the growth performance of *I. punctatus*. On the other hand, the result from the same study validates that *I. punctatus*, fed with a Basal-F diet, resulted in no significant differences with the growth, FCR, and survival in a RAS system. Additionally, the result from this study shows differences within both systems used to rear the *I. punctatus* fingerlings.

Fish reared in the IPRS system had a better performance in terms of final weight, FCR, and survival as compared to fish fed using the Basal-F and the Basal-CFP diets, in comparison with the RAS system where the Basal-F diet has a less significant impact to the animals. Considering the experiment's methodology during the data analyses on SAS software, for the IPRS system, one of the replicates for the Basal-LO was eliminated from the study due to a size inconsistency of the animals, likely due to fish being left in the system. This generated an unequal number of replicates for the calculations. It is also important to mention that the feeding

techniques were different between trials. The fish received feed twice daily for the RAS system, in contrast with the once-per-day ad libitum feeding used for the IPRS system, providing a difference in the feed intake. An important observation is that the commercial feed used as commercial reference in the RAS system presented the poorest performance of all the diets. For a better understanding, the numbers in Table 1. show that the Basal-F, Basal-CFP, and Basal-LO diets have superiority in terms of quality nutrients, helping in the growth of the fish.

The low performance produced by the commercial reference could be linked to the presence of antinutrient factors and or low digestibility, which impact the metabolism of the channel catfish, as Lim et al., 1998 mentioned. The lack of a processing method for dealing with those substances generated a problem for the animal, making it more challenging to obtain the full benefits of a diet. Another experiment conducted by Grieshop et al., 2003 demonstrated how solvent-extracted soybean meal is used because of the availability and low prices. Still, the same solvent-extracted soybean can negatively impact the production performance of channel catfish, as we can observe in our trial. Studies have reported that fermented soybean meal can be utilized as an alternative protein source in Nile Tilapia (El-Dakar et al., 2023), but the growth parameters did not improve as expected. Zhang et al., 2021 reported that pearl gentian grouper juveniles had an unfavorable growth compared to other soybean products. These experiments have confirmed that the growth performance in fish fed with fermented soybeans is not optimal and likely species specific.

Blood parameters are a useful measure of physiological disturbances in intensively farmed fishes and can deliver essential information for diagnosis and prognosis of diseases (Dal'Bó et al., 2015; Tavares-Dias & Moraes, 2007a). All the blood parameters evaluated in these trials include albumin, alkaline phosphatase, alanine aminotransferase, amylase, blood urea

nitrogen, calcium, phosphorous, glucose, sodium, potassium, total protein, and globulin had comparable results among the experimental treatments ($P > 0.05$) except for creatinine and total bilirubin, ($P < 0.05$). The RAS system presented a small variability for the biochemical factor of creatinine ($p < 0.0482$), and both systems, RAS ($p < 0.0037$) and IPRS (0.0086), exposed another small variability on the VetScan test performed, but this time with total bilirubin. The study conducted on Dojo loach (*Misgurnus anguillicaudatus*) demonstrated that exogenous factors, such as management, diseases, and stress can induce major changes in blood composition; including fluctuations in the concentrations of cortisol, glucose, cholesterol, and other components as response to handling and hypoxic stress (Zhou et al., 2009). Agreeing with Lermen et al., 2004 a wide range of environmental stressors like captivity, hypoxic environment, starvation, and transport can generate stress and affect the hormone and glucose levels. It is very likely that during the blood collecting step involving transporting the fish, transferring from one tank to another and applying the anesthesia generated stress in the channel catfish fingerlings. A higher value of bilirubin can be interpreted as a consequence of an increasing level of hemoglobin, bilirubin is a breakdown product of the hemoglobin and can be expected to increase proportionally with higher levels of hemoglobin (de Souza & Bonilla-Rodriguez, 2007; McKenzie et al., 2002). Hemoglobin is critical in fish adaptation as they constitute an interface between the organism of the fish and the environment (Landini et al., 2002), acting as response to any possible stress condition to the fish. Therefore, external conditions impacting the physical condition in the channel catfish fingerlings could lead to an increase in the concentrations of hemoglobin, which could be interpreted as an increase for total bilirubin as well. For the subtle increase of creatinine levels, we need to understand that creatinine is the final product of energy in the muscle tissues, which comes from creatine, and the concentration of this compound in

blood changes depending on the level of muscle activity. Generally, fish eliminates creatinine through the kidneys, so using creatinine content levels can be helpful to measure the filtration efficiency of the fish (Pastorino et al., 2022). Presumably, the content level of creatinine in the channel catfish could be the result of a stress condition generated during the sampling, like the two other factors previously mentioned.

The detection of changes in blood and serum are compared using reference intervals for all the variables which are defined by upper and lower limits that help to cover the values obtained for healthy individuals in the reference population, which is defined as a number of individuals with specific criteria where there is an absence of diseases (Tavares-Dias & Moraes, 2007b). For channel catfish (*Ictalurus punctatus*) some red blood cells (Breazile et al., 1982) and blood biochemistry ranges and means have been determined (Bentinck-Smith et al., 1987; Hrubec & Smith, 1999). According to Tavares-Dias & Moraes, 2007a once the blood sample is collected from the fish, the blood immediately begins to change and could impact the results of the test, for this reason the samples must be handled and received an appropriate and standardized methodology for the collection, transportation, and analysis. Additionally Hrubec & Smith, 1999 demonstrated on their study that some changes appear to occur rapidly in fish serum, even when the blood is handled in an appropriate manner, it is highly recommended that blood chemistry values be determined from plasma. Comparing previous studies, we can assume that the small variabilities in the blood parameters from the RAS and IPRS can be the result of a mishandling during the collection of the blood samples which appears that the human manipulation has a physiological effect on the fish (Zhou et al., 2009) but according to the results obtained in koi (*Cyprinus carpio*) by Tripathi et al., 2003 the nutritional status, age, and environmental conditions can be another factors responsible for changes in blood cell indices in

fish. Although, the serum biochemical parameters are not commonly used as a diagnostic tool due to the lack of suitable references for clinical interpretation (Tavares-Dias & Moraes, 2007a). Based on the results of this study, we could determine that an improper human manipulation of the fish generates a negative impact in the physiological process of the channel catfish.

For this reason, after the different analyses were performed, the data was analyzed and the Basal-LO and Basal-CFP outcomes with the best results for the growth performance, feed intake, and survival rate. The best results were obtained within the indoor system, where most of the production parameters were under control. On the other hand, the results from the outdoor trial presented some differences compared to the indoor system, the external conditions could be considered a game changing factor in the production. The best results for the outdoor trial were produced by the Basal-F and Basal-CFP diets.

Plant-based proteins are presented as a reliable replacement for animal-based proteins, because of their nutritional profile, low-cost production, and availability. Although plant-based ingredients are not exempt from problems, the presence of anti-nutritional factors and amino acid imbalance complicated the use of them in diet formulations. Soybean is one of the plant-based ingredients that stand out from the other ones due to the low anti-nutritional factors and a better amino acid profile. To promote the use of plant-based ingredient, especially soybean in the industry several processing methods have been developed to convert soybeans into a better product for aquatic species. Modern technologies like genetic modifications, use of fermenting process produced by microorganisms, and comixing with ethanol byproducts have improved the nutritional profile for soybean. The main objective of this study was to evaluate the effect of partial or total replacement of solvent-extracted soybean meal with fermented soybean meal, low oligosaccharide content soybean meal, and soybean meal combined corn-fermented protein,

promising protein sources obtained from the different processing methods, which could be used to produce commercial feed for aquatic species, including channel catfish. For this experiment, two aquaculture rearing systems were used to evaluate the experimental diets over a 12-week period. After the trial was completed, different analyses were performed to measure the nutritional and growth performance. Results demonstrated that the use of extracted- solvent soybean meal replaced by low oligosaccharide content and extracted-solvent soybean meal mixed with corn fermented protein generated better results for channel catfish fingerlings in terms of growth performance, feed intake, and survival rate. The importance of finding new novel ingredients for aquaculture industry, ensure its sustainability and development for the next years, in this way the use of new processing methods for soybean assure a steady protein supply. The implementation and development of new processing techniques will positively impact aquaculture production worldwide.

6. Conclusion

This study demonstrates that a solvent-extracted soybean meal used in the basal diet can be blended with corn fermented protein or completely replaced using fermented soybean meal or low-oligosaccharide when formulating production diets for catfish without affecting the growth performance, and survival. Identifying and using these alternative soybean products is critical since the current limitation with animal-based products, and their prices greatly emphasize diversifying the ingredient sources in the catfish industry. Additionally, this research demonstrated an excellent performance in the catfish fingerlings' growth, survival, and feed utilization. Further studies will be recommended to understand better how these new soybean alternatives could impact some physiological properties of the fish. Obtaining a better understanding of the biological mechanisms of the channel catfish under new novel ingredients.

7. References

- Abass, N. Y., Ye, Z., Alsaqufi, A., & Dunham, R. A. (2022). Comparison of growth performance among channel-blue hybrid catfish, ccGH transgenic channel catfish, and channel catfish in a tank culture system. *Scientific Reports*, *12*(1), 740. <https://doi.org/10.1038/s41598-021-04719-1>
- Baker, K. M., Utterback, P. L., Parsons, C. M., & Stein, H. H. (2011). Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. *Poultry Science*, *90*(2), 390–395. <https://doi.org/10.3382/ps.2010-00978>
- Bedford, M. R. (1995). Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology*, *53*(2), 145–155. [https://doi.org/10.1016/0377-8401\(95\)02018-U](https://doi.org/10.1016/0377-8401(95)02018-U)
- Bentinck-Smith, J., Bealeau, M. H., Waterstrat, P., Tucker, C. S., Stiles, F., Bowser, P. R., & Brown, L. A. (1987). Biochemical Reference Ranges for Commercially Reared Channel Catfish. *The Progressive Fish-Culturist*, *49*(2), 108–114. [https://doi.org/10.1577/1548-8640\(1987\)49<108:BRRFCR>2.0.CO;2](https://doi.org/10.1577/1548-8640(1987)49<108:BRRFCR>2.0.CO;2)
- Boyd, C. E., Torrans, E. L., & Tucker, C. S. (2018). Dissolved Oxygen and Aeration in Ictalurid Catfish Aquaculture. *Journal of the World Aquaculture Society*, *49*(1), 7–70. <https://doi.org/10.1111/jwas.12469>
- Breazile, J. E., Zinn, L. L., Yauk, J. C., Mass, H. J., & Wollscheid, J. (1982). A study of haematological profiles of channel catfish, *Ictalurus punctatus* (*Rafinesque*). *Journal of Fish Biology*, *21*(3), 305–309. <https://doi.org/10.1111/j.1095-8649.1982.tb02835.x>

- Dal'Bó, G. A., Sampaio, F. G., Losekann, M. E., Queiroz, J. F. D., Luiz, A. J. B., Wolf, V. H. G., Gonçalves, V. T., & Carra, M. L. (2015). Hematological and morphometric blood value of four cultured species of economically important tropical foodfish. *Neotropical Ichthyology*, 13(2), 439–446. <https://doi.org/10.1590/1982-0224-20140115>
- de Souza, P. C., & Bonilla-Rodriguez, G. O. (2007). *Fish Hemoglobins*.
- Dunham, R. A., Brummett, R. E., Ella, M. O., & Smitherman, R. O. (1990). Genotype-environment interactions for growth of blue, channel and hybrid catfish in ponds and cages at varying densities. *Aquaculture*, 85(1–4), 143–151. [https://doi.org/10.1016/0044-8486\(90\)90013-D](https://doi.org/10.1016/0044-8486(90)90013-D)
- El-Dakar, A., Elgamal, A., Abd ElBaky, M., Shalaby, S., Mohammed, A. S., & Abdel-Aziz, M. F. (2023). *Evaluation of Fermented Soybean Meal by Bacillus Subtilis Bacteria and Corn Gluten as Plant Protein Sources in Fish Meal Free Diets for Nile Tilapia, Oreochromis Niloticus*. <https://doi.org/10.2139/ssrn.4426973>
- Engle, C. R., Hanson, T., & Kumar, G. (2022). Economic history of U.S. catfish farming: Lessons for growth and development of aquaculture. *Aquaculture Economics & Management*, 26(1), 1–35. <https://doi.org/10.1080/13657305.2021.1896606>
- Galkanda-Arachchige, H. S. C., Hussain, A. S., & Davis, D. A. (2021). Fermented corn protein concentrate to replace fishmeal in practical diets for Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*, 27(5), 1640–1649. <https://doi.org/10.1111/anu.13303>
- Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogh, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., J Souza, E., Stone, D., Wilson, R., & Wurtele, E. (2007). Expanding the utilization of

- sustainable plant products in aquafeeds: A review. *Aquaculture Research*, 38(6), 551–579. <https://doi.org/10.1111/j.1365-2109.2007.01704.x>
- Gaylord, I. G., & Gatlin, D. M. (2000). Assessment of Compensatory Growth in Channel Catfish *Ictalurus punctatus* R. and Associated Changes in Body Condition indices. *Journal of the World Aquaculture Society*, 31(3), 326–336. <https://doi.org/10.1111/j.1749-7345.2000.tb00884.x>
- Goda, A. M., El-Haroun, E. R., & Kabir Chowdhury, M. A. (2007). Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. *Aquaculture Research*, 38(3), 279–287. <https://doi.org/10.1111/j.1365-2109.2007.01663.x>
- Grieshop, C. M., Kadzere, C. T., Clapper, G. M., Flickinger, E. A., Bauer, L. L., Frazier, R. L., & Fahey, G. C. (2003). Chemical and Nutritional Characteristics of United States Soybeans and Soybean Meals. *Journal of Agricultural and Food Chemistry*, 51(26), 7684–7691. <https://doi.org/10.1021/jf034690c>
- Hatch, U., Hanson, T. R., Zidack, W., Lovell, R. T., & Li, M. (1998). Economic analysis of alternative protein levels in channel catfish feeds. *Aquaculture Economics & Management*, 2(1), 13–22. <https://doi.org/10.1080/13657309809380210>
- Hayward, J. W., & Hafner, F. H. (1941). The Supplementary Effect of Cystine and Methionine Upon the Protein of Raw and Cooked Soybeans as Determined with Chicks and Rats. *Poultry Science*, 20(2), 139–150. <https://doi.org/10.3382/ps.0200139>
- Hegde, S., Kumar, G., Engle, C., Hanson, T., Roy, L. A., Van Senten, J., Johnson, J., Avery, J., Aarattuthodi, S., Dahl, S., Dorman, L., & Peterman, M. (2022). Economic contribution of

- the U.S. catfish industry. *Aquaculture Economics & Management*, 26(4), 384–413.
<https://doi.org/10.1080/13657305.2021.2008050>
- Herman, E. M., & Schmidt, M. A. (2016). The Potential for Engineering Enhanced Functional-Feed Soybeans for Sustainable Aquaculture Feed. *Frontiers in Plant Science*, 7.
<https://doi.org/10.3389/fpls.2016.00440>
- Hrubec, T. C., & Smith, S. A. (1999). Differences between Plasma and Serum Samples for the Evaluation of Blood Chemistry Values in Rainbow Trout, Channel Catfish, Hybrid Tilapias, and Hybrid Striped Bass. *Journal of Aquatic Animal Health*, 11(2), 116–122.
[https://doi.org/10.1577/1548-8667\(1999\)011<0116:DBPASS>2.0.CO;2](https://doi.org/10.1577/1548-8667(1999)011<0116:DBPASS>2.0.CO;2)
- Imorou Toko, I., Fiogbe, E. D., & Kestemont, P. (2008). Growth, feed efficiency and body mineral composition of juvenile vundu catfish (*Heterobranchus longifilis*, Valenciennes 1840) in relation to various dietary levels of soybean or cottonseed meals. *Aquaculture Nutrition*, 14(3), 193–203. <https://doi.org/10.1111/j.1365-2095.2007.00518.x>
- Jakobsen, G. V., Jensen, B. B., Knudsen, K. E. B., & Canibe, N. (2015). Improving the nutritional value of rapeseed cake and wheat dried distillers grains with solubles by addition of enzymes during liquid fermentation. *Animal Feed Science and Technology*, 208, 198–213. <https://doi.org/10.1016/j.anifeedsci.2015.07.015>
- Kumar, D., & Singh, V. (2019). Bioethanol Production From Corn. In *Corn* (pp. 615–631). Elsevier. <https://doi.org/10.1016/B978-0-12-811971-6.00022-X>
- Kumar, K., Gambhir, G., Dass, A., Tripathi, A. K., Singh, A., Jha, A. K., Yadava, P., Choudhary, M., & Rakshit, S. (2020). Genetically modified crops: Current status and future prospects. *Planta*, 251(4), 91. <https://doi.org/10.1007/s00425-020-03372-8>

- Landini, G. F., Schwantes, A. R., & Schwantes, M. L. B. (2002). Astyanax scabripinnis (Pisces: Characidae) hemoglobins: structure and function. *Brazilian Journal of Biology*, 62(4a), 595–599. <https://doi.org/10.1590/S1519-69842002000400006>
- Lermen, C. L., Lappe, R., Crestani, M., Vieira, V. P., Gioda, C. R., Schetinger, M. R. C., Baldisserotto, B., Moraes, G., & Morsch, V. M. (2004). Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*. *Aquaculture*, 239(1–4), 497–507. <https://doi.org/10.1016/j.aquaculture.2004.06.021>
- Liener, I. E. (1981). Factors affecting the nutritional quality of soya products. *Journal of the American Oil Chemists' Society*, 58(3Part2), 406–415. <https://doi.org/10.1007/BF02582390>
- Lim, C., Klesius, P. H., & Higgs, D. A. (1998). Substitution of Canola Meal for Soybean Meal in Diets for Channel Catfish *Ictalurus punctatus*. *Journal of the World Aquaculture Society*, 29(2), 161–168. <https://doi.org/10.1111/j.1749-7345.1998.tb00975.x>
- Liti, D. M., Waidbacher, H., Straif, M., Mbaluka, R. K., Munguti, J. M., & Kyenze, M. M. (2006). Effects of partial and complete replacement of freshwater shrimp meal (Caridinea niloticus Roux) with a mixture of plant protein sources on growth performance of Nile tilapia (*Oreochromis niloticus* L.) in fertilized ponds. *Aquaculture Research*, 37(5), 477–483. <https://doi.org/10.1111/j.1365-2109.2006.01450.x>
- Mckenzie, S., Deane, E. M., & Burnett, L. (2002). Haematology and Serum Biochemistry of the Tammar Wallaby, *Macropus eugenii*. *Comparative Clinical Pathology*, 11(4), 229–237. <https://doi.org/10.1007/s005800200024>

Meyer, F. P., Gray, D. L., Mathis, W. P., Martin, J. M., & Wells', B. R. (n.d.). *PRODUCTION AND RETURNS FROM THE COMMERCIAL PRODUCTION OF FISH IN ARKANSAS DURING 1966.*

Mugwanya, M., Dawood, M. A. O., Kimera, F., & Sewilam, H. (2023). Replacement of fish meal with fermented plant proteins in the aquafeed industry: A systematic review and meta-analysis. *Reviews in Aquaculture*, *15*(1), 62–88. <https://doi.org/10.1111/raq.12701>

N. Ruiz, C.M. Parsons, H. H. Stein, C.N. Coon, J.E. van Eys, and R.D. Miles. (2020). *A review: 100 Years of Soybean Meal.*

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(7851), 551–563. <https://doi.org/10.1038/s41586-021-03308-6>

Nelson, B. (n.d.). *PROPAGATION OF CHANNEL CATFISH IN ARKANSAS.*

Overland, M., Granli, T., Kjos, N. P., Fjetland, O., Steien, S. H., & Stokstad, M. (2000). Effect of dietary formates on growth performance, carcass traits, sensory quality, intestinal microflora, and stomach alterations in growing-finishing pigs. *Journal of Animal Science*, *78*(7), 1875. <https://doi.org/10.2527/2000.7871875x>

Pangeni, D., Jendza, J. A., Anil, L., Yang, X., & Baidoo, S. K. (2017). Effect of replacing conventional soybean meal with low-oligosaccharide soybean meal on growth performance and carcass characteristics of wean-to-finish pigs. *Journal of Animal Science*, *95*(6), 2605. <https://doi.org/10.2527/jas2016.1164>

Pastorino, P., Bergagna, S., Vercelli, C., Pagliasso, G., Dellepiane, L., Renzi, M., Barbero, R., Re, G., Elia, A. C., Dondo, A., Barceló, D., & Prearo, M. (2022). Changes in Serum

- Blood Parameters in Farmed Rainbow Trout (*Oncorhynchus mykiss*) Fed with Diets Supplemented with Waste Derived from Supercritical Fluid Extraction of Sweet Basil (*Ocimum basilicum*). *Fishes*, 7(2), 89. <https://doi.org/10.3390/fishes7020089>
- Perez, Karni R. (2006). *Fishing for gold: The story of Alabama's catfish industry*.
- Robinson, E. H., & Li, M. H. (2020). Channel catfish, *Ictalurus punctatus*, nutrition in the United States: A historical perspective. *Journal of the World Aquaculture Society*, 51(1), 93–118. <https://doi.org/10.1111/jwas.12657>
- Shell, E.W. (1993). *Development of Aquaculture An Ecosystems Perspective*.
- Tavares-Dias, M., & Moraes, F. R. (2007a). Haematological and biochemical reference intervals for farmed channel catfish. *Journal of Fish Biology*, 71(2), 383–388. <https://doi.org/10.1111/j.1095-8649.2007.01494.x>
- Tavares-Dias, M., & Moraes, F. R. D. (2007b). Leukocyte and thrombocyte reference values for channel catfish (*Ictalurus punctatus* Raf), with an assessment of morphologic, cytochemical, and ultrastructural features. *Veterinary Clinical Pathology*, 36(1), 49–54. <https://doi.org/10.1111/j.1939-165X.2007.tb00181.x>
- The State of World Fisheries and Aquaculture 2020*. (2020). FAO. <https://doi.org/10.4060/ca9229en>
- Tripathi, N. K., Latimer, K. S., Lewis, T. L., & Burnley, V. V. (2003). Biochemical reference intervals for koi (*Cyprinus carpio*). *Comparative Clinical Pathology*, 12(3), 160–165. <https://doi.org/10.1007/s00580-003-0495-x>
- USDA. (2022). *Agricultural Competition: A Plan in Support of Fair and Competitive Markets*.
- Waldrop, J. E., & Smith, R. D. (n.d.). *An Economic Analysis of Producing Pond-Raised Catfish for Food in Mississippi, A January 1980 Update*.

- Williams, P. (2022). Corn fermented protein a commercially viable alternative protein for aquaculture and livestock. *Animal - Science Proceedings*, 13(1), 87–88.
<https://doi.org/10.1016/j.anscip.2022.03.118>
- Xiang, D., Luo, M., Jiang, F., Wen, Z., Chen, X., Wang, X., Xu, X., Wei, W., & Xu, J. (2023). Safety assessment of subchronic feeding of insect-resistant and herbicide-resistant transgenic soybeans to juvenile channel catfish (*Ictalurus punctatus*). *Scientific Reports*, 13(1), 5445. <https://doi.org/10.1038/s41598-023-31072-2>
- Yamamoto, F. Y., Huang, J., Suarez-Barazeta, C. C., Craig, S. R., Older, C. E., Richardson, B. M., Santana, T. M., Griffin, M. J., Reifers, J. G., Goodman, P. M., & Gatlin Iii, D. M. (2024). Exploring the nutritional value of corn fermented protein as a replacement for soybean meal in diets for juvenile channel catfish (*Ictalurus punctatus*): Impacts on production performance, intestinal health, and disease resistance. *Aquaculture*, 587, 740824. <https://doi.org/10.1016/j.aquaculture.2024.740824>
- Zhang, W., Tan, B., Deng, J., Dong, X., Yang, Q., Chi, S., Liu, H., Zhang, S., Xie, S., & Zhang, H. (2021). Mechanisms by Which Fermented Soybean Meal and Soybean Meal Induced Enteritis in Marine Fish Juvenile Pearl Gentian Grouper. *Frontiers in Physiology*, 12, 646853. <https://doi.org/10.3389/fphys.2021.646853>
- Zhou, X., Li, M., Abbas, K., & Wang, W. (2009). Comparison of haematology and serum biochemistry of cultured and wild Dojo loach *Misgurnus anguillicaudatus*. *Fish Physiology and Biochemistry*, 35(3), 435–441. [https://doi.org/10.1007/s10695-008-9268-](https://doi.org/10.1007/s10695-008-9268-4)

Table 1. Composition (g/100g as is) of practical diets formulated to contain 32% protein and 6% lipids on an as is basis. Diets were commercially produced by Optimal Aquafeed Inc. (Omaha, NE, U.S.)

	Basal	Basal-F	Basal-LO	Basal-CFP
Soybean Meal (SBM) ¹	56.4	-	-	44.5
Fermented SBM ²	-	43.0	-	-
Low Oligosaccharide SBM ³	-	-	45.0	-
Corn Fermented Protein ⁴	-	-	-	10.0
Poultry Meal	8.0	8.0	8.0	8.0
Menhaden Fish Oil	2.0	2.0	2.0	2.0
Soy Oil	1.4	0	0.8	0.4
Corn	20.3	34.7	31.9	22.8
Wheat Mids.	10.0	10.0	10.0	10.0
Premix	0.5	0.5	0.5	0.5
CaP-dibasic	1.8	1.8	1.8	1.8

¹ Solvent Extracted Soybean Meal, Bunge Limited (Chesterfield, MO, U.S.)

² Fermented Soybean Meal, HP300, Hamlet Inc (Findlay, OH, U.S.)

³ Low Oligosaccharide Soybean Meal, Bright Day, Benson Hill (St. Louis, MO, U.S.)

⁴ Corn Fermented Protein CFP-GT33 proprietary blend.

Table 2. Proximate composition and amino acid profile (g/100g dry Weight) of the four experimental diets used during a 12-week trial to feed channel catfish (*Ictalurus punctatus*) fingerlings conducted in RAS and IPRS systems.

	Basal	Basal-F	Basal-LO	Basal-CFP
Crude protein	36.42	35.8	35.48	35.93
Moisture*	7.56	7.36	10.35	8.05
Acid Hydrolysis Fat	6.02	8.19	7.35	8.43
Crude Fiber	4.37	4.41	3	4
Ash	6.94	6.8	6.55	6.48
Arginine	2.56	2.49	2.55	2.37
Histidine	0.97	0.96	0.95	0.95
Isoleucine	1.7	1.64	1.62	1.61
Leucine	2.81	2.81	2.72	2.99
Lysine	2.27	2.12	2.17	2.08
Methionine	0.57	0.58	0.55	0.6
Phenylalanine	1.76	1.74	1.73	1.74
Threonine	1.35	1.36	1.33	1.35
Tryptophan	0.46	0.43	0.45	0.43
Valine	1.85	1.84	1.79	1.78
Alanine	1.79	1.84	1.74	1.9
Aspartic Acid	3.81	3.6	3.74	3.5
Cysteine	0.58	0.55	0.53	0.57
Glutamic Acid	6.41	6.12	6.31	6.22
Glycine	1.89	1.9	1.86	1.84
Hydroxylysine	0.01	0.03	0.03	0.03
Hydroxyproline	0.22	0.23	0.24	0.25
Lanthionine	0.08	0.12	0.11	0.09
Ornithine	0.06	0.09	0.06	0.06
Proline	1.98	2.01	1.98	2.09
Serine	1.42	1.45	1.43	1.45
Taurine	0.24	0.24	0.22	0.26
Tyrosine	1.24	1.23	1.21	1.26

*Expressed on an as-is basis.

Table 3. Response of channel catfish (*Ictalurus punctatus*) fingerlings (mean initial weight 2.14 ± 0.03 g) offered one of five practical diets over a 12-week growth trail conducted in a recirculating aquaculture system. Four diets were open formulations using solvent-extracted soybean meal [(SBM), Basal], Fermented soybean meal (Basal-F), low oligosaccharide soy (Basal-LO), and SBM in combination with Corn fermented (Basal-CFP). A commercial 32% floating catfish feed was also included as a reference (commercial reference).

	Basal	Basal-F	Basal-LO	Basal-CFP	Commercial Reference	PSE ¹	p-value
Biomass (g)	1065.1 ^{bc}	1066.2 ^{bc}	1305.3 ^a	1181.9 ^{ab}	932.7 ^c	43.57	0.0870
Final Weight(g)	53.8 ^{bc}	54.0 ^{bc}	64.9 ^a	60.6 ^{ab}	47.9 ^c	2.43	0.0017
Weight gain(g)	51.7 ^{bc}	51.9 ^{bc}	62.8 ^a	58.5 ^{ab}	45.8 ^c	2.42	0.0016
Weight gain (%)	2405.8 ^{bc}	2406.3 ^{bc}	2998.9 ^a	2746.3 ^{ab}	2119.7 ^c	114.29	0.0007
Survival (%)	98.7 ^a	98.75	91.2 ^a	97.5 ^a	97.5 ^a	1.36	0.9265
FCR⁴	1.20 ^a	1.14 ^{ab}	1.04 ^b	1.11 ^{ab}	1.25 ^a	0.04	0.0070
IPF² (%)	1.7	2.2	2.1	2.0	1.1	0.17	0.0012
HSI³ (%)	1.7	2.0	1.7	1.9	2.4	0.14	0.0122

Values represent the means of three replicate groups of fish. Data was subjected to one-way ANOVA followed by a Tukey's Test to determine significant differences between treatments.

¹Pooled Standard Error of Treatment Means

²Intraperitoneal Fat Index

³Hepatosomatic Index

⁴Feeding Conversion Ratio

Table 4. Response of channel catfish (*Ictalurus punctatus*) fingerlings (mean initial Weight 32.56 ± 0.72 g) offered one of four practical diets over a 12-week growth trial conducted in an IPRS system. Four diets were open formulations using solvent-extracted soybean meal [(SBM), Basal], Fermented soybean meal (Basal-F), low oligosaccharide soy (Basal-LO), and SBM in combination with Corn fermented (Basal-CFP).

	Basal	Basal-F	Basal-LO	Basal-CFP	PSE³	p-value
Biomass (kg)	54.97	63.06	48.93	58.79	4.55	0.2490
Final Weight (g)	113.4	130.4	120.9	128.6	8.82	0.8276
Weight gain (g)	81.83	98.54	87.75	95.70	7.73	0.7326
Weight gain (%)	276	309	266	290	18.74	0.4426
Survival (%)	78.1	81.3	70.5	75.5	6.78	0.7304
FCR¹	2.0	1.6	2.2	1.9	0.19	0.3056
ANPR² (%)	30.4	41.0	35.8	38.5	1.88	0.0198

Values represent the means for four experimental diets with three replicates each and are evaluated with an ANOVA test to find the differences between the variances using a Post Hoc Tukey Test

¹Feeding Conversion Ratio

²Apparent Net Protein Retention

³Pooled Standard Error

Table 5. Water quality parameters were recorded over a 12-week trial for channel catfish (*Ictalurus Punctatus*) fingerlings fed with experimental diets containing different soybean meal treatments as a substitute for a basal diet and performed into RAS and IPRS systems, respectively.

Parameters	RAS¹ System	IPRS² System
D.O.⁴ (mg/L)	6.81 ± 0.05 (8.7 – 4.7)	9.16 ± 0.04 (16.9 – 4.4)
Temperature (°C)	25.67 ± 0.06 (27.3 – 23.2)	20.15 ± 0.11 (30.7 – 10.9)
Salinity (ppt)	2.02 ± 0.12 (5.0 – 0.1)	0.2 ± 0.001 (0.4 – 0.1)
pH	7.53 ± 0.08 (8.5 – 6.8)	8.31 ± 0.61 (9.9 – 7.4)
TAN³ (mg/L)	0.4 ± 0.19 (5.0 – 0.0)	0.3 ± 0.01 (1.6 – 0.0)
Nitrite (mg/L)	0.17 ± 0.04 (1.1 – 0.0)	0.08 ± 0.001 (0.3 – 0.0)

Values represent the mean ± standard error, and values in parenthesis represent the minimum and maximum water quality readings.

¹Recirculatory Aquaculture System

²In-Pond Raceway System

³Total Ammonia Nitrogen

⁴Dissolved Oxygen

Table 6. Compiled statistical analysis results for bloodwork values obtained using a portable chemistry analyzer VetScan2 for channel catfish (*Ictalurus punctatus*) fingerlings offered one of four practical diets over a 12-week growth trial in the RAS system.

	Basal	Basal-F	Basal-LO	Basal-CFP	Commercial Reference	PSE	p-value
ALB (g/L)	1.8	1.8	1.9	2.1	1.9	0.08	0.6003
ALP (U/I)	84.0	89.7	97.3	83.0	87.7	2.01	0.2798
ALT (U/I)	18.3	21.0	16.0	16.0	27.7	2.77	0.058
AMY (U/I)	79.0	82.3	81.0	41.0	58.3	8.50	0.394
TBIL (µmol/l)	0.3	0.3	0.4	0.4	0.3	0.02	0.0037
BUN (mmol urea/l)	1.0	1.3	1.3	1.0	1.0	0.17	0.6554
CA⁺⁺ (mmol/l)	12.4	12.4	12.4	12.7	13.3	0.45	0.8428
PHOS (mmol/l)	16.1	15.6	17.0	12.9	13.3	0.67	0.0817
CRE (µmol/l)	0.2	0.4	0.2	0.1	0.2	0.07	0.0482
GLU (mg/dl)	79.7	82.7	84.3	68.7	78.3	4.03	0.8955
NA⁺ (mmol/l)	143.0	142.3	142.0	141	140.3	2.22	0.6882
K⁺ (mmol/l)	0.9	1.2	1.2	0.7	0.9	0.20	0.5262
TP (g/l)	3.5	3.5	3.5	3.6	3.6	0.11	0.5870
GLOB (g/l)	1.7	1.7	1.6	1.5	1.7	0.08	0.4108

ALB, Albumin; **ALP**, Alkaline Phosphatase; **ALT**, Alanine Aminotransferase; **AMY**, Amylase; **TBIL**, Total Bilirubin; **BUN**, Blood Urea

Nitrogen; **CA⁺⁺**, Calcium; **PHOS**, Phosphorous; **CRE**, Creatinine; **GLU**, Glucose; **NA⁺**, Sodium; **K⁺**, Potassium, **T.P.**, Total Protein; **GLOB**, Globulin.

Table 7. Compiled statistical analysis results for bloodwork values obtained using a portable chemistry analyzer VetScan2 for channel catfish (*Ictalurus punctatus*) fingerlings offered one of four practical diets over a 12-week growth trial in an IPRS system.

	Basal	Basal-F	Basal-LO	Basal-CFP	PSE	p-value
ALB (g/L)	2.1	2.2	2.3	2.2	0.08	0.3968
ALP (U/l)	23.3	24.7	26.7	29.3	6.36	0.2453
ALT (U/l)	13.7	14.3	15.7	16.3	4.58	0.8986
AMY (U/l)	61.7	53.7	52.7	71.7	9.18	0.4133
TBIL (μmol/l)	0.4	0.5	0.4	0.4	0.03	0.0086
BUN (mmol urea/l)	1.0	1.7	1.0	1.0	0.18	0.0519
CA⁺⁺ (mmol/l)	11.4	11.5	11.7	11.1	0.11	0.82
PHOS (mmol/l)	9.4	9.4	9.2	8.6	0.50	0.8015
CRE (μmol/l)	0.5	0.5	0.4	0.4	0.04	0.743
GLU (mg/dl)	28.7	23.3	32.7	24.7	5.33	0.4066
NA⁺ (mmol/l)	137.3	137.7	137.0	138.3	1.02	0.9765
K⁺ (mmol/l)	0.9	0.9	0.8	1.03	0.16	0.8954
TP (g/l)	3.9	4.0	4.0	3.9	0.07	0.8764
GLOB (g/l)	1.9	1.8	1.8	1.8	0.06	0.7051

ALB, Albumin; **ALP**, Alkaline Phosphatase; **ALT**, Alanine Aminotransferase; **AMY**, Amylase; **TBIL**, Total Bilirubin; **BUN**, Blood Urea Nitrogen; **CA⁺⁺**, Calcium; **PHOS**, Phosphorous; **CRE**, Creatinine; **GLU**, Glucose; **NA⁺**, Sodium; **K⁺**, Potassium, **T.P.**, Total Protein; **GLOB**, Globulin.

Figure 1. Proximate analysis for the experimental diets tested on the trial at the end of the 12-week period for (*Ictalurus punctatus*), fed with diets containing different soybean products replacing or comixing with solvent-extracted soybean meal and cultured in an IPRS system.

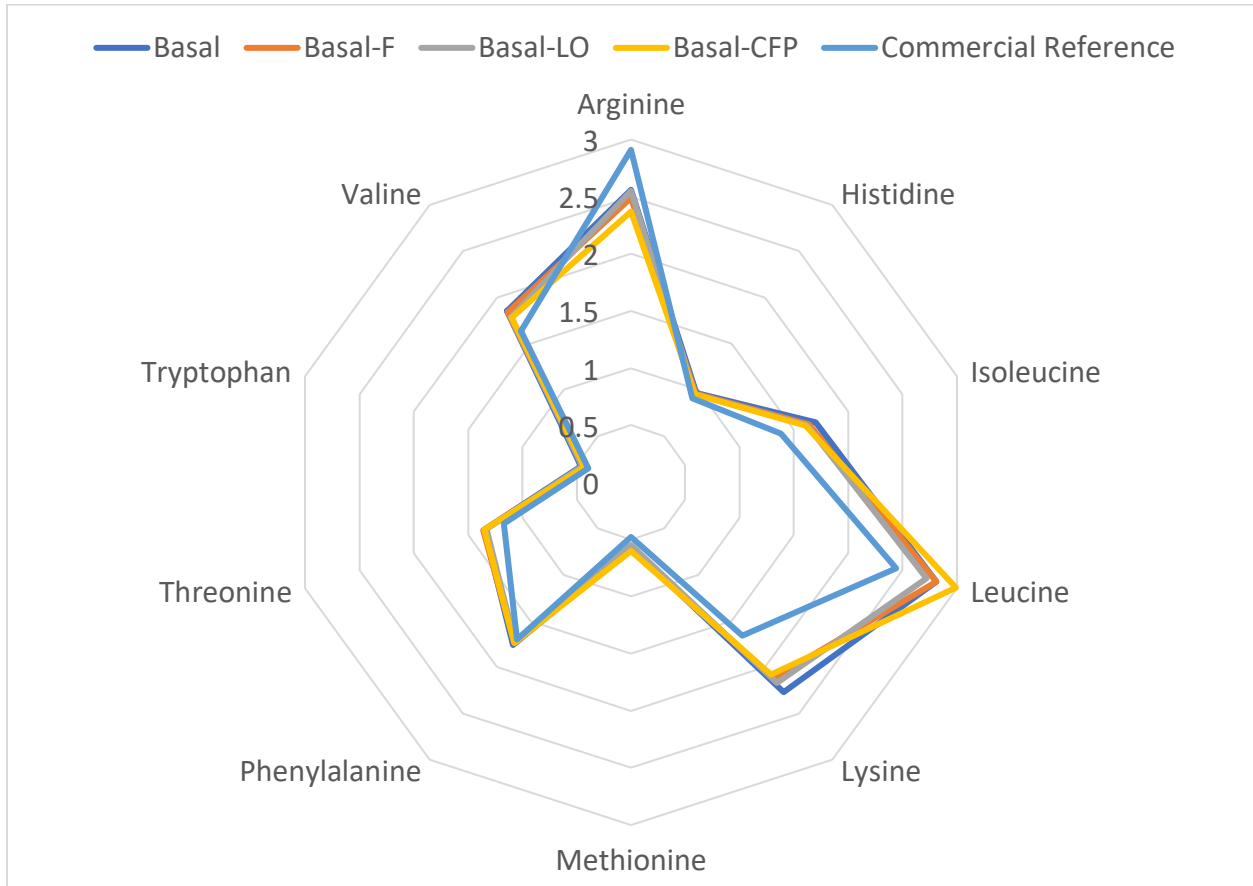


Figure 2. Final Weight obtained for experimental diets tested on the trial at the end of the 12-week period for (*Ictalurus punctatus*), fed with diets containing different soybean products replacing or comixing with solvent-extracted soybean meal and cultured in a RAS system.

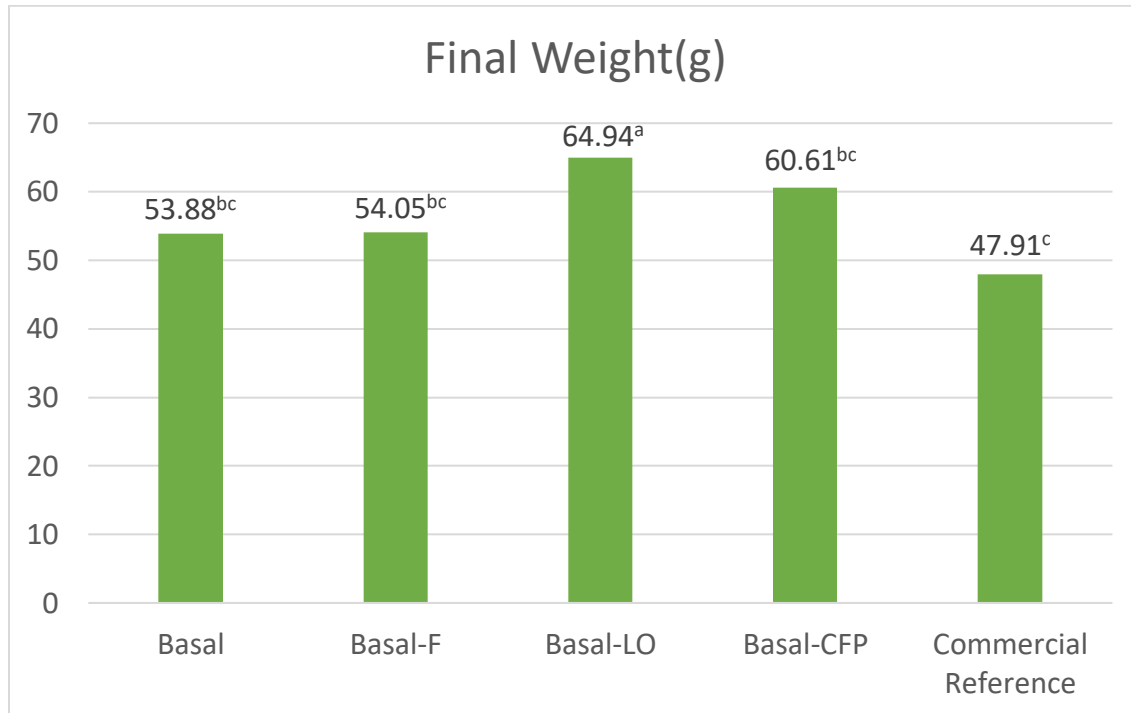


Figure 3. Feeding Conversion Ratio obtained for experimental diets tested on the trial at the end of the 12-week period for (*Ictalurus punctatus*), fed with diets containing different soybean products replacing or comixing with solvent-extracted soybean meal and cultured in a RAS system.

