

Extruded aquatic feed - Impact of ingredients and processing on physical quality of feed,
animal performance and environmental footprint

by

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AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
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Manhattan, Kansas

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Abstract

This dissertation presents a comprehensive investigation into the sustainability of extruded aquafeeds, focusing on the interplay between raw ingredient selection, physical feed characteristics, and extrusion processing parameters. The research specifically addresses the efficiency of production, physical quality, nutritional performance, and environmental footprint of feeds designed for key aquaculture species: Nile tilapia, Pacific white shrimp, and rainbow trout. A core objective was to evaluate grain sorghum and its derivatives as sustainable alternatives to conventional ingredients such as wheat and fishmeal. The studies explored the impact of various factors, including ingredient particle size, thermal and mechanical energy inputs during extrusion and drying, and the use of different cereal grain types and compositions. The primary aim was to identify optimal processing conditions that not only maximize pellet quality, feed digestibility, and animal growth performance but also minimize production costs and environmental impacts. Through an integrated approach combining processing trials, *in vivo* growth and digestibility assessments, detailed energy balance analyses, and cradle-to-gate life cycle assessments, this research provides a holistic understanding of how feed formulation and manufacturing choices influence the entire aquafeed production chain.

Key findings demonstrate that grain sorghum is a viable alternative to wheat in aquafeeds, maintaining pellet quality and producing comparable animal performance while offering environmental benefits. Mechanical energy during processing of tilapia feeds for sorghum-based formulations ranged from 267.6 to 358.8 kJ/kg and 290.5 to 351 kJ/kg for wheat-based diets, with more intense particle size reduction increasing mechanical energy. For

shrimp feeds, reducing the amount of preconditioning for both wheat and steam based diets resulted in higher mechanical energy inputs (228-260.4, and 237-288 kJ/kg, respectively). For sorghum-based diets specifically, it was found that gelatinization of feed was primarily affected by preconditioning inputs, with higher steam input leading to a greater degree of gelatinization (83-90.5%), while wheat-based feeds had similar gelatinization across all inputs (95-98%). For both wheat and sorghum-based diets for trout, processing under similar conditions resulted in similar bulk density numbers (372 and 382 g/L), floating percentage (~100%), pellet durability (86 and 87%), and water stability (~91%). Growth studies for sorghum-based feeds resulted in feed conversion ratios of 1.03-1.1, 1.44-1.51, and 0.96 for tilapia, shrimp, and trout. For wheat, these values were 1.11-1.13, 1.46-1.52, and 0.86 for tilapia, shrimp and trout. For all species except rainbow trout, sorghum-based diets exhibited improved feed conversion ratios. The research also highlighted that the choice of cereal grain significantly influences the environmental footprint, with sorghum-based feeds generally exhibiting lower environmental burdens. Net global warming potential values (kg CO₂ eq/ton of animal growth) for sorghum and wheat-based diets were, respectively, 90.18 and 120.8 (trout feed), 160.4-224.4 and 240.79-303.52 (tilapia feeds), and 185.4-188.8 and 243.7-263.12 (shrimp feeds). It was found that while the main driver of global warming potential came from the choice of cereal grain used, total emissions were also drive by processing choices, with more intense processing such as increased particle size reduction (tilapia) and more intense preconditioning (shrimp) resulting in a larger environmental footprint. Furthermore, the inclusion of sorghum distillers dried grains (sDDGs) presents a

sustainable protein source, though its environmental impacts necessitate careful formulation and processing to mitigate increased burdens from upstream agricultural activities.

Ultimately, this dissertation provides evidence supporting the development of cost-effective, high-quality, and environmentally responsible aquafeeds that effectively utilize drought-tolerant, lower-input grains like sorghum. This contributes significantly to enhancing the resilience and sustainability of the global aquaculture industry, addressing challenges posed by rising ingredient costs and the increasing demand for sustainable ingredient sources.

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Chapter 1 - Introduction

The global demand for high-quality protein continues to accelerate as the population increases, pushing food systems to evolve to meet this challenge. Fish consumption has grown by an average of 3% annually between 1961 and 2019, nearly twice the rate of global population growth (FAO, 2022). Aquatic species currently provide 17% of the world's animal protein intake, with over 3 billion people relying on fish for at least 20% of their daily protein (FAO, 2022). Aquaculture, the farming of aquatic animals, has emerged as a major contributor to this protein demand, producing 88 million tons of seafood in 2020, accounting for nearly 50% of global seafood supply (FAO, 2020). Unlike capture fisheries, which have largely plateaued since the 1990s, aquaculture has seen sustained annual growth of 5.3% from 2001 to 2018 (FAO, 2020), and it is predicted to produce over 93 million tons by 2030 (World Bank, 2013).

Feeding this rapidly growing industry presents both economic and technical challenges. Feed is typically the largest operational cost for aquaculture, comprising 50–70% of total production expenses (FAO, 2010). Currently, 70% of aquaculture operations rely on commercial compound feeds (Global Feed Survey Archives, 2023). From 2021 to 2022, the aquafeed industry outpaced nearly all other livestock sectors with a 2.72% growth rate, compared to 1.27% for poultry and 0.31% for layers, while other sectors declined (Global Feed Survey Archives, 2023). These trends underline the critical importance of developing cost-effective and sustainable feed strategies.

Historically, fishmeal has been the cornerstone protein ingredient in aquatic feeds due to its nutritional profile and digestibility (Miles & Chapman, 2006). However, limited availability, rising prices, and ecological concerns over overfishing have led to extensive research into alternative protein and energy sources. Plant proteins and co-products such as distillers dried grains with solubles (DDGS), particularly from corn, have demonstrated potential for use in aquatic species (Garza de Yta et al., 2012; Sookying & Davis, 2012). As ethanol production from grain sorghum (*Sorghum bicolor*) expands, the inclusion of sorghum DDGS (sDDGS) in aquafeeds is drawing increasing attention. Adedeji et al. (2017) found that sDDGS could be included at levels up to 40% in Pacific white shrimp (*Litopenaeus vannamei*) diets without negatively impacting growth, survival, or feed conversion ratios, though some changes in pellet durability and bulk density were observed.

Beyond protein sources, carbohydrates play an important role in aquatic feeds despite the lack of a strict dietary requirement in many fish and shrimp species. Carbohydrates, such as starches found in cereal grains, act as an immediate energy source and enable protein-sparing effects that reduce overall feed costs (Shiau & Peng, 1993; Wang et al., 2015; Cruz-Suarez et al., 1994; Cuzon et al., 2004). Additionally, starch serves a critical functional role in feed manufacturing, improving pellet durability and water stability through gelatinization (Stone et al., 2003; Romano & Kumar, 2019). Wheat has become a preferred starch source due to its high digestibility and its gluten content, which further strengthens pellets (Draganovic et al., 2013; Tiarniyu & Solomon, 2012).

However, wheat's high cost, environmental impact, and reliance on irrigation and nitrogen inputs make it a less sustainable long-term option. Furthermore, consumer resistance to genetically modified organisms (GMOs) globally, particularly in the European Union, restricts the use of more commonly used grains like corn (Science, 2023). Grain sorghum represents a viable alternative. It is a non-GMO, drought-tolerant crop with lower water and nitrogen requirements, better yields under stress than maize, and high starch content (70–72%) alongside moderate protein and fiber content (Knudsen et al., 1988; van Oosterom et al., 2021; Hadebe et al., 2017).

Sorghum's compositional attributes make it particularly suited for extrusion, the primary method of aquafeed production. Extrusion involves combining ingredients with moisture, heat, steam, and mechanical energy to form a viscous dough that is shaped through die holes, yielding fully cooked pellets (Riaz, 2000). Compared to pellet mills, extrusion allows for better control over cooking, energy use, and final pellet characteristics, including floating or sinking ability, expansion, and durability (Robinette, 1977; Monti et al., 2016). The extrusion process relies on two key energy inputs: specific mechanical energy (SME) and specific thermal energy (STE). SME is generated through friction and shear within the extruder, while STE is delivered through steam in the preconditioner (Pacheco et al., 2018; Kantrong et al., 2018).

Preconditioning plays a critical role by hydrating and softening feed ingredients, beginning starch gelatinization and improving process stability (Guy, 2001; Gibson & Alavi, 2014). The degree of gelatinization can affect digestibility, pellet quality, and the extrusion

process itself. For instance, increasing preconditioner temperature has been shown to improve starch gelatinization and pellet durability (Lundblad et al., 2011; Lundblad et al., 2012). Moreover, diet composition affects energy absorption, flow through the extruder, and mechanical durability.

Pellet quality is also influenced post-extrusion during drying, a process that stabilizes moisture levels but can be energy-intensive. Parameters such as air temperature, humidity, and retention time affect moisture loss, fines, and pellet durability (Haubjerg & Veje, 2018). Pellets that are overdried become brittle and produce fines, while those insufficiently dried may be prone to microbial growth. Optimal drying conditions can improve both energy efficiency and final product integrity (Dai et al., 2015; Kasapis, 2005).

As fish feed is produced, the choices made during processing have direct environmental impacts. It is estimated that feed production systems can contribute up to 64% of the total greenhouse gas emissions from aquaculture (Snyder et al., 2009). Grain production systems are a significant source of nitrate, phosphorous and ammonia emissions, and can contribute to acidification and eutrophication. Due to this, it is necessary to conduct an estimation of environmental impacts through a Life Cycle Assessment (LCA). LCAs are a methodology that are used to assess environmental impacts through a product life cycle or a set system boundary, typically including raw materials, production, and waste management (Finnveden et al., 2009). The optimization of aquatic feed production is crucial, as LCA studies often indicate that aquatic feed processing is

the main contributor to negative environmental impacts at commercial farms (Pennells et al., 2025)

Research gaps remain in several areas: the functional and nutritional performance of sorghum relative to wheat in aquatic diets, the impact of sDDGS inclusion on extrusion energy use, and how drying conditions affect pellet durability and fines production. Additionally, species-specific responses to carbohydrate digestibility, such as in rainbow trout, which show limited ability to metabolize large amounts of starch (Wang et al., 2024; Geurden et al., 2018; Lyons et al., 2024), must be considered.

1.1 Overall Objectives

The overall objective of this body of research is to comprehensively evaluate how different raw ingredient choices, ingredient physical characteristics, and extrusion processing parameters influence the production efficiency, physical quality, nutritional performance, and environmental sustainability of extruded aquafeeds formulated for key species such as Nile tilapia, Pacific white shrimp, and rainbow trout. Central to these studies is the investigation of sorghum and its derivatives as alternative carbohydrate and protein sources compared to more traditional ingredients like wheat and fishmeal. The research systematically examines factors such as ingredient particle size, thermal and mechanical energy inputs during extrusion and drying, and variations in grain type and composition to identify optimal processing conditions that maximize pellet quality, feed digestibility, and animal growth performance while minimizing feed costs and environmental impacts.

By integrating processing trials with growth and digestibility assessments, energy balance analyses, and cradle-to-gate life cycle assessments, this research aims to provide a holistic understanding of how feed formulation and manufacturing choices interact across multiple scales, from raw material characteristics through processing efficiencies to sustainability outcomes. Ultimately, the goal is to support the development of cost-effective, high-quality, and environmentally responsible aquafeeds that leverage drought-tolerant, lower-input grains like sorghum, thereby enhancing the resilience and sustainability of aquaculture production systems in the face of rising ingredient costs and increasing global demand for sustainable protein.

1.1.1 Feed Processing

Feed processing of aquatic feeds was examined across several chapters (Chapters 2, 3 5, and 8) to evaluate how different raw materials and processing conditions affect pellet quality and energy efficiency. In Chapter 2, the focus was on how ingredient particle size (fine, medium, and coarse grinds of sorghum and wheat) impacted extrusion parameters such as specific mechanical energy (SME), pellet expansion, and bulk density, as well as pellet quality metrics like durability, water stability, and water absorption. The aim was to determine optimal grind sizes that enhance both feed quality and processing efficiency while supporting sorghum's potential as a lower-cost, drought-tolerant alternative to wheat.

Chapter 3 expanded this focus to examine how varying levels of preconditioner thermal energy influenced the physical quality of sinking shrimp feed. By adjusting the

specific thermal energy (STE) input, the study assessed how starch gelatinization and other extrusion outcomes varied between wheat and sorghum. Higher preconditioning intensities were expected to boost gelatinization, particularly for sorghum, reducing SME demand and improving final pellet quality.

Chapter 5 further explored how different grain types and starch contents impact extrusion dynamics in rainbow trout feeds. By comparing energy usage (STE, SME), pellet durability, floatability, and expansion, this chapter highlighted how starch composition and kernel properties alter processing behavior.

Chapter 8 continued this trajectory by incorporating sorghum distillers dried grains (sDDGs), measuring how rising sDDG levels and adjusted preconditioning impacted both extrusion energy and pellet characteristics such as water stability and expansion.

1.1.2 Aquatic Animal Performance

Growth performance and nutrient digestibility were analyzed in Chapters 2, 4, and 5. In Chapter 2, Nile tilapia were fed diets with varying particle sizes of sorghum and wheat to evaluate how physical feed properties, modified through grind size, impacted in-vivo outcomes. Parameters such as weight gain, feed conversion ratio (FCR), and apparent digestibility coefficients were tracked to connect processing adjustments to biological responses.

Chapter 4 focused on Pacific white shrimp and assessed how thermal energy during extrusion influenced starch gelatinization and, in turn, shrimp growth and nutrient

digestibility. The hypothesis was that sorghum's performance could improve with higher thermal inputs, making it competitive with wheat despite its typically lower starch digestibility.

In Chapter 5, rainbow trout served as the model species for evaluating how different cereal grains, including three sorghum varieties and wheat, influenced feed intake, FCR, and tissue yields. Differences in starch type (amylose vs. amylopectin) were examined for their impact on digestibility and energy availability, with a particular focus on whether low amylose content sorghum varieties could enhance growth performance relative to conventional red sorghum or wheat-based diets.

1.1.3 Drying of Aquatic Feeds

The drying of aquatic feeds was researched in Chapters 6 and 8. Chapter 6 concentrated on energy use during the post-extrusion drying stage. It quantified how dryer feed rates, used to simulate changes in bed depth, influenced thermal efficiency, energy consumption, and pellet uniformity in sorghum-based feeds. For wheat-based diets, different drying temperatures were tested to determine their effects on gas usage, moisture removal, and final pellet quality.

This study developed an energy balance model to track steam, air, and feed contributions to total drying energy and linking thermal processing choices directly to final pellet attributes like bulk density and durability. Trade-offs between throughput, energy

efficiency, and product quality were analyzed to inform practical decisions in commercial aquatic feed drying systems.

Chapter 8 also included a drying component, investigating how rising inclusion levels of sorghum distillers dried grains (sDDGs) affected drying efficiency and product uniformity. By tracking changes in moisture loss and thermal input, the study examined how sDDG-rich formulations responded to post-extrusion drying, particularly when preconditioning parameters were adjusted. This provided insights into how sDDG levels interact with drying conditions to influence both pellet integrity and thermal load, adding to the broader understanding of sustainable processing with alternative ingredients.

1.1.4 Drying of Aquatic Feeds

Environmental impact assessments were performed in Chapters 7 and 8 using cradle-to-gate life cycle assessment (LCA) methodologies. Chapter 7 evaluated the full environmental profile of wheat- and sorghum-based extruded feeds formulated for tilapia, trout, and shrimp. By integrating detailed processing energy data, particularly grinding and extrusion SME and STE, along with grain production inputs, this study calculated metrics like global warming potential, freshwater ecotoxicity, eutrophication, and smog formation.

The LCA used one metric ton of animal weight gain as its functional unit, allowing for a direct, performance-linked environmental comparison between cereal grains. Sensitivity analyses explored how variations in steam use, grain yield, and electricity impacted outcomes.

Chapter 8 extended the LCA approach to assess how increasing levels of sDDGs in extruded aquafeeds altered the environmental footprint. The study evaluated operational energy changes and linked them to shifts in GWP, eutrophication, and toxicity metrics, offering a holistic view of sDDG feasibility from both an economic and sustainability standpoint. Thermal process inputs during both extrusion and drying were factored into the LCA, ensuring a complete energy-environment linkage.

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Chapter 2 - Impact of particle size on physical quality and in-vivo performance of grain sorghum-based aquatic feed for tilapia

Oreochromis niloticus

Abstract

This research focused on grain sorghum as an ingredient in floating tilapia feed and studied the impacts of particle sizes on final product qualities and animal performance. As particle size of the diets decreased, extruded floating feed expansion increased, and bulk density decreased off the extruder (452.5 to 367 g/L). Grain source also impacted pellet durability, with sorghum-based diets having a statistically significant higher PDI than wheat diets on average (98.76% vs 98.39%). Tilapia fed the sorghum-based diet with a medium grind had the lowest feed conversion ratio (1.03), while fish fed the wheat-based fine grind diet had the highest (1.13). These results indicate that grain sorghum can successfully be incorporated into Nile tilapia diets with positive effects on both physical feed qualities, as well as growth rates of the fish.

2.1 Introduction

Aquaculture is defined as the rearing of aquatic animals for food purposes. Globally, the production of aquatic animals in 2020 was 178 million tons, with 88 million (49%) produced through aquaculture [41]. Total aquatic production is valued at USD 406 billion, with aquaculture's share of that total being USD 265 billion. Aquaculture's share of the total production is rapidly growing; capture fisheries have largely held steady since around 1990, so the growth in recent years has come from aquaculture. Overfishing of wild

fish stocks has led to a rapid increase in aquaculture production to meet the growing demand for animal protein. Aquatic animals (farmed and caught) provide 17% of global animal protein, and over 3 billion people rely on fish for at least 20% of their daily protein intake [42]. From 1961-2019, global fish consumption has increased at an average annual rate of 3.1%, twice that of the average annual population growth (1.6%) [42]. This increase is higher than that of all other animal protein foods, including meat and dairy products, which increased by approximately 2.1% annually [41]. It is likely that aquaculture production will sustain the world's population growth as it is one of the few food sectors that outpace population growth rates. Because of that, the World Bank predicts aquaculture production will reach 93.6 million tons in 2030 [9]. More recently, world production of aquatic animals grew at an average rate of 5.3% from 2001-2018 [41].

Compound feeds for aquaculture vary in ingredient usage and production methods. Traditionally, most feeds are produced using a pellet mill or extruder. Pellet sizes vary; micro feed diameters under 1.5 mm present unique processing problems, while more traditional feeds can range from 2mm to 12 mm in diameter. Feeds for aquaculture rely heavily on fishmeal for protein requirements and for its “large quantities of energy per unit weight” [25], but due to high costs and limited supplies due to increasing demand and overfishing, replacing fishmeal with alternative protein and energy sources is vitally for the long-term stability of the industry. Much of the research focus has been alternative plant proteins, but this research is focused on cereal grains as a carbohydrate and energy source. Carbohydrates have a ‘protein-sparing’ effect in aquatic diets, as the use of a non-protein energy source to meet basic energy needs enables dietary protein to be ‘spared’

and used for growth and tissue repair [37]. As more and more aquatic feed is created through extrusion, starch content is especially important due to its role as a binding agent and assisting with important pellet qualities such as durability and water stability [33]. Currently, the industry has a wide-spread anti-GMO sentiment; this has prevented the use of traditional cereal grains that are commonly used in animal feeds, such as corn. Because of this, wheat is a very common ingredient in aquatic feed formulations; not only does it provide a source of energy in the diet, but it has important functional properties as well through the inclusion of starch. As most aquatic feed is produced through extrusion, starch serves an important role in processing by binding the pellet together and preventing disintegration in the water, thereby preserving water quality and preventing health issues in the animal. Unfortunately, wheat is an expensive ingredient, and the demand for feed is rising quickly. Currently, 70% of global aquaculture relies on commercial compound feeds, with only 27.8% of the industry raising fish without traditional feeds. From 2021 to 2022, the aquatic feeds industry grew 2.72%; poultry grew at 1.27%, layers at 0.31%, and all other food animals experienced negative growth [17].

Potential cost savings exist if commonly used grains such as wheat are replaced with a cheaper and more sustainable source of starch such as grain sorghum. With the increasing number and severity of drought conditions, as well as the declining water levels in aquifers such as the Ogallala, promoting drought-tolerant crops such as sorghum is of vital importance. At low water use, sorghum has been shown to have better yields per acre than maize [43]. Additionally, sorghum is a non-GMO grain, which is important to consider when assessing its value. Globally, GMO's are divisive, facing particularly strong

opposition in the European Union and this stance has only gotten worse over the last 15 years [40].

Grain sorghum is high quality energy source that has the potential to be a key component of fish feeds across a wide range of species, but more research is needed to evaluate the nutritional benefits, cost of feed production, and processing parameters. Nutritional content of sorghum varies across varieties, but accurate measurements exist; starch (70.1-72%), crude protein (11.0-12.8%), total dietary fiber (8.4-10.9%), fat (3.2-3.5%), and ash content (1.5-1.9%) [21]. The high starch content is ideal for aquatic feed applications. Limited research has been done on the use of sorghum in tilapia diets, but some research does exist for shrimp. Research conducted at Kansas State University has shown grain sorghum DDG's to be a viable ingredient in aquatic feed for shrimp [4]. As stated, wheat is a common ingredient in aquaculture feeds for various reasons; these reasons range from being the most readily available ingredient at hand, to the binding capability in extruded or pelleted products, and its status as a non-GMO grain.

Particle size after grinding is an important consideration when producing aquatic feed. Discussions with industry have led us to understand that producers prefer a finely ground particle size because of the commonly held belief that finely ground ingredients have a positive correlation with digestibility and homogeneity of the diet. This correlation does exist in traditional livestock such as swine [22], but research that supports this belief is limited in the aquatic industry. Feed mills would prefer to grind to the largest possible particle size to reduce production costs and for the benefits in material handling that come with larger particle sizes. A 2020 review by Glencross et al. [16] of ingredient strategies in

the industry found that “most studies proved little indication of particle size for any of the ingredients use.” Further, particle size of the raw material being processed is important. It can affect the rate of starch gelatinization during processing and other downstream qualities such as pellet expansion and density. Typically, smaller particle sizes lead to a greater degree of starch gelatinization than it does for larger particle sizes [10].

Extrusion is quickly becoming the primary processing technology for producing aquatic feeds. As feeding strategies evolve, floating feed is becoming more important for producers to be able to judge animal feed consumption and to minimize the negative impacts on water quality. The raw material is combined with processing inputs such as heat, water, steam, pressure and mechanical shear. The mixed diet is transformed into a viscous dough before exiting the extruder through the die holes, resulting in a completely cooked pellet. This energy transfer is relatively fast and is what sets extrusion processing apart from traditional feed production methods such as a pellet mill [32].

Energy transferred into the material is composed of two forms: specific mechanical energy (SME) and specific thermal energy (STE). These two inputs are important metrics for energy use per kg of feed and drives the extrusion cooking process [26]. SME is defined as the energy produced by the friction generated between the material, screw profile, and barrel [20]. STE primarily occurs in the preconditioner, and only in the barrel to a lesser amount. It is a result of the temperature change and heating of the raw diet through the addition of steam in the preconditioner barrel [29].

Aquatic feeds have an additional parameter to consider that traditional livestock feeds lack; the density of the feed and its ability to sink or float accordingly. Depending on

the aquatic species being fed, sinking or floating requirements must be met to deliver the feed to the animal in the quickest manner possible. Pellet expansion is the natural outcome of the extrusion process due to the amounts of heat and pressure involved at the die; yet the amount of expansion is controllable. Expansion depends on three primary components: the diet composition, thermal and mechanical energy inputs, and the level of pressure at the die. As the feed exits the die, the molten mash goes from a highly pressurized environment to an ambient environment with very little pressure; this leads to an instantaneous pressure drop causing the superheated liquid water inside the dough to flash evaporate into steam, causing the pellet to expand rapidly. As finer particle size has been shown to lead to a great amount of starch gelatinization, smaller particle sizes should also lead to more expansion of the pellet.

Based on this review, it was hypothesized that a more intense particle size reduction would lead to an increase in mechanical energy usage during extrusion due to the increase in viscosity during processing, as well as a greater degree of pellet expansion in both wheat and sorghum-based diets. Limited research exists regarding grain-sorghum based feeds in aquaculture diets, but due to its nutritional profile and ample starch content, it was hypothesized that sorghum-based diets would perform similarly to wheat-based diets fed to Nile tilapia. The specific goals of this study was to evaluate the impact of sorghum inclusion on the extruder performance, pellet qualities and growth performance in Nile tilapia, and to determine how particle size reduction impacts extrusion mechanical energy, pellet qualities and growth performance in sorghum and wheat-based diets for Nile Tilapia.

2.2 Materials and Methods

2.2.1 Experimental Design

The experiment was designed as a 2x3 factorial with two grain types (wheat and red sorghum) and 3 hammermill screen sizes (0.61 mm, 1.02 mm, 1.27 mm) creating 6 experimental treatments; a coarse, medium and fine grind for both wheat and sorghum based diets.

2.2.2 Formulas

All diets, both wheat and sorghum-based, were formulated to be nutritionally complete and balanced, with approximately 36% crude protein and 8% crude fat according to the dietary requirements of Nile Tilapia, as shown in Table 2.1. Grain sorghum was sourced from a regional sorghum processor (Nu-Life Market, Scott City, KS, USA). The Empyreal 75 (corn protein concentrate) was sourced from a food ingredient corporation (Cargill, Inc., Wayzata, MN, USA). Vitamin and mineral premixes were sourced from an aquatic nutrition company (Ziegler Bros, Inc., Gardners, PA, USA), and all other ingredients were sourced through a local feed mill (Fairview Mills, Seneca, KS, USA). Major ingredients were sampled and analyzed for percent dry matter, crude protein (AOAC 990.03, nitrogen combustion method, [6]), fat (AOAC 920.39, ether extract method [6]), ash (AOAC 942.05 [6]), and total dietary fiber (AACC method 32-07 [1]) through proximate analysis conducted by SDK Laboratories (Hutchinson, Kansas). Formulas were then adjusted to account for the true nutritional value of the major ingredients to achieve similar crude protein and lipid contents. Because of this, ingredient inclusion levels were slightly different in each formula to account for the nutritional differences between wheat and sorghum. Sorghum

was included at 32.24%, while wheat was included at 32.32%. Soy oil inclusion was 3.52% in the wheat-based diet, and 3% in the sorghum-based diet. Soybean meal inclusion was 44.0% and 44.6% in the wheat and sorghum-based diets, respectively, to account for protein differences in the whole grains.

2.2.3 Grinding, Energy Collection, and Mixing

Whole grains (wheat and sorghum) were ground using a pilot scale hammer mill (Model D Comminuter, Fitzpatrick, Westwood MA, USA). The initial grain grinding was done using a 1.65 mm screen with round openings to break the grain kernels. A second grind followed using a screen matching the desired particle size. Both grains were ground using a 1.27 mm screen for a coarse grind, a 1.02 mm screen for a medium grind, and a 0.61 mm screen for a fine grind. Energy used during the grinding process was collected using a three-phase power quality logger (Fluke Corporation, Everett, WA, USA). Grain grinding was done in 200-pound batches to provide replication. The ground grains were then mixed with the other ingredients in a double ribbon mixer (Wenger, Sabetha, KS, USA). Diets were mixed for 3 minutes for mix uniformity. The complete mixed diets were post-ground through the same hammermill using the same screens as stated previously in order to provide a uniform particle size within each diet.

Energy data was collected and analyzed. Energy use in kWhr was divided by throughput in lbs/hr to get kW/lb. This was then further converted into kJ/kg to match energy data that was gathered in the extrusion stage.

2.2.4 Particle Size

Particle size analysis was done using a rotating-tapping sieve system (Ro-Tap, Tyler Co., Mentor, OH, USA) according to the standard ASAE S319.2 [7] method of determining fineness of feed materials by sieving. The Ro-Tap test is done using a series of sieves with decreasing screen size (beginning with a #6 screen and ending with a #270 screen) in the sieve stack. One hundred grams of material were placed on the top sieve and the shaker is turned on and run for 10 minutes. The material that remains on top of each sieve post-test is weighed and recorded for the calculations to be performed. This gives an estimate of particle size distribution, standard deviation, cumulative distribution, and estimated particles per gram. The geometric mean diameter (dgw) of the samples were calculated.

2.2.5 Feed Rate Calibration

Prior to extrusion, flow rate of each formulation delivered by the volumetric feeding system of the extruder was calibrated at three feeder screw speeds, 8, 12, and 15 rpm. Measurements were done in duplicate and averaged over the high, medium, and low hopper fill levels of the live-bottom feed hopper. Formulations were processed using a 37.3 kW (50 hp) pilot-scale single screw extruder (X-20, Wenger Manufacturing, Sabetha, KS, USA) with a screw diameter of 82.6 mm (3.25 in) and L/D ratio of 10:1. The extruder barrel consisted of 6 heads divided into three heating zones, with the inlet head having a smooth liner and rest of the heads having spiral liners. The screw profile (Figure 2.1) had single-flighted full pitch screws in the feed intake section, transitioned to double-flighted half pitch screws for creating compression, and ended with a cone screw at the discharge for directing flow into the die opening. Four steam locks were interspersed between the screw

elements to provide resistance to flow, increase material fill and lead to desired mechanical energy input.

2.2.5 Extrusion

During the extrusion experiments raw material mash in the feed hopper was maintained at medium fill level. The feeder screw speed was set at 10 rpm, which corresponded to dry mash feed rate of 76.25-86.6 kg/h as obtained from the calibration for each formulation. The mash was conditioned to 98-99°C in a Wenger model 2 DDC pre-conditioner, with water and steam injection at 10.34-11.91 kg/h and 12.32-12.85 kg/h, respectively. Water was injected into the extruder barrel at an average rate of 10 kg/h, to achieve an in-barrel moisture of 39-37% wb. The extruder barrel temperatures were set at 50, 70 and 90°C from inlet to discharge, and the screw speed at 414 rpm. A circular cross-section die with 2 mm diameter with nine openings was used to shape the product exiting the extruder, which was cut at a speed of 2141 rpm using a rotary knife system with 6 hard blades. A thermocouple probe was inserted into the material stream immediately downstream of the discharge screw to measure the die temperature. Each formulation was extruded for 25 min. Process data were recorded using a computerized data acquisition system, with the exception of die temperature and extruder motor power consumption data that were recorded using a video camera and transcribed every two minutes. The extruded pellets were conveyed using a negative pressure pneumatic system to a pilot-scale Wenger series 4800 two-pass gas-fired continuous dryer and single-pass cooling

system. Drying temperature and time were 104.4°C (220°F) and 16 min, while cooling was done with room temperature air at for 5 min.

2.2.6 Specific Mechanical Energy

Specific mechanical energy (SME) transferred from the screw to the material during the extrusion process was calculated as described below from the power consumed by the extruder motor measured using a wattmeter [14].

$$SME \left(\frac{kJ}{kg} \right) = \frac{W - W_0}{m_f} \quad (2.1)$$

where W is the power consumed during processing (kW), W_0 is power consumed at no load with the screw running and no material flowing through (kW) and m_f is the dry feed rate or rate of material delivered by the feeder screw in kg/sec as determined using volumetric feed delivery calibration.

2.2.7 Rapid Visco Analysis

The pasting properties of formulations were studied using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Waltham, MA, USA) by measuring viscosity changes in slurry form during a controlled heating and cooling process with the application of moderate shear. AACC standard method 76-21.02 was followed for the test (AACC Method 76-21.02 STD1). Briefly, ~3g of each sample was combined with ~25 ml deionized water to create a 14% (w/v basis) suspension and placed in the RVA. Stirring was carried out throughout the test as specified in the standard. The temperature cycle involved holding at 50°C for 1 min, heating 95°C at 12.2°C per min, followed by holding again at 95°C for 2.5 min, and cooling to 50°C at 11.8°C per min before a final holding period for 2 min.

Parameters such as pasting temperature and peak viscosity were recorded using the pasting profiles that were generated from each test. RVA analyses were conducted in duplicate for each formulation.

2.2.8 Bulk Density

Extruded product bulk density (g/L) was measured by recording the mass of pellets filling a 1-liter cup to the brim. Three replicate measurements were made for both wet product directly off the extruder discharge and dry product after the dryer.

$$\text{Bulk Density} = \frac{\text{mass (g)}}{\text{volume (L)}} \quad (2.2)$$

2.2.9 Pellet Diameter and Length

Twenty representative pellets per treatment were randomly selected and measured with a digital caliper. Diameters and lengths were measured in millimeters (mm) and averaged.

Pellet Durability

Pellet durability indexes of the dried products were determined using the tumble box method as described in Maichel [23]. 500 grams of screened pellets are placed in a metal container with dimensions equaling 30 cm by 30 cm by 12 cm. The box also contains a baffle that is 23 cm long, 5 cm wide, and is centered inside the box diagonally. The box is rotated at 50 rpm for 10 minutes, and then the pellets are removed and screened again.

The equation for pellet durability is:

$$\text{Durability} = \frac{\text{Weight of pellets after tumbling}}{\text{Weight of pellets before tumbling}} \quad (2.3)$$

2.2.10 Water Absorption

Water absorption of the dried pellet was measured as follows. Ten pellets were randomly selected, weighed, and soaked in 100 ml of water in a glass beaker at room temperature for twenty minutes. At the end of the soaking time, the water was gently decanted, and excess water was blotted off the surface of the pellet using a paper towel. The wet sample was then weighed. Water absorption was defined as the difference between the two weights as a percentage.

$$\% \text{ Water Absorption} = \frac{(\text{Post soak weight}) - (\text{Pre soak weight})}{\text{Pre soak weight}} \times 100 \quad (2.4)$$

2.2.11 Water Stability

Water stability of the dried pellet was measured using a modified method of the version described by Adedeji et al. [4]. 5 grams of each treatment were weighed and placed in a glass beaker. Each container was filled with 100 ml of distilled water at room temperature. The pellets were left to soak for one hour, gently decanted from the container, and dried in a convection oven for 24 hours at 90°C. Weight was collected post-drying, and the difference in mass pre-soak is defined as how water stable it is, or how much mass remains after being soaked in water.

$$\text{Water Stability} = \frac{\text{Post soak weight}}{\text{Pre soak weight}} \times 100 \quad (2.5)$$

2.2.12 Sinking Percentage

Fifty pellets of each treatment were placed in excess water at room temperature. After 1 minute, the pellets that sank below the surface of the water were counted.

2.2.13 Growth and Digestibility

A 12-week growth trial was conducted to compare the effects of different ingredient particle sizes in wheat and sorghum-based practical diets for Nile tilapia. Diets were top-coated with 2% fish oil (based on final, coated weight) prior to feeding. Proximate composition analysis for all experimental diets were analysed at MidWest Laboratories, Inc., Omaha, NE, USA (Table 2.2 and 3).

A growth trial was conducted at E.W. Shell Fisheries Center at Auburn University, Auburn, Alabama in an indoor recirculation system for both trials. Twenty-four 57-L glass rectangular tanks connected to a common reservoir tank (800-L) were used, with twenty juvenile Nile tilapia (mean initial weight = 3.83 ± 0.03 g) that were batch-sorted (group weighed) to uniform size and randomly stocked into each tank. Four replicate groups per dietary treatment were randomly assigned and offered to fish divided into two equal feedings by weight per day. Fish were counted and weighed every other week to adjust the daily feed ration, which was calculated based on expected growth, body weight, and feed response. At the end of the 12-week growth trial, fish were counted, and the group was weighed by replicate tank to determine mean final biomass, final weight, survival, percent weight gain, and feed conversion ratio (FCR). Fish were packed in sealed bags and stored in a freezer (-20°C) for proximate analysis.

Apparent digestibility coefficient for dry matter, protein, energy, and amino acids were determined by using Titanium oxide as an inert marker (1% of the coated weight). Digestibility coefficients of test diets were determined using groups of 20 fish weighing approximately 81 g. Fish were allowed to acclimate to the test diets before starting

the collection of feces. Prior to each feeding the tanks and fecal settling chambers (FSC) were cleaned. Fish were offered two feedings and all feces collected using a settling system. Samples were collected for several days until a suitable quantity was obtained for analyses (~1 g dry weight). Daily samples were pooled by tank and three replicate aquaria (n=4) were utilized for each treatment. Feces were stored in sealed plastic containers and stored in a freezer. Moisture (AOAC 930.15), dry matter, crude protein (AOAC 990.03), total energy, and amino acids were determined for the fecal and diet samples according with established procedures. Total energy content was calculated using a micro-calorimetric adiabatic calorimeter bomb using benzoic acid as standard (Model 1425, Parr Instrument Co. Moline, IL, USA). Titanium oxide content analysis followed Short et al. [38] procedures. Apparent digestibility coefficients of the dry matter, protein and energy for each diet were calculated according to Cho et al [11] using the following formulas:

$$ADMD (\%) = 100 - [100 \times (\% TiO_2 \text{ in feed} / \% TiO_2 \text{ in feces})] \quad (2.6)$$

$$APD \text{ or AED } (\%) =$$

$$100 - [100 \times ((\%TiO_2 \text{ in feed} \div \%TiO_2 \text{ in feces}) \times (\% \text{ nutrient feces} \div \% \text{ nutrient feed})] \quad (2.7)$$

Where ADMD is apparent dry matter digestibility, APD is apparent protein digestibility, AED is apparent energy digestibility, and TiO₂ is titanium oxide.

Water Analysis

Dissolved oxygen (DO) was maintained near saturation using air stones in each culture tank via a common airline connected to a regenerative blower. Dissolved oxygen, salinity and water temperature were measured twice daily using a YSI-2030 Pro digital

oxygen/temperature meter (YSI Corporation, Yellow Springs, Ohio, USA), and total ammonia N (TAN) and nitrite-N were measured twice per week using YSI 9300 photometer (YSI, Yellow Springs, OH). The pH of the water was measured two times per week during the experimental period using a pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA).

During the growth period for Trial 1, DO, temperature, salinity, pH, total ammonia nitrogen (TAN), and nitrite were maintained within acceptable ranges for freshwater fish culture at 6.42 ± 0.06 mg/L, 27.52 ± 1.21 °C, 3.36 ± 0.06 g/L, 6.33 ± 0.16 , 0.91 ± 0.22 mg/L, and 0.41 ± 0.24 mg/L respectively.

2.2.14 Statistical Analysis

Statistical analysis was done using SAS software (SAS, Cary, NC, USA) and a Two-Way ANOVA model with Tukey's Test to determine the p-values, significant differences (significance level of 5%) and the interaction effects.

All growth and digestibility data were analyzed using SAS (V9.4, SAS Institute, Cary, NC, USA). Growth indices of fish were analyzed using one-way ANOVA to determine significant differences ($P < 0.05$) among treatments followed by Tukey's multiple comparison test to evaluate significant differences between treatment means.

2.3 Results and Discussion

2.3.1 Grinding and Particle Size

Average particle sizes were impacted by the grain that was being ground as seen in Table 2.4. No specific particle size was targeted; instead, grinding conditions were kept the same across all treatments, resulting in differences in final particle sizes as each grain ground differently. Wheat ground through a 0.61, 1.02, 1.27 and 1.65-mm hammermill

screen size resulted in average particle size of 196, 255, 289, and 380 microns, respectively. Sorghum ground through the same screens (finest to coarsest) resulted in average particle sizes of 240, 290, 348, and 390 microns.

Particle size between the mixed wheat and sorghum-based diets when ground through the same screen differed as seen in Table 2.5, largely due to the differences in grinding of the raw grains. The Tilapia-Wheat (TW) and the Tilapia-Sorghum (TS) diets ground through the smallest hammermill screen size (0.61 mm) had average particle sizes of 203 and 270 microns, respectively. That difference also existed in diets ground through the 1.02 mm screen, but particle size differences disappeared when comparing the diets ground through the 1.27 mm screen.

As a result of this, particle sizes are not directly comparable across all treatments. The sorghum-based diet ‘fine’ grind resulted in a particle size of 270 microns, whereas the wheat-based diet with the most similar particle size distribution was the ‘medium’ grind through the 1.02 screen. Ultimately, three distinct particle sizes were created for each grain, which was the goal of this study. Particle size is an important component in the swine and broiler industry, having ramifications on animal health, growth rates, and fecal production [28], but that relationship has never been established in aquatic species such as tilapia regarding raw ingredient particle size. Previous research that refers to ‘particle size’ does exist [8], but it is important to note that those studies use particle size to refer to the size of the extruded pellet, not the size of the ground ingredients. Additionally, grinding of the whole grain before extrusion is critical; without it, product will not be able to exit the smaller die. Having a uniform particle size also helps ensure a uniform mix when blending

the diets [5]. Large particles tend to self-segregate during agitation, preventing a uniform mix. Due to the small pellet sizes necessary to feed aquatic species, a very small particle size is required to ensure a smooth extrusion process. Large particles can block and plug the die holes at the end of the extruder, leading to increased pressures, decreased throughput and ultimately, complete extruder stoppage resulting in time lost through repair and increased costs.

2.3.2 Rapid Visco Analysis

RVA provides an idea of how easily a material gelatinizes. Pasting properties of raw materials are indicators of how the starch transforms during the extrusion process of cooking and shearing. A higher RVA pasting temperature indicates that the starch is more resistant to gelatinization [36]. As the RVA tests progress, starch granules present in the grain absorb water, and as heat is applied, swell rapidly (Hung & Morita, 2005). The temperature at which viscosity rapidly increases is the pasting temperature. Grain sorghum pasting temperatures (in °C for fine, medium, and coarse grind) were 76.6, 79.8, and 80.7; wheat pasting temperatures were 67.8, 68.5 and 69.35, respectively, for each grind size. These results are similar to existing literature; Pourmohammadi et al. [31] reported a pasting temperature of 71.3°C for native wheat, with no indication of particle size. Maichel [23] reported pasting temperatures of approximately 78-79°C for grain

sorghum. Particle size did have an impact on both pasting temperatures and peak viscosities, as shown in Figure 2.2.

2.3.3 Extrusion Processing

Specific mechanical energy (SME) is the main mechanism behind product expansion and the degree of product cook. It is defined as the amount of energy per unit of mass (kJ/kg). SME values ranged from 358.8 to 267.6 kJ/kg depending on the particle size and grain source. For both grain sources, as particle size increased, SME values decreased; 351 to 290.5 for wheat diets, 358.8 to 267.6 for sorghum diets. Extrusion processing parameters can be found in Table 2.6. Coarser particle sizes lead to a lower SME because it is likely that the coarseness of the particle inhibits water absorption in the barrel, generating a lower viscosity, which in turn also lowers the motor load and then the SME. This was found by Mathew [24] when working with corn in pet food; Mathew found that a coarser, or larger, ingredient particle size lowered the dough viscosity which lowered the motor load which, consequently, resulted in a lower SME. Finer particle sizes create a more uniform dough and more viscosity, leading to increased SME and more product expansion. Die temperatures also follow this trend, with finer particle sizes having a higher die temperature due to the increased friction and energy present in the barrel. Except for coarsely ground diets, sorghum-based diets generated more mechanical energy than wheat-based diets. Notably, this runs counter to what is expected based on particle size analysis results. The finest ground sorghum diet had a particle size of 270 microns in comparison to the 203 microns of the finest ground wheat diet; if SME simply increased as particle sizes decreased, sorghum-based diets would be expected to have a lower SME.

Since this is not the case, it is likely that there is some underlying mechanism regarding ground sorghum rheology that is increasing the SME during the extrusion process. RVA results further confirm this; as particle size decreased, pasting temperatures decreased as water was more easily able to penetrate the granules due to increased surface area. Similarly, peak viscosities increased in both wheat and sorghum grains as the particle size decreased. This trend replicates what was seen in the SME calculations; as particle size decreased, viscosity of the raw material increases, which leads to higher mechanical energy inputs due to frictional energy from the screw.

2.3.4 Extrudate Properties

Bulk Density measurements off the extruder (OE) were taken by filling a 1-liter cup (known weight) over the rim of the cup, then striking off the excess feed that is over the rim of the cup. The full cup was weighed on a scale and the weight was recorded in grams/Liter (g/L). The mass is in grams, and the known volume is in Liters. As particle size increased, bulk density decreased. Finer particle sizes lead to greater expansion, greater pellet diameter, and a lower bulk density. Significant differences were found between treatments in off the extruder (OE) bulk density, pellet diameter, and pellet length. These values are summarized in Table 2.7. Sorghum had a larger bulk density (g/L) and a smaller pellet diameter and expansion (in mm) than wheat diets, except for the fine grind. Pellet diameter and expansion greatly increased at that point, with bulk density drastically decreasing. This is likely due to how the starch granules are present in grain sorghum. Starch exists and is tightly bound in the protein matrix within the grain [35]. It is likely that as particle size decreased, more of the starch was released from that matrix and was able to fully hydrate

and gelatinize, leading to a greater expansion upon exiting the die. Sectional Expansion Index is a useful way to quantify the degree of expansion in the radial direction when compared to the cross section of the die used for processing. It is a direct measure of expansion as opposed to a measurement of a physical pellet. For wheat, little variation or trends existed with SEI ranging from 1.85 to 2.05, despite the differences in SME across the three grind sizes. For sorghum however, as the particle size decreased, SEI went up (1.45 to 2.15). This follows and confirms previous data; finely ground sorghum diets had the lowest bulk density, the largest pellet diameter and length, and the highest SME, all of which indicate a greater degree of expansion.

2.3.5 Pellet Durability

PDI's were determined using the standard tumbler box method modified with the addition of three 1/2" hexagonal nuts, as the extruded feeds were smaller than the screen size available for the Holmen Air Tester. Particle size had no impact on PDI; it is likely that the extrusion process creates sufficient gelatinization for pellet durability across all particle sizes. Grain source, however, did impact particle size. Although the numbers are similar, there was a statistically significant difference in PDI; wheat diets had an average PDI of 98.39% and sorghum diets had an average PDI of 98.80%. PDI results are shown in Table 2.8. Pellet durability index is an important indicator of feed quality as the generation of fines is a significant problem in the industry due to widespread use of pneumatic conveying [2], which in turn contributes to poor water quality, increased costs and labor at the farm, and decreased health and potential mortality issues for the fish [3]. Feed handling in aquaculture differs from traditional animal feeding. Traditionally, fines were

generated through abrasion in the bags as they were handled. For aquaculture, most feed is pneumatically conveyed to the fish; this generates more fines through significant impact force [2]. Several methods exist to measure PDI; unfortunately, none of them provide a complete picture. The tumbler box test is the industry standard domestically, but it has trouble differentiating between extruded aquatic feeds due to the increased binding ability of the extruder. The Holmen air test is another, but a significant number of aquatic feeds are smaller than the screen size present, rendering the test unable to be used in many cases. A new method for testing durability is called the DORIS (Durability on a Realistic Test [3], but use of it is very regional, and it is largely unknown. While the differences in results in this study were small, they were statistically significant. More importantly, this difference appeared with the tumbler test, which has historically had trouble differentiating between aquatic feeds [39]. It is likely that if the feed was large enough to be tested in a Holmen, or if access to a DORIS tester was possible, this difference would be even greater.

2.3.6 Water Absorption

Water absorption is an important metric, representing possible nutrient loss through leeching and increased pellet size as water is absorbed, causing possible feeding issues. While there was not a significant interaction between grain source and particle size, individual interactions were significant. Wheat-based diets were more prone to water absorption than sorghum-based diets, with significant differences in the amount absorbed (334% to 256%), and as the particle size decreased, water absorption significantly increased (258% to 329%). This data is shown in Table 2.9.

Feeds were missing the 2% of fish oil that was top coated before feeding. It is likely that, as oil is hydrophobic, water absorption values would decrease. Differences in water absorption between treatments are also attributable to the ingredient particle size. A finer particle size in both grains led to a more expanded pellet, and as more expanded pellets have more air pockets in the pellet, more water penetrated the pellet. Additionally, wheat starch is easier to access – it is not bound in the protein matrix like sorghum is. Because the starch is freer, more water is absorbed into the starch than in sorghum, leading to higher water absorption percentages.

Another factor leading to wheat-based feeds absorbing more water is the gluten present in the grain, unlike in sorghum which is gluten-free. Haraszi et al. [19] found that as more gluten was added to bread dough, water absorption increased. Similarly, literature shows that as sorghum flour was added to a wheat-based bread dough, water absorption decreased [27].

2.3.7 Water Stability

Water stability was conducted by determining the loss in mass after a feed was soaked for a set period. If a feed had 60% of its pre-soak mass post soak and drying, then it was determined to be 60% water stable. There were no significant differences between treatments. All diets were physically water stable (~80%) to an acceptable degree after one hour of soak time. All wheat-based diets were 82% water stable. Sorghum based diets increased in water stability as the grind size decreased from coarse to medium to fine; 79, 80, and 82%, respectively. While this appears to be a trend, it was not statistically significant. The starch in sorghum is tightly bound in a particularly strong protein matrix

[35]. It is likely that as the grind size decreased, more and more of the starch was freed from said matrix, enabling a better cook and gelatinization of the pellet during extrusion.

Additionally, tested feeds did not have the 2% inclusion of top-coated fish oil that was applied immediately before feeding trials commences. It is likely that, as oil is hydrophobic, water stability values would increase after oil application.

2.3.8 Sinking Percentage

Aquatic feeds are unique in that, depending on the species being fed, various degrees of floating or sinking feed is preferred. Tilapia will consume both floating and sinking feed, but farmers prefer floating feed to monitor feed intake. All feeds ranged from 98-100% floating, indicating a consistent extrusion process with no deviations.

2.3.9 Growth and Digestibility

The growth and digestibility trials were conducted at Auburn University without any water quality problems or disease occurrences. Water quality was within all suitable ranges for Nile Tilapia. For the growth trial, there were significant differences in final biomass, final weight, and weight gain, but not for weight gain (%), total feed per fish, Feed Conversion Ratio (FCR), and survival as show in Table 2.10. Final biomass values ranged from 1778.6 to 1469.3 grams. Final biomass values were higher for sorghum-based feeds (1778.6-1617.3 g) than they were for wheat-based feeds (1590.9-1469.3 g). Feeds with the smallest raw ingredient particle size had the lowest biomass, with the medium grind diets having the highest final biomass. This trend (sorghum diets outperforming wheat diets; medium grind having the highest values) also exists for final weight and weight gain. Final weight values ranged from 88.93 to 74.42 g, and weight gain values were from 85.08 to

70.61 g. While not statistically significant, these same trends (sorghum over wheat) existed in weight gain by % (2218 to 2031 vs 1985 to 1850), total feed per fish (87 to 84.51 g vs 85.31 to 79.36 g). For sorghum, the highest weight gain was observed in fish fed S1.02, the medium grind. For wheat, W1.02 fish had the highest weight gain for fish fed wheat sources. Conversely, the lowest fish growth performance was observed in fish fed the finest grind, S0.61 and W0.61. There were no significant differences in FCR (1.03 to 1.13) and fish survival (99-100%) between treatments. Highest growth performance was observed in fish fed S1.02, and the lowest performance was observed in fish fed W0.61.

Proximate analysis of the whole-body composition of the tilapia was conducted. As shown in Table 2.11, there were no significant differences between moisture content, crude protein, crude fat, and ash content on a wet basis. Values ranged from 66.18-70.15% for moisture content, 15.4-16.08% for crude protein, 10.03-13.4% for fat, and 2.24-3.53% for ash content. Previous research by Hafedh [18] has shown that dietary protein levels can affect body composition of tilapia. The diet was formulated with 36% crude protein and whole-body composition is in line with what was expected.

Digestibility results (Table 2.12) showed no significant differences between the apparent digestibility coefficients of dry matter (ADMD) of the diets, regardless of grain source or particle size. ADMD values ranged from 77.92 to 81.11%.. There was a significant difference between apparent energy digestibility (AED). Values ranged from 87.15 to 84.09%, with the medium grind of the wheat reporting the highest value (87.15%) and the coarse grind of the sorghum diet reporting the lowest (84.09%). There is a limited amount of research regarding sorghum as an aquafeed ingredient, although there has been

extensive research using sorghum in traditional livestock [34]. Fitzner et al. [13] found that extrusion processing improved nutrient digestibility and performance in finishing pigs. When compared to wheat, research indicates sorghum has a lower digestible protein (wheat – 10.1%; sorghum – 7.3%); increased total digestible nutrients (78 vs 86%, respectively); and more metabolizable energy (2820 vs 3093 kcal/kg). Apparent protein digestibility (APD) was not significantly impacted by grain source or particle size, with values ranging from 92.86 to 91.52%, indicating that it is likely that extrusion processing improved the protein digestibility as shown in other livestock industries. Digestible energy did increase in sorghum diets as the raw ingredient particle size decreased. This is in line with other research. A study by Bazolli et al. [10] looked at sorghum digestibility and particle size in dog diets and found that as particle size decreased, the apparent digestibility of energy and protein increased. It is likely that protein digestibility values were not significant due to a combination of extrusion processing increasing the digestibility and diets not being dependent upon grain as a protein source due to the inclusion of other dietary protein sources.

Aquatic species are very dependent upon protein for growth and normal physiological function. Fish nutrition textbooks show that there are 10 nutritionally essential amino acids (AAs) in tilapia; arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine [15]. The limiting AA in sorghum is lysine [30]. Lysine is present in fishmeal and in soybean meal, but soybean meal can be deficient in methionine [12]. Apparent amino acid digestibility values for the previously listed

essential amino acids were similar across all diets, regardless of grain source or particle size as shown in Table 2.13. Tilapia fed the sorghum-based diets grew significantly more than wheat-based diets, primarily due to increased feed intake. Particle size did not improve final biomass or feed conversion ratios, indicating that grinding to a fine particle size is not necessary from an animal performance viewpoint.

2.4 Conclusions

Overall, these results are very promising for the future of sorghum in aquatic feeds. Sorghum inclusion resulted in no negative impact on physical feed qualities, and in the case of water absorption and pellet durability, it improved physical quality through reduced water absorption and improved durability. Particle size was shown to have significant impact on the physical qualities of the feed, but limited effects on growth rates, offering feed producers a significant opportunity to lower costs by reducing their grinding steps. Finally, dietary sorghum inclusion of approximately 30% did not negatively impact growth rates in juvenile tilapia. In fact, across all particle sizes, sorghum performed better than wheat diets, indicating that grain sorghum is a viable and promising alternative to wheat in tilapia diets. While FCR values were not significantly different, final weights and weight gain per fish were significantly different demonstrating the success of sorghum as an aquatic feed ingredient. Wheat based diets showed slightly higher apparent digestibility of energy, but growth rates and digestibility are not always linked as shown by fish fed the sorghum-based diets having higher final weights. Apparent protein digestibility was not impacted by dietary inclusion of sorghum at approximately 30%.

In conclusion, the results obtained from this study indicate that sorghum holds great promise for the future of aquatic feeds. Its ease of grinding improved physical feed qualities, and the positive impact on growth rates in tilapia make it a viable and promising alternative to wheat. Finally, excessive particle size reduction did not improve fish growth rates or digestibility, offering producers the opportunity to save time and money through improved manufacturing practices.

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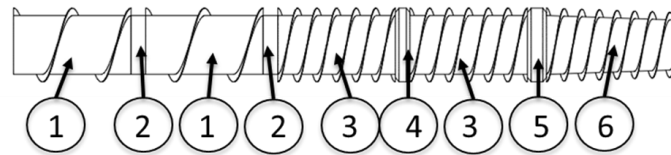
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2.6 Figures and Tables

Figure 2.1 X-20 Screw Profile



Number	Description
1	Single Flight
2	Spacer
3	Double Flight
4	Small Steam lock
5	Medium Steam lock
6	Cone, Double flight

Figure 2.2: RVA of Ground Wheat and Sorghum

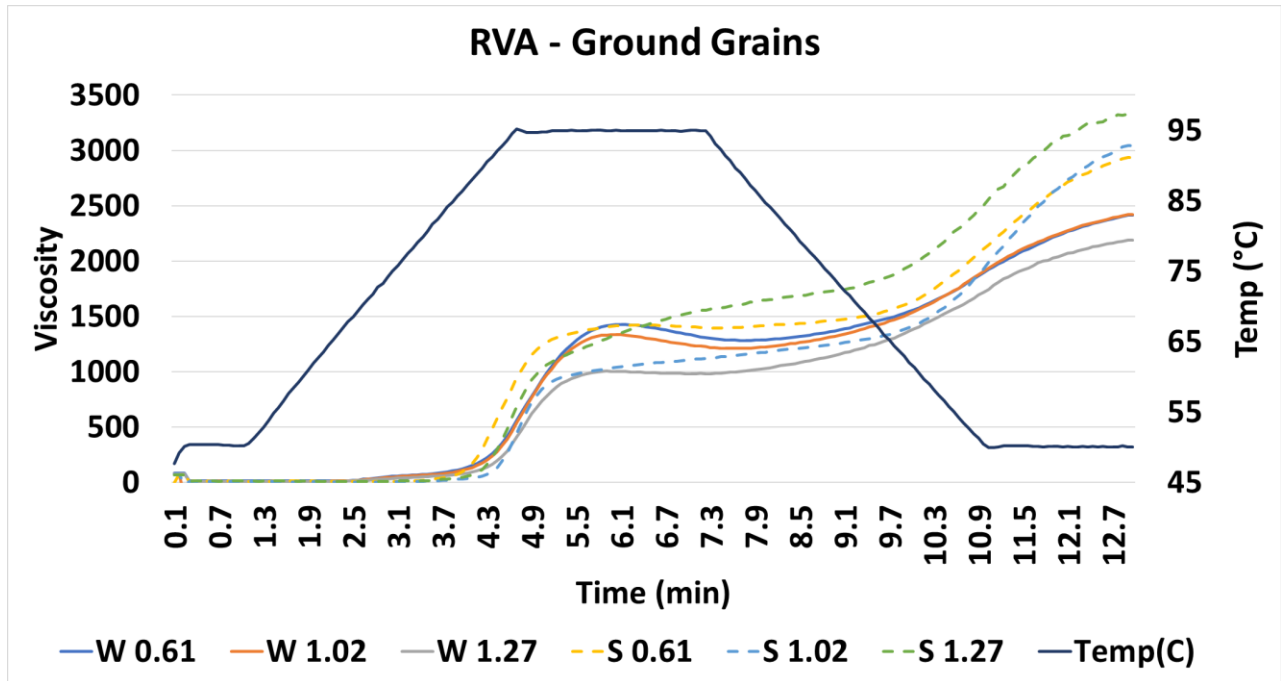


Table 2.1: Dietary formulation for extruded tilapia feed. Percentages of soybean meal, soy oil, and ground grain differed slightly between the two diets.

Ingredients	%
Menhaden Fishmeal	6
Soybean Meal	44/44.60
Corn Protein Concentrate	9
Menhaden Fish Oil	2
Soy Oil	3.52/3.00
Soy Lecithin	1
Ground Wheat/Sorghum	32.32/32.24
Mineral Premix	0.07
Vitamin Premix	0.04
Choline Chloride	0.2
Rovimix Stay-C 35%	0.1
Dicalcium Phosphate	1.75
Total	100

Table 2.2: Proximate and mineral composition of practical tilapia diets with different particle sizes of sorghum and wheat. Values represent the mean of two duplicates.

Proximate composition (g/100 g as is)	S 0.61	S 1.02	S 1.27	W 0.61	W 1.02	W 1.27
Protein (crude) %	37.7	38.25	38.35	37.65	37.9	38.35
Moisture %	5.55	5.425	6.115	4.33	4.78	5.04
Fat %	7.4	7.57	7.465	5.175	7.8	7.395
Ash %	7.23	7.225	7.25	6.94	7.365	7.285
Sulfur %	0.365	0.37	0.355	0.355	0.37	0.365
Phosphorus %	1.03	1.02	0.97	0.945	1.04	1.04
Potassium %	1.31	1.3	1.24	1.25	1.32	1.275
Magnesium %	0.21	0.21	0.2	0.19	0.2	0.2
Calcium %	0.945	0.93	0.855	0.825	0.89	0.95
Sodium %	0.06	0.07	0.07	0.07	0.07	0.07
Iron %	215	218.5	201	189.5	206.5	211.5
Manganese %	50.5	48.45	46.45	54.55	56.85	55.65
Copper %	41.445	45.55	38.7	43.2	42.85	39.7
Zinc %	70.3	72.25	66.15	73.45	74.65	71.05

Table 2.3: Amino acid composition of practical tilapia diets with different particle sizes of sorghum and wheat. Values are the means of two duplicates.

Proximate composition (g/100 g as is)	S 0.61	S 1.02	S 1.27	W 0.61	W 1.02	W 1.27
Alanine	2.09	2.18	2.16	1.91	1.87	1.93
Arginine	2.12	2.14	2.07	2.19	2.14	2.16
Aspartic Acid	3.39	3.5	3.38	3.34	3.34	3.37
Cysteine	0.53	0.58	0.56	0.59	0.57	0.58
Glutamic Acid	7.12	7.44	7.3	7.53	7.5	7.61
Glycine	1.51	1.57	1.58	1.59	1.56	1.58
Histidine	0.87	0.91	0.88	0.88	0.88	0.89
Hydroxylysine	0.02	0.02	0.03	0.03	0.02	0.02
Hydroxyproline	0.11	0.16	0.16	0.11	0.08	0.12
Isoleucine	1.71	1.82	1.76	1.72	1.77	1.71
Lanthionine	0	0	0	0	0	0
Leucine	3.53	3.69	3.6	3.33	3.25	3.36
Lysine	1.87	1.95	1.87	1.91	1.92	1.9
Methionine	0.63	0.65	0.63	0.62	0.61	0.63
Ornithine	0.03	0.03	0.03	0.03	0.03	0.03
Phenylalanine	1.87	1.95	1.9	1.86	1.85	1.88
Proline	2.1	2.21	2.16	2.19	2.14	2.16
Serine	1.55	1.64	1.61	1.53	1.53	1.62
Taurine	0.17	0.16	0.16	0.17	0.17	0.16
Threonine	1.29	1.35	1.31	1.27	1.28	1.32
Tryptophan	0.43	0.42	0.4	0.46	0.46	0.46
Tyrosine	1.35	1.22	1.21	1.38	1.21	1.25
Valine	1.78	1.83	1.77	1.78	1.76	1.74

Table 2.4: Particle size of wheat and sorghum ground through different hammer mill screen sizes. Values represent the percentage of material that remained on the sieve post-test.

Micron Size (%)	Wheat				Sorghum			
	0.61	1.02	1.27	1.65	0.61	1.02	1.27	1.65
2380	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1680	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1191	0.00	0.31	0.00	2.84	0.00	0.10	0.00	1.20
841	0.43	0.60	3.46	16.86	0.30	0.32	3.50	18.00
594	0.53	7.37	18.99	19.17	1.29	12.45	27.01	24.37
420	6.76	22.39	20.32	16.06	15.25	25.62	23.40	15.60
297	23.96	18.12	14.47	11.64	24.70	16.34	11.80	8.60
212	16.93	12.85	10.49	8.40	18.65	11.56	7.38	6.15
150	10.85	8.34	6.40	5.44	19.84	13.74	7.04	5.36
103	26.37	20.06	8.38	7.08	11.79	12.13	12.00	10.75
73	9.13	6.10	10.06	6.97	4.51	4.62	4.14	4.74
53	4.89	3.71	7.15	5.40	3.49	2.98	3.61	4.74
37	0.17	0.18	0.31	0.15	0.17	0.13	0.12	0.46
Average Particle Size	196	255	289	380	240	290	348	390

Table 2.5: Particle size analysis of wheat and sorghum-based tilapia diets ground through three different hammermill screen sizes.

Micron Size	Tilapia - Wheat			Tilapia - Sorghum		
	0.61	1.02	1.27	0.61	1.02	1.27
2380	0.00	0.00	0.00	0.00	0.00	0.00
1680	0.00	0.00	0.00	0.00	0.00	0.00
1191	0.00	0.00	0.00	0.00	0.00	0.00
841	0.00	0.48	1.73	0.52	0.23	1.92
594	0.35	6.50	21.30	1.52	8.75	22.04
420	7.52	22.40	23.40	10.98	24.70	22.40
297	22.68	18.16	14.74	28.61	18.18	13.30
212	17.26	14.44	11.33	33.69	20.29	11.76
150	18.20	16.46	10.65	16.04	18.02	12.30
103	25.40	16.46	12.97	7.08	8.41	14.49
73	4.75	3.65	3.19	1.21	1.28	1.54
53	3.80	1.28	0.60	0.23	0.07	0.18
37	0.04	0.18	0.08	0.13	0.06	0.08
Average Particle Size	203	266	334	270	303	335

Table 2.6: Processing settings for extruded tilapia feed on a Wenger X-20 single screw extruder.

	Wheat			Sorghum		
	0.61	1.02	1.27	0.61	1.02	1.27
Feeder RPM	10	10	9	9	9	9
<i>Preconditioner</i>						
Cylinder Speed	400	400	400	400	400	400
Steam Flow (kg/hr)	12.85	12.79	12.78	12.32	12.34	12.85
Water Flow (kg/hr)	10.34	11.91	11.76	11.14	11.25	11.55
Discharge Temp (°C)	98	99	99	99	98	98
<i>Extrusion</i>						
Screw Speed	414	414	414	414	414	414
Motor Load %	50	47	46	48	47	46
Water Flow (kg/hr)	9.81	9.45	9.14	12.34	8.91	10.73
<i>Temperature</i>						
Zone 1 °C	49	48	49	49	48	49
Zone 2 °C	74	74	74	74	74	74
Zone 3 °C	89	89	89	88	89	89
Die Temp °C	129	123	120	124	122	119
Conehead Pressure PSI	250	250	250	250	250	250

Table 2.7: Physical properties of extruded tilapia feed with different grains and grind sizes.

	Wheat			Sorghum		
	0.61	1.02	1.27	0.61	1.02	1.27
Pellet Diameter (mm)	2.72(B)	2.86(A)	2.73(C)	2.93(A)	2.57(C)	2.41(D)
Pellet Length (mm)	4.06(c)	4.19(c)	4.04(c)	6.28(a)	4.86(b)	4.89(b)
OE Bulk Density (g/L)	444.00(a)	423.00(a)	430.50(a)	367.25(b)	435.00(a)	452.50(a)

Pellet Diameter; particle size*grain P-value = <.0001

Pellet Length; particle size*grain P-value = <.0001

Off Extruder (OE) Bulk Density; particle size*grain P-value = 0.0031

Table 2.8: Pellet durability as measured by the rotating tumbler box method.

Pellet Durability Index	
Wheat	98.39 (a)
Sorghum	98.76 (b)
Grain factor P-value = 0.0104	

Table 2.9: Water absorption of extruded tilapia feed with different grain sources and particle sizes.

Grain Source	Wheat	334.43 (a)
	Sorghum	255.67 (b)
Particle Size	Fine	329.28 (a)
	Medium	297.55 (b)
	Coarse	258.32 (c)

Grain source P-value = <.0001; particle size P-value = <.0001

Table 2.10: Response of juvenile Nile tilapia (mean initial weight 3.83 ± 0.03 g) fed diets with different extrusion sizes of sorghum and wheat within a 12-week period. Values represent the means of four replicates.

Diet		Final Biomass (g)	Final weight (g)	Weight Gain (g)	Weight Gain (%)	Total feed per fish (g)	FCR ¹	Survival (%)
D1	S 0.61	1617.3 ^{ab}	81.94 ^{ab}	78.10 ^{ab}	2031	84.51	1.09	99
D2	S 1.02	1778.6 ^a	88.93 ^a	85.08 ^a	2218	86.41	1.03	100
D3	S 1.27	1640.5 ^{ab}	83.09 ^{ab}	79.20 ^{ab}	2036	87.00	1.10	99
D4	W 0.61	1469.3 ^b	74.42 ^b	70.61 ^b	1850	79.36	1.13	99
D5	W 1.02	1590.9 ^{ab}	80.63 ^{ab}	76.77 ^{ab}	1985	85.31	1.11	99
D6	W 1.27	1509.8 ^{ab}	75.49 ^{ab}	71.75 ^{ab}	1918	80.89	1.13	100
PSE ²		29.96	1.52	1.51	37.96	0.92	0.01	0.39
p-value ³		0.0278	0.0454	0.0452	0.0815	0.0716	0.2409	0.8424

¹FCR = Feed conversion ratio = feed offered / (final weight-initial weight).

²PSE = Pooled standard error.

³One-way analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$). Tukey's multiple comparison test was used to determine statistically significant differences between treatment means when there was statistical significance in the ANOVA test ($n = 4$), represented by values with different letters.

Table 2.11: Whole-body composition (on wet weight basis) of tilapia fed diets containing different extrusion sizes of sorghum and wheat. Values represent means of four replicates.

Diet		Moisture %	Protein (crude) %	Fat %	Ash %
D1	S 0.61	68.05	15.40	12.83	3.21
D2	S 1.02	66.18	16.08	13.40	3.19
D3	S 1.27	69.25	16.08	11.38	2.89
D4	W 0.61	69.65	15.55	12.13	2.24
D5	W 1.02	70.15	15.85	10.03	3.11
D6	W 1.27	68.53	15.78	11.33	3.53
PSE ²		0.66	0.21	0.48	0.18
p-value ³		0.3534	0.9409	0.0661	0.5173

²PSE = Pooled standard error.

³One-way analysis of variance (ANOVA) was used to determine significant differences ($P < 0.05$). Tukey's multiple comparison test was used to determine statistically significant differences between treatment means when there was statistical significance in the ANOVA test ($n = 4$), represented by values with different letters.

Table 2.12: Apparent digestibility coefficients of dry matter (ADMD), protein (APD), energy (AED) of the diets offered to Tilapia

Diets		ADMD	AED	APD
D1	S 0.61	78.84±3.31	85.75±0.40ab	92.12±1.28
D2	S 1.02	79.56±0.67	85.53±0.96ab	92.65±0.45
D3	S 1.27	77.92±0.57	84.09±1.03b	91.52±0.69
D4	W 0.61	79.01±0.67	86.10±1.54ab	92.79±0.57
D5	W 1.02	81.11±0.78	87.15±1.00a	92.86±0.24
D6	W 1.27	78.52±0.88	86.00±0.88ab	92.38±0.67

Note: Values from each diet are means and *SD* of four tanks. Values within column with different superscripts are significantly different ($p < .05$) based on one-way ANOVA followed by Tukey's multiple comparison test.

Table 2.13: Apparent amino acid (AA) digestibility for the diets offered to Tilapia

AA	S 0.61	S 1.02	S 1.27	W 0.61	W 1.02	W 1.27
Alanine	92.28±1.63	93.23±0.89	92.47±1.09	93.622±0.55	93.57±0.31	93.46±0.50
Arginine	96.48±0.43	96.36±0.53	96.02±0.29	96.85±0.36	96.73±0.25	96.24±0.31
Aspartic Acid	95.39±0.86	95.67±0.33	95.00±0.38	95.96±0.25	95.80±0.12	95.42±0.34
Cysteine	93.09±1.31	93.92±0.11	93.09±0.60	94.75±0.15	94.37±0.10	94.16±0.46
Glutamic Acid	96.03±0.69	96.41±0.38	95.57±0.54	97.29±0.25	97.14±0.18	96.97±0.30
Glycine	89.14±1.89	89.77±2.43	89.85±1.27	90.91±0.81	91.06±0.68	90.15±0.87
Histidine	95.14±0.81	95.62±0.39	95.16±0.41	96.06±0.40	95.98±0.16	95.72±0.17
Hydroxylysine	62.74±15.16	69.13±9.34	76.03±4.18	82.53±3.98	74.11±4.32	73.06±6.81
Hydroxyproline	59.26±8.78	72.98±9.57	72.70±3.67	66.57±4.37	57.55±4.83	62.82±3.08
Isoleucine	93.42±1.25	94.44±0.25	93.66±0.49	94.68±0.55	94.90±0.24	94.47±0.36
Leucine	93.63±1.26	94.50±0.46	93.66±0.75	94.90±0.51	94.97±0.25	94.93±0.33
Lysine	96.07±0.66	96.35±0.29	96.28±0.19	96.37±0.26	96.26±0.11	95.87±0.16
Methionine	95.19±0.95	95.67±0.48	95.09±0.38	96.01±0.60	95.67±0.11	95.82±0.30
Ornithine	89.72±3.13	93.18±0.22	92.64±0.19	93.00±0.22	90.64±3.33	90.98±3.89
Phenylalanine	93.80±1.11	94.47±0.38	93.72±0.55	94.86±0.49	94.87±0.27	94.74±0.26
Proline	92.53±1.18	93.00±0.97	92.14±0.91	94.31±0.63	94.33±0.24	93.93±0.36
Serine	93.97±1.17	94.91±0.61	94.19±0.72	95.20±0.20	95.07±0.15	94.89±0.36
Taurine	84.01±3.73	82.46±1.30	81.01±1.10	83.68±1.06	85.03±0.94	82.18±1.88
Threonine	89.86±2.17	91.06±0.61	90.26±0.64	90.87±0.42	90.90±0.30	90.81±0.30
Tryptophan	95.61±0.33	95.62±0.14	95.16±0.33	95.99±0.68	95.99±0.26	95.91±0.53
Tyrosine	94.82±0.98	94.13±0.28	93.38±0.48	95.55±0.40	95.00±0.53	94.83±0.44
Valine	92.90±1.51	93.99±0.20	93.16±0.69	94.56±0.66	94.50±0.34	94.29±0.50
Sum AA	94.03±1.09	94.57±0.54	93.88±0.60	95.18±0.40	95.11±0.16	94.82±0.34

Note: Values from each diet are means and $\pm SD$ of (n=4) tanks.

Chapter 3 - Impact of different levels of preconditioner thermal energy on total extrusion processing energy inputs and the physical quality of sorghum-based sinking shrimp feed

Abstract

This study examined the impact of extrusion processing conditions on the physical qualities of wheat and sorghum-based feeds for aquaculture. Specific thermal energy (STE) and mechanical energy (SME) were calculated to determine their impact on starch gelatinization, bulk density, water stability, and other feed qualities. Results indicated that sorghum-based feeds required higher thermal energy values to achieve optimal starch gelatinization compared to wheat-based feeds, which had consistently higher starch gelatinization regardless of STE inputs or decreased total energy inputs. Reduced STE values resulted in increased SME values and a lower degree of gelatinization in sorghum-based diets, which in turn led to increased bulk densities and decreased water stability. Pellet durability was not impacted by energy inputs and was high for all treatments. These findings underscore the importance of energy use in extrusion processing, particularly for sorghum-based feeds where increased thermal energy was necessary to ensure adequate starch gelatinization and water stability. These insights are essential for optimizing feed formulation and extrusion processing to enhance feed qualities and improve sustainable feed manufacturing practices in aquaculture.

3.1 Introduction

As the global population continues to grow, the demand for high-quality protein grows with it, which has led to a 3% annual increase in seafood consumption between 1961 and 2019, a rate that is nearly double that of the population growth (FAO, 2022). Global aquaculture production reached 88 million tons of seafood in 2020, accounting for roughly 50% of all seafood consumed, and this share is continuing to rise due to concerns over depleted ocean stocks. Consequently, production has accelerated and increased at an average rate of 5.3% annually from 2001 to 2018 (FAO, 2020).

Pacific white shrimp are among the most extensively farmed shrimp species in aquaculture (FAO, 2022). Despite aquatic animals, such as shrimp, not having specific carbohydrate requirements, cereal grains are often included in shrimp feed formulations (Romano & Kumar, 2019). Carbohydrates can serve as an energy source for many crustaceans, thereby reducing the amount of dietary protein necessary in the diet (Wang et al., 2015). Importantly, carbohydrate sources can enhance pellet qualities such as water stability and durability by acting as a binder through starch gelatinization (Stone et al., 2003).

Wheat starch has been shown to improve pellet quality by enhancing pelletability and water stability (Tiarniyu & Solomon, 2012), and vital wheat gluten was demonstrated to create a strong pellet with a non-porous surface (Draganovich et al., 2013). The combination of starch and gluten has cemented wheat as the preferred starch source in shrimp feed in terms of pellet quality. Unfortunately, wheat is more costly than other grains

and it has a high environmental impact due to its high inputs such as nitrogen and water use through irrigation.

Grain sorghum, a highly drought-tolerant non-GMO crop, is a promising alternative. When exposed to drought and water stress, wheat suffers high yield loss and crop failure (Hadebe et al., 2017). Under low water conditions, sorghum yields per acre are higher than maize yields per acre (van Oosterom et al., 2021). It is among the five cereal crops produced worldwide, with the U.S. being the largest producer (8.1 metric tons in 2023). It has a high starch (70-72%) and protein (11-12.8%) content (Knudsen et al., 1988). This makes it ideal for extruded feed applications as the high starch content helps hold the pellet together, subsequently improving its water stability (Romano & Kumar, 2019).

Extrusion processing is now the primary method for producing aquatic feeds, offering many advantages over older methods such as pellet milling. Extrusion applies much higher levels of thermal energy, pressure, and moisture, which leads to increased starch gelatinization (Adedeji et al., 2021; Robinette, 1977). Extrusion processing involves preconditioning and mechanical energy through the use of screws (Riaz, 2000). In single-screw extrusion, thermal energy primarily takes place in the preconditioner (Monti et al., 2016; Pachecho et al., 2018). Preconditioning mixes the raw material with water and steam, preheating it and beginning the cooking process and starch gelatinization. The cooking process, and subsequent starch gelatinization, is then completed by mechanical energy in the barrel through the use of rotating screws (Levine, 2014). The thermal energy during preconditioning hydrates and softens the raw materials, which in turn improves final product quality and extruder stability (Guy 2001; Gibson & Alavi, 2013).

Steam conditioning, as well as diet composition, has been shown to have a large impact on pellet quality (Behnke, 1996). Steam conditioning of wheat-based diet fed to nursery pigs led to a higher dry matter digestibility when compared to the non-processed mash, but starch and protein digestibility were largely unaffected; as steam conditioning intensified, however, bulk density of the feed decreased, while pellet durability index and starch gelatinization increased (Lundblad et al., 2011). Ileal digestibility of starch, total indispensable amino acids, lysine, arginine, isoleucine, and threonine was improved through steam conditioning, but there was no evidence to show that increasing precondition temperatures from 47° to 90°C improved digestibility (Lundblad et al., 2012). As starch in cereal grains has been shown to improve physical qualities of extruded feed, the hypothesis of this study was that increasing levels of thermal energy through an increased preconditioner temperature would lead to a higher degree of gelatinization in the final product, resulting in improved pellet qualities such as water stability and durability. The objective of this study was to evaluate the effect of three levels of thermal energy in wheat and sorghum-based diets on the physical qualities of extruded sinking feed.

3.2 Materials and Methods

3.2.1 Experimental Design and Diets

The experiment was designed as a 2x3 factorial using two grain types and three different levels of thermal energy in the preconditioner. The grains selected were whole wheat and burgundy sorghum. Thermal energy was adjusted by varying the steam and water addition to achieve a preconditioner downspout temperature of approximately 95°C, 80°C, and 60°C for a total of six experimental diets.

Diets were formulated to be isonitrogenous and isolipidic with 36% crude protein and 8% crude fat. Dietary formulations and ingredient sources are shown in Table 3.1. All major ingredients underwent analysis for moisture content (AOAC 930.15, oven method), crude protein (AOAC 990.03, nitrogen combustion method), fat (AOAC 920.39, ether extract method), ash (AOAC 942.05, revised ignition method) and total dietary fiber (AACC method 32-07, Lee method) at SDK Laboratories (Hutchinson, Kansas). Formulas were adjusted based on this in order to achieve balanced protein and lipid contents in both the wheat and sorghum-based diets.

3.2.2 Grinding and Mixing

A pilot-scale hammer mill (Model D Comminutor, Fitzpatrick, Westwood, MA, USA) was used to grind the whole grains and mixed diet. Whole grains were initially ground through a screen with a 1.65 mm hole size; a secondary grain grinding step was performed using a 1.02 mm screen. The diets were mixed in a double-ribbon batch blender (Wenger Manufacturing, Sabetha, KS, USA) for 3 minutes each. After mixing, a post-batch grind was performed through the same hammer mill with a 1.02 mm screen.

3.2.3 Particle Size Analysis

Particle size analysis was performed using the ASAE S319.2 standard method (ASAE, 1995) with a rotating-tapping sieve stack (Ro-Tap, Tyler Co., Mentor, OH, USA). The largest sieve used in the stack is a #6 screen and the finest is a #270 screen. Approximately one hundred grams is placed in the top of the sieve stack attached to a shaker which is then turned on for ten minutes. The material on each sieve is measured and the geometric mean diameter (dgw) as well as the geometric standard deviation (sgw) are calculated. The

percentage of particles that pass through each sieve are also calculated. Dgw is calculated in microns, but the sgw is unitless; multiplying and dividing the dgw by the sgw will give you the dgw for the 84th and 16th percentile, respectively. Particle size results are shown in Table 3.2 and Table 3.3.

3.2.4 Feed Rate Calibration

Prior to extrusion, flow rate of each formulation delivered by the volumetric feeding system of the extruder was calibrated at three feeder screw speeds, 12, 15, and 18 rpm. Measurements were done in duplicate and averaged over both high and low fill levels of the live-bottom feed hopper. Formulations were processed using a 37.3 kW (50 hp) pilot-scale single screw extruder (X-20, Wenger Manufacturing, Sabetha, KS, USA) with a screw diameter of 82.6 mm (3.25 in) and L/D ratio of 8:1. The extruder barrel consisted of 6 heads divided into three heating zones, with the inlet head having a smooth liner and rest of the heads having spiral liners. The screw profile (Figure 3.1) had single-flighted full pitch screws in the feed intake section, transitioned to double-flighted half pitch screws for creating compression, and ended with a cone screw at the discharge for directing flow into the die opening. Two spacers and two steam locks were interspersed between the screw elements to provide resistance to flow, increase material fill and lead to desired mechanical energy input.

3.2.5 Extrusion

During the extrusion experiments raw material mash in the feed hopper was maintained at medium fill level. The feeder screw speed was set at 14 rpm, which corresponded to dry mash feed rate of 123.9 – 124.6 kg/h as obtained from the calibration

for each formulation. The mash was conditioned to 58-98°C in a Wenger model 2 DDC pre-conditioner, with water and steam injection at 11-21 kg/h and 5-15 kg/h, respectively. Water was injected into the extruder barrel at an average rate of 11 kg/h, to achieve an in-barrel moisture of 30-31% wb. The extruder barrel temperatures were set at 50, 70 and 90°C from inlet to discharge, and the screw speed at 445 rpm. A drilled die plate with 90 holes of 1.7 mm diameter was used to shape the feed exiting the extruder, which was cut at a speed of 1012 rpm using a rotary knife system with 6 hard blades. A thermocouple probe was inserted into the material stream immediately downstream of the discharge screw to measure the die temperature. Each formulation was extruded for 35 min. Process data were recorded using a computerized data acquisition system, with the exception of die temperature and extruder motor power consumption data that were recorded using a video camera and transcribed every two minutes. The extruded feeds were conveyed using a negative pressure pneumatic system to a pilot-scale Wenger series 4800 two-pass gas-fired continuous dryer and single-pass cooling system. Drying temperature and time were 104.4°C (220°F) and 16 min, while cooling was done with room temperature air at for 5 min.

3.2.6 Specific Mechanical Energy and Thermal Energy

Specific mechanical energy (SME) transferred from the screw to the material during the extrusion process was calculated as described below from the power consumed by the extruder motor measured using a wattmeter (Flory et al., 2023).

$$SME \left(\frac{kJ}{kg} \right) = \frac{W - W_0}{m_f} \quad (3.1)$$

where W is the power consumed during processing (kW), W_0 is power consumed at no load with the screw running and no material flowing through (kW) and m_f is the dry feed rate or

rate of material delivered by the feeder screw in kg/sec as determined using volumetric feed delivery calibration.

Specific thermal energy (STE) input during the extrusion process was calculated for selected treatments from net heat transferred to the material due to steam absorbed during preconditioning, as follows.

$$STE \left(\frac{kJ}{kg} \right) = \frac{Q_{thermal}}{m_f} \quad (3.2)$$

where $Q_{thermal}$ is the net rate of thermal energy or heat absorbed from steam (kJ/s), which was calculated using the below equation from the enthalpies of steam injected and unabsorbed steam lost to atmosphere at the preconditioner discharge.

$$Q_{thermal} \left(\frac{kJ}{s} \right) = (m_s \cdot h_s - m_s \cdot 4.18 \cdot T_r) - (m_{sl} \cdot h_{sl} - m_{sl} \cdot 4.18 \cdot T_r) \quad (3.3)$$

where h_s and h_{sl} are enthalpies of steam injected and steam lost to atmosphere (kJ/kg), respectively, as obtained from the steam tables, m_s and m_{sl} are rates of steam injection and loss (kg/s), respectively, the constant 4.18 is the specific heat of water at room temperature in kJ/kg°C and T_r is room temperature (°C). The rate of steam loss from the preconditioner was calculated using mass balance as described below.

$$m_{sl} \left(\frac{kg}{s} \right) = (m_f + m_s + m_w) - m_f \left(\frac{1-X_{wf}}{1-X_{wpc}} \right) \quad (3.4)$$

where m_w is the water injection rate into the preconditioner (kg/s) and the second term on the right of the equation is the rate of conditioned mass exiting the preconditioner with X_{wf} and X_{wpc} representing the fractional wet basis (or as is) moisture contents of the feed material and the conditioned mass, respectively, measured using oven drying method (135°C, 2 h).

3.2.7 Starch Gelatinization

Starch gelatinization was determined through two methods and in two processing locations. Samples of the mash diet were collected after exiting the preconditioner but before entering the extruder barrel. Extruded product was collected immediately after exiting the die and being cut by rotating knives. Differential scanning calorimetry was performed on the base dry mix and the preconditioned sample to determine the degree of gelatinization as described in Maichel (2021). Approximately 10 mg of the sample (dry basis) is placed in a stainless-steel pan with deionized water on a 1:2 feed/water basis. The sample is allowed to equilibrate overnight. Analysis was performed using a Q100 DSC (TA Instruments, New Castle, DE, USA). The pan is heated in the test chamber from 10° to 160°C at a rate of 10°C/min. The onset, peak, and conclusion gelatinization temperatures, as well as the enthalpy of gelatinization (ΔH). Degree of gelatinization was determined through the following equation:

$$\text{Degree of Gelatinization} = \frac{\Delta H_0 - \Delta H_1}{\Delta H_0} \times 100 \quad (3.5)$$

Where ΔH_0 is the gelatinization enthalpy of the raw diet (J/g) and ΔH_1 is the gelatinization enthalpy of the preconditioned product (J/g). All measurements were performed in duplicate. Starch gelatinization of the extruded feed was determined using a modified version of AOAC 996.11 and the YSI application note 319 by MidWest Laboratories (Omaha, NE, USA).

3.2.8 Bulk Density and Expansion

Extruded product bulk density (g/L) was measured by recording the mass of pellets filling a 1-liter cup to the brim. Three replicate measurements were made for both wet product directly off the extruder discharge and dry product after the dryer.

3.2.9 Piece Measurements

For each treatment, ten bags of pellet samples were collected evenly spaced in time during extrusion and numbered. From each bag, ten pellets were randomly selected and their individual mass, diameters at two perpendicular axes and thickness were measured. These data were used for calculating sectional expansion ratio (ER), specific length (L_{sp}) and piece density ([2]) as described below (Karkle et al., 2012) and an overall average, standard deviation and coefficient of variation calculated for each treatment based on averages for each of the 10 sampling bags.

$$ER = \frac{d_k^2}{d_{die}^2} \quad (3.6)$$

where d_k is the average of the two pellet diameter measurements (cm) and d_{die} is the extruder die diameter (cm).

$$L_{sp} \left(\frac{cm}{g} \right) = \frac{l_k}{m_k} \quad (3.7)$$

where l_k is the pellet thickness (cm) and m_k is the pellet mass (g).

$$\rho \left(\frac{g}{cm^3} \right) = \frac{m_k}{\frac{\pi}{4} d_k^2 \cdot l_k} \quad (3.8)$$

where the denominator on the right of the equation is the pellet volume assuming a cylindrical shape and all symbols are as defined above.

3.2.10 Void Fraction/Porosity

Void fraction of the pellets was measured using a Quantachrome Instruments gas pycnometer (Ultrapyc 1200e). Each sample was ground through a grain grinder for 20 seconds to break the closed cellular structure. This ground product was tested in triplicate using the pycnometer to determine the solid density of the samples. Void fraction was then calculated by dividing the calculated piece density (equation 9) by the solid density obtained in this test.

3.2.11 Floating/Sinking Percentage

Fifty representative pellets from each experimental treatment were immersed in excess room temperature water for one minute. The number of floating and sinking pellets was counted by hand to calculate the percentage of pellets that sank. This was done in duplicate.

3.2.12 Pellet Durability Index

Pellet durability indexes (PDI) of the dried, extruded pellets was determined using the tumble box method as described in Maichel (2021). The test was modified to use two ½” hexagonal nuts. 500 grams of pellets were sifted using a #16 screen and placed in a tumble box that measures 30 cm by 30 cm by 12 cm with a 23 cm x 5 cm baffle that is diagonally centered. The tumble box is rotated at 50 rpm for 10 minutes before the pellets are removed and sifted again. Diets were tested in duplicates. Pellet durability is defined as:

$$Durability = \frac{\text{Weight of pellets after tumbling}}{\text{Weight of pellets before tumbling}} \quad (3.9)$$

3.2.13 Water Stability

Pellet water stability was calculated using the following equation:

$$Water\ Stability = \frac{\text{Post soak weight}}{\text{Pre soak weight}} \times 100 \quad (3.10)$$

This was a modified version of Adedeji et al. (2017). 5 grams from each diet were collected and placed into a glass beaker with 100 ml of room temperature distilled water. After one hour, the pellets were decanted from the beaker and dried in a convection oven at 90°C for 24 hours. Water stability is defined as the weight of the pellets post-drying divided by the weight of the initial pellets. Diets were tested in duplicate.

3.2.14 Statistical Analysis

Statistical analyses were performed on SAS software (SAS Institute, Inc, Cary, NC, USA). Two-way ANOVA ($P < 0.05$) using the general linear model was employed to determine statistical difference between treatment means with the fixed effects being grain choice and process energy. Tukey's post-hoc test ($P < 0.05$) was performed for pairwise comparison between means after a significant ANOVA result.

3.3 Results and Discussion

3.3.1 Particle Size

The cumulative particle size distributions for the raw shrimp feed formulations are shown in Figure 3.2. The particle sizes of the wheat-based formulation had a distribution very similar to one based on sorghum, with the latter being slightly coarser as can be inferred from the lower slope of its cumulative distribution curve. The average particle size

(dgw) and 84th percentile particle size of the sorghum formulation was slightly higher (276 μm and 497 μm , respectively) than that for the wheat formulation (253 μm and 453 μm , respectively). These small differences are probably due to different post-grinding particle size distributions of wheat and sorghum grains. The particle size distribution of the raw mixes can impact water and heat absorption characteristics, starch gelatinization, flow and mechanical energy generation and also film forming or gas holding capability of the matrix, all of which affecting both the process and product quality. In this study, however, particle size was not such a critical factor due to similarities between the two formulations.

3.3.2 Feed Rate Calibration

The feed rate calibration data are shown in Table 3.2. As expected, the flow rate delivered by the feeder screw increased with its speed. When the feeder screw was raised from 12 rpm to 15 rpm (25% increase), the average increase in feed rate for wheat and sorghum formulations were higher (27.7% and 29.6%, respectively) than what was expected on volumetric capacity basis alone. This is probably because increase in mechanical conveying speed typically leads to decrease in specific flow energy and improvement in flowability of most grain-based particulate materials (Alavi and Ambrose, 2016; McGuire et al., 2022; Graff et al., 2024). Similar observation was made, although relatively less pronounced, when the feeder screw speed was further increased by 20% (from 15 rpm to 18 rpm) with the corresponding feed rates increasing by an average of 21.9% and 20.6%, respectively for wheat and sorghum-based formulations. In general, at any given feeder screw speed the average flow rates delivered for the wheat formulation

were very similar and only slightly higher than the sorghum formulation (102.0 – 158.8 kg/h and 100.6 – 157.3 kg/h, respectively). This corresponded well with the similar particle size distribution results described earlier, as particle size can impact flow of granular materials considerably (McGuire et al., 2022). Typically, coarser particle size leads to better flowability of grain-based mixes. The minor deviation from this trend in the current results can point to differences in surface chemistry of the particles which impacted the inter-particle cohesion. The feeding bin fill level had a clear impact, with higher fill leading to increase in flow rate, irrespective of feeder screw speed and formulation type. This was expected due to greater compaction at the bottom of the bin with more material fill, which in turn increases the bulk density and thus the mass flow without change in volumetric conveying capacity of the screw. The slightly smaller coefficient of variation of feed rate with respect to bin fill level observed for the wheat formulation (4.5 – 5.2%) versus sorghum formulation (4.7 – 5.3%) points to marginally less cohesion in the former, and is line with the earlier discussion on minor differences in flow rate between the two.

3.3.3 Extrusion Processing

Preconditioner steam and water injection rates were adjusted so that their ratio, which in this study will be called preconditioning intensity, varied between low (0.29 – 0.34) with a target preconditioner discharge temperature of 60°C, medium (0.52-0.63) with target of 80°C and high (1.30-1.51) with 95°C as the target, while keeping the sum of the two flows constant at 26-27 kg/h. The corresponding treatments are labeled as W60, W80 and W95 or S60, S80 and S95, respectively, with the letters W and S referring to wheat and sorghum-based formulations, respectively.

The primary energy sources in extrusion processing are mechanical energy generated by the extruder screws and thermal energy generated by the addition of steam in the preconditioner (Karkle et al., 2012; Monti et al., 2016; Maichel, 2021; Graff et al., 2024). The specific thermal energy and specific mechanical energy data are shown in Table 3.4. The absolute value of STE and also its relative contribution to total specific energy (% STE) increased with preconditioning intensity for both wheat-based (87.2 - 220.5 kJ/kg; 23-47%) and sorghum-based treatments (92.4 - 266.8 kJ/kg; 22-51%). The increase in STE with the stepwise increase in preconditioning intensity was higher than what was expected from just the proportional increase in steam injection, probably because of better absorption of steam and reduction in the percentage lost to atmosphere leading to improvement in preconditioning efficiency. It should also be noted that STE was in general greater in sorghum-based treatments than wheat, even though steam loss percentage was higher in the former, as it took slightly higher steam injection to reach the target preconditioner discharge temperatures for the sorghum formulation. This difference could be due to material properties such as heat capacity. Steam preconditioning is an important processing step for aquatic feed production in both pellet milling and extrusion. However, to the best of knowledge of the authors, only one other study reported specific thermal energy input during aquatic feed processing, but did not provide any methodology details (Samuelsen and Oterhals, 2016). There are also few studies that discuss STE in extrusion processing of dog or cat food, which is an application similar to aquatic feed in terms of the process, extrusion equipment and general composition of diets (Monti et al., 2016; Pacheco et al., 2018; Maichel, 2021). Compared with the data from this work, a higher

range of STE was reported in these studies (161- 430 kJ/kg), which could be due to differences in raw material characteristics and variables such as feed rate, mash moisture content, preconditioning efficiency and target discharge temperature. Differences in calculation methodology and collection of process data might also have an important role.

Specific mechanical energy is generated in extrusion primarily due to the friction between the material, screw surface and barrel wall, and is impacted by various factors including viscosity of the material, hardware parameters and process conditions (Rokey, 2000; Agbisit et al., 2007; Kantrong et al., 2018). Specific mechanical energy was higher for sorghum-based treatments (255.9 – 318.0 kJ/kg) as compared to wheat (244.5 – 285.5 kJ/kg). Process parameters such as raw material feed rate, total moisture injection and extruder screw speed were kept constant throughout the experiment, so differences in SME could be due to material properties of sorghum versus wheat, including viscosity building characteristics. Interestingly, even after a wide survey very few studies were found that reported SME data for extrusion of aquatic feed, leave alone sinking aquatic feed, in particular (Pennells et al., 2025). Most literature on aquatic feed focuses on nutritional aspects and in vivo trials with animals, some on physical quality of feeds but not on specific mechanical energy that is an important aspect of processing (Singh et al., 2019; Delgado et al., 2021; Cheng et al., 2023; Gamero-Barraza et al., 2024; Zhang et al., 2025; Chen et al., 2025). In addition, preconditioning using steam and water is integral to the extrusion process for commercial aquatic feed production. This is also a standard feature for research based on pilot-scale extrusion equipment as summarized below, but not studies using smaller lab-scale extrusion equipment. In an earlier study by this group

involving processing of sinking shrimp feed with different formulations using pilot-scale single screw extrusion, much lower SME input was observed ranging from 48.8 - 55.5 kJ/kg (Adedeji et al., 2015). Similarly, a relatively low SME range of 59.1 – 135 kJ/kg was reported for pilot-scale twin screw aquafeed extrusion, with a decreasing trend accompanying increase in the extruder barrel steam to water injection ratio (Sameulsen and Oterhals, 2016). The use of a wattmeter for calculation of SME in the current study is a more reliable and direct technique than indirect methods based on measurement of electrical current that might have been the cause of lower SME calculations in previous research. In another pilot-scale study, a decrease in SME was observed with increase in feed rate and in-barrel moisture during twin screw extrusion of generic aquatic feed (Ma et al., 2023). The extremely low SME range of 9 – 14 kJ/kg that was reported however points to some kind of a measurement error. A few lab-scale extrusion studies on aquatic feed processing have reported SME in a similar range (125-396 kJ/kg) as the current research, but these data are not very applicable considering the smaller scale and also absence of preconditioning step (Singh and Muthukumarappan 2016; Martin et al 2019).

A clear decreasing trend in SME was also observed, as STE increased with rise in preconditioning intensity. This inverse relationship between SME and STE was found in the case of both sorghum and wheat-based treatments. Preconditioning transfers heat and moisture to the raw material, which in turn partially gelatinizes the starch and softens and plasticizes the material before it is discharged into the extruder barrel (Strahm, 2000). This plasticizing effect can reduce the mechanical energy during extrusion as the material is no longer as abrasive (Levine, 2014; Pacheco et al., 2018). A negative correlation ($r^2 = 0.774$)

between SME and STE was also observed previously for aquatic feed extrusion (Sameulsen and Oterhals, 2016).

The total specific energy (TSE), which is the sum of STE and SME, increased with preconditioning intensity for both sorghum and wheat-based treatments. Thus, the increase in STE more than compensated for the decrease in SME. The TSE was higher for sorghum-based treatments (410 – 523 kJ/kg) than wheat-based treatments (373 – 472 kJ/kg), which was not surprising as both STE and SME were higher for the former. The sorghum-based treatments consequently had higher die temperature as compared to wheat (118 – 131 °C versus 110 – 127 °C, respectively). Die temperature increased with preconditioning intensity for both sorghum and wheat treatments, which was also consistent with the TSE input trends.

3.3.4 Starch Gelatinization and Degradation

Data for starch gelatinization onset and peak temperatures and degree of gelatinization of raw formulations and preconditioned mash during processing of aquatic feed are shown in Table 3.4. The sorghum formulation had significantly higher starch gelatinization onset and peak temperatures (74.5 and 79.2 °C, respectively) than the wheat formulation (65.3 and 70.6 °C, respectively). This was expected as previous studies have shown that isolated sorghum starches have higher gelatinization onset and peak temperatures (59.6 - 68.4 °C and 68.3 – 74.6 °C, respectively) than isolated wheat starches (54.7 – 55.2 °C and 60.3 – 61.8 °C, respectively) (Boudries et al., 2009; dos Santos et al., 2022; Chen et al., 2023; Lacerda et al., 2024). The gelatinization onset and peak temperatures in the aquatic feed formulations were higher than the corresponding

starches because of the presence of various other ingredients such as fish meal, soybean meal and rice bran, and also other components in the ground cereal grain such as fiber, protein and lipids that might interfere with the phenomena of plasticization, hydration and heat absorption contributing to gelatinization (Sun et al., 2014; Mohamed and Rayas-Duarte, 2003; Bondt et al, 2020; Chen et al., 2023). The enthalpy of gelatinization in the above studies has been reported to be similar for isolated sorghum and wheat starches (8-10 J/g). Considering ~23% grain content in the aquatic feed formulations and ~70% starch content in the two grains (Chen et al., 2019; Shevkani et al, 2017), the calculated enthalpy would be approximately 1.5 J/g for the two mixes. However, the actual enthalpy of gelatinization was higher (1.8-1.9 J/g) probably due to the same reasons mentioned above in the context of gelatinization temperatures.

Preconditioning and intensity of the hydrothermal treatment did not significantly impact the onset and peak temperatures of starch gelatinization for either sorghum or wheat formulations. This implied there was no annealing effect on starch due to water and steam addition during preconditioning, unlike what has been reported for certain thermal treatments involving excess water (Park et al., 2024). The relatively low hydration during the preconditioning process is probably the main reason for the absence of annealing. However, preconditioning did lead to partial gelatinization of the starch in both formulations, as can be seen from the lower gelatinization enthalpy of the wheat and sorghum based preconditioned mash (1.0 and 1.1-1.3 J/g, respectively) as compared to the raw formulations. The corresponding post-preconditioning degree of gelatinization was calculated to be 49.3-49.7% and 29.9-38.5%, respectively for wheat and sorghum

formulations. It is noteworthy that the degree of gelatinization for sorghum-based preconditioned mash was lower despite up to 21% higher STE as compared to the wheat-based treatments. This resistance to gelatinization in the case of sorghum is consistent with literature and is due to starch granules being embedded in a dense protein matrix and the surrounding protein bodies that act as a barrier (Ocheme et al., 2015). Also, higher preconditioning intensity and STE led to a significant increase in degree of gelatinization of the sorghum formulation but had no effect on the wheat formulation. This is because even with the least STE input, the lower gelatinization onset temperature for wheat starch led to maximum transformations allowable given the limited moisture and residence time during preconditioning.

The subsequent thermo-mechanical treatment during extrusion led to complete gelatinization of extruded aquatic feed pellets, irrespective of the formulation type or preconditioning intensity. Thus, the SME input even though variable depending on treatment and the additional residence time inside the extruder barrel were adequate to bring gelatinization to completion. However, the extent or degree of starch degradation, as measured by susceptibility of extruded pellets to hydrolysis by α -amylase, did vary depending on the treatment (Table 3.5). The degree of degradation for sorghum-based pellets (83.0 – 90.5%) was significantly lower ($p < 0.0001$) as compared to wheat-based pellets (94.6 – 98.8%) even though the SME and total specific energy input or TSE for the former were higher by up to 15% and 11% higher, respectively. Similar to gelatinization the susceptibility to enzymatic hydrolysis, commonly referred to as starch digestibility, is negatively impacted by starch-protein interactions in sorghum. Starch granules are

embedded in a protein matrix and the two adhere tightly in the sorghum endosperm. The inter- molecular crosslinks between the prolamin proteins or kafirins decrease the digestibility of both the protein and the starch granules enmeshed in it (Rooney and Pflugfelder, 1986; Wong et al., 2009). Higher preconditioning intensity (and total specific energy input) led to significant increase in degree of starch degradation in both sorghum and wheat pellets ($p = 0.0003$), which was also consistent with die temperature trends. These differences due to preconditioning intensity were however less pronounced in wheat as the degradation was already high even at the lowest intensity, leading to significant interaction between grain type and thermal energy ($p = 0.0075$). Research has found that complete starch hydrolysis is more difficult to achieve in sorghum (Ezeogu et al., 2005); this is theorized to be due to the cross-linking of proteins in the endosperm of the sorghum grain (Zhang & Hamaker, 1998; Dudou et al., 2003). Wheat starch does not have this cross-linking of proteins in the endosperm; therefore, it stands to reason that gelatinization is relatively easier to achieve, and total process energy would have less of an impact than it would in sorghum-based feeds.

3.3.5 Pellet Macrostructure

The wet pellet or pre-dryer bulk density and dry pellet or post-dryer bulk density data are shown in Table 3.6. The wet bulk density of sorghum-based treatments was not significantly different than wheat treatments ($p = 0.0528$) but the former was slightly higher (506 – 524 g/L versus 493 – 514 g/L), implying lesser expansion to some degree despite the higher TSE and die temperatures. The lesser starch degradation in sorghum-based treatments probably led to reduced film forming ability and matrix extensibility and thus

lower expansion of pellets at the extruder discharge (Maichel, 2021). Wet bulk density was significantly affected by thermal energy ($p = 0.0124$), with the overall decreasing trend with increase in preconditioning intensity pointing to more expansion due to higher TSE and die temperatures. This effect was more pronounced in the case of sorghum treatments as can be seen from the significant interaction between grain type and thermal energy input ($p = 0.0316$). The dry bulk density of both wheat and sorghum-based pellets (590 – 615 g/L and 620 – 627 g/L, respectively) was much higher than the wet density as the loss in mass due to water removal was more than compensated by the substantial amount of shrinkage in volume during drying. The broad trends in bulk density with respect to grain type and thermal energy were maintained even after drying.

Dry pellet macrostructure data based on individual piece measurements and statistical analyses are shown in Table 3.7. As compared to the 1.70 mm die opening, the pellet diameters ranged from 1.91 to 2.14 mm depending on the treatment (data not shown). This indicated combined effect of die swell due to elastic forces in the melt and limited degree of expansion or porosity induced by water vapor flash off at the die discharge in order to reduce buoyancy and allow the pellets to sink, as appropriate for shrimp feed. The corresponding radial expansion ratio (ER) data followed similar pattern as bulk density with a slight but significantly higher expansion for wheat-based treatments as compared to sorghum (ER of 1.42 – 1.58 vs 1.26 – 1.37, respectively) and a significant impact of preconditioning intensity with a general increasing trend between lowest and highest thermal energy input. Specific length (L_{sp}), which is a measure of longitudinal expansion (in the direction of extrusion), had in general an inverse pattern as compared to

radial expansion with significantly lower range observed for wheat-based treatments as compared to sorghum (365.3 – 402.8 mm/g versus 392.0 – 407.4 mm/g, respectively) and a general decreasing trend with respect to lowest and highest thermal energy input. The opposite trends of ER and L_{sp} might be due to impact of melt elasticity that tends to promotes radial expansion at the expense of longitudinal. However, piece density (P) and void fraction (VF) data followed the overall expansion trends described previously with respect to bulk density and expansion ratio, indicating the predominance of expansion in the radial direction. Piece density was lower and VF higher as for wheat treatments as compared to sorghum (0.767 – 0.790 g/cm³ versus 0.790 – 0.876 g/cm³ and 0.437 – 0.460 versus 0.375 – 0.494, respectively), indicating higher expansion for former. It should be noted that the increase in preconditioning intensity and resultant total specific energy and die temperature led to conflicting impact on die swell and water vapor flash off. The former tends to decrease due to lower melt elasticity while the latter increases. These opposing effects made the expansion trends with respect to thermal energy not as clear as expected, as can be seen from bulk density and piece-wise macrostructure data discussed above. In any case, the main primary goal for shrimp feed processing from a point of view of physical quality of pellets is to minimize expansion in order to maximize sinking characteristics while maintaining durability and water stability. These aspects are discussed next.

Pellet diameters, length, sectional expansion ratios (ER) and specific lengths are shown in Table 3.10. There were significant differences in expansion when comparing treatments; all sorghum-based diets had a higher dry bulk density than the wheat-based

diets. Total energy transfer, in the form of mechanical and thermal energy, from the extruder to the molten mash is what drives expansion (Monti et al, 2016). In sorghum-based diets, bulk density increased as thermal energy was reduced, which led to an overall reduction in total process energy. In wheat-based diets, this trend is less clear; dry bulk density was highest in the diet with the lowest thermal energy input (W60), while the diet with the lowest bulk density was the W80 diet. As stated, total energy values for wheat-based diets were 465 kJ/kg in the high thermal energy treatment, and 375 kJ/kg in the low thermal energy treatment. Despite this difference in total energy use, wet bulk density values for wheat-based feeds were not statistically different from each other. This is most likely due to the differences in starch between the two grains.

Minimal radial expansion occurred in all treatments, with SEI values ranging from 1.26 to 1.58. Specific length, a measure of longitudinal expansion, ranged from 365.3 to 407.36 mm/g. Statistical analysis showed that specific length and piece density were significantly different each other when comparing wheat to sorghum; process energy did not significantly impact longitudinal expansion or density. Conversely, there was an interaction between process energy and grain type with radial expansion (SEI); in both grains, SEI decreased as overall energy input decreased. However, this decrease was only significantly different in the wheat-based diets (1.58 to 1.43); in sorghum-based diets, there was a decreasing trend, but not to a significant degree (1.32 to 1.26). Porosity of the pellets, or the void fraction percentage, ranged from 38.15 to 49.44%. These values were significantly different from each other; however, it is important to note that shrimp feed is not necessarily an expanded product. It is more likely that what we are measuring is the

inadvertent swelling of the pellet as it exits the extruder; shrimp feed is highly dense and compact due to the necessity of it needing to sink. A highly porous product is counterproductive to that. Analysis showed that generally speaking, as process energy was reduced, porosity of the pellets increased in both grains, but to a greater extent in the sorghum-based diets. This is most likely due to the increased mechanical energy; the reduction in thermal energy led to an increase in mechanical energy in all diets which lead to the creation of internal porosity, but not necessarily increased expansion as the total energy inputs remained lower.

3.3.6 Floating/Sinking Percentage

There were no significant differences among diets with regard to sinking percentage. All diets, with the exception of W80 and W60, had sinking percentages that ranged from 84.7 to 88%. W80 and W60 had sinking percentages of approximately 79 and 81%, respectively. This data is shown in Table 3.11.

3.3.7 Pellet Durability Index

Pellet durability results are shown in table 3.12; they ranged from 98-99% signifying the extruded pellets are of high physical quality. Pellet binding qualities typically are influenced by the degree of starch gelatinization (Loar & Corzo, 2011); despite differences in gelatinization in both the preconditioned mash and in the extruded pellets, there was no significant differences between treatments in pellet durability.

3.3.8 Water Stability

Water stability values ranged from 80% to 59% as shown in table 3.13; there were no significant differences in water stability among the wheat-based feeds, and water

stability values decreased in sorghum-based feeds as both overall process energy and the subsequent degree of starch gelatinization decreased. As thermal energy was reduced, mechanical energy increased, but not enough to prevent the decrease of overall process energy use. In wheat-based diets, this reduction of energy use did not significantly impact the water stability of the feed; in sorghum-based diets, this reduced energy did result in significant differences. It was observed that as thermal energy reduced, starch gelatinization of the sorghum-based feeds in the preconditioner decreased. As the starch granules present in sorghum are trapped in a protein matrix (Zarei et al., 2022), it seems likely that increased thermal energy is needed to fully gelatinize sorghum-based feeds and improve the water stability of them. It is possible that increased mechanical energy alone could improve the water stability of the feed, but mechanical energy is the primary driver of pellet expansion, and as shrimp require a sinking feed, more mechanical energy and pellet expansion would not be desirable.

It is important to note that overall, process energy use was higher in sorghum-based feeds than in wheat-based feeds; however, this did not translate to a higher degree of gelatinization nor a higher water stability. Within each grain-based feed, however, process energy values mirrored water stability values, with the effect being more visible in the sorghum-based feeds.

3.4 Conclusion

Results showed that sorghum-based feeds required higher thermal energy for optimal starch gelatinization and subsequent water stability when compared to wheat-based feeds, where consistent gelatinization and water stability was achieved regardless

of thermal energy input. Reduced preconditioning temperatures led to increased mechanical energy demands and an overall lower extrusion process energy use. Sorghum-based feeds exhibited increased bulk densities and decreased water stability with lower thermal energy, indicating that increased heat during preconditioning is needed to fully gelatinize the starch that is entrapped in the protein matrix. Despite varying energy inputs, pellet durability values were high across all treatments. Overall, maintaining adequate thermal energy is crucial for improving the gelatinization and water stability of sorghum-based feeds, while wheat-based feeds showed more stable feed qualities regardless of energy inputs.

3.5 References

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3.6 Figures and Tables

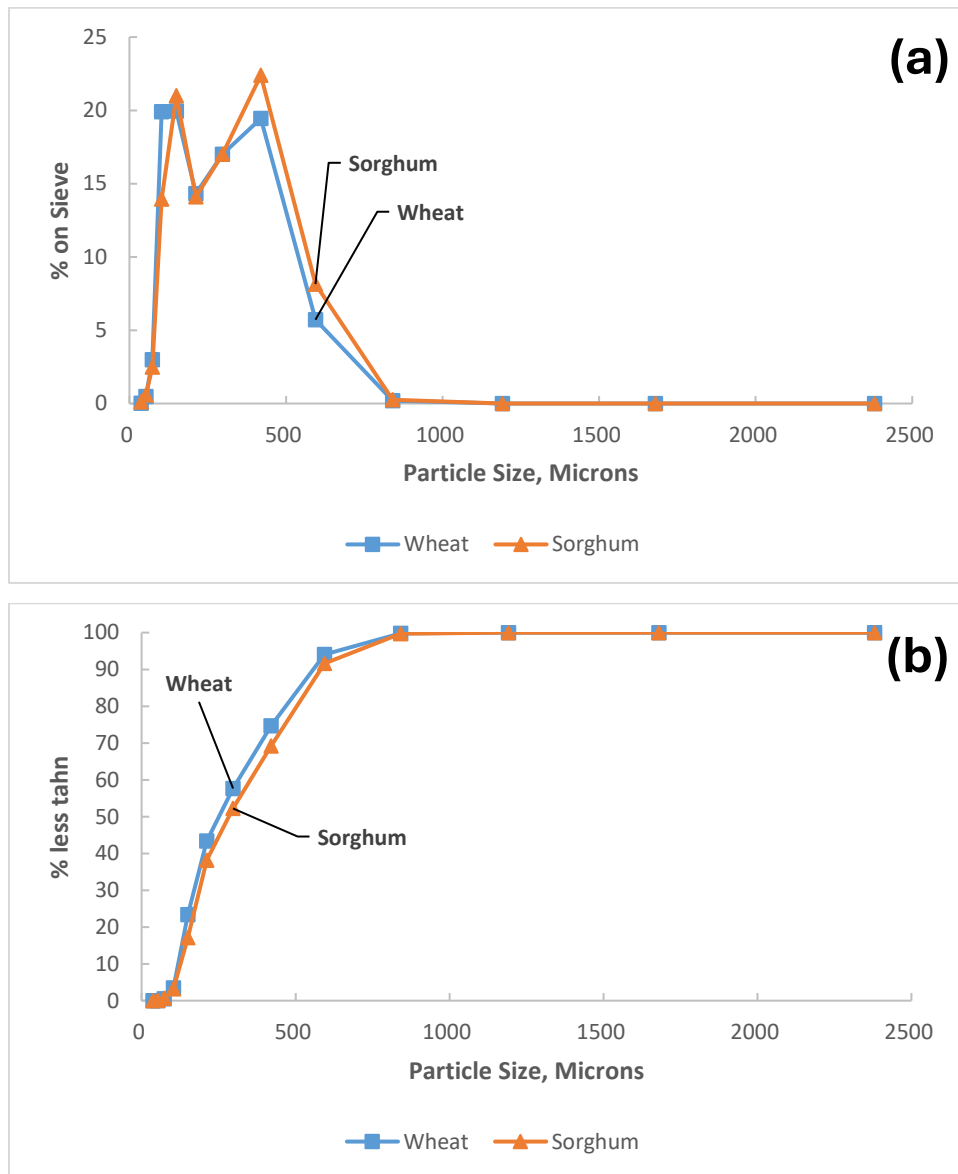
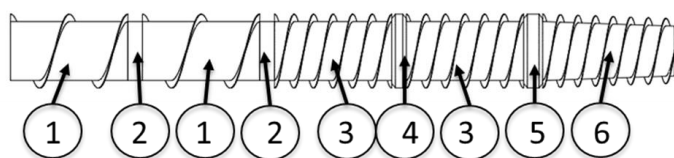


Figure 3.1: Particle size distribution of mixed diets with (a) the percentage of diet remaining on sieve and (b) the percentage of diet less than the corresponding microns.



Flight Location	Description
1	Single Flight, Full Pitch Screw
2	Small Steam Lock
3	Double Flight, 1/2 Pitch Screw
4	Medium Steam Lock
5	Large Steam Lock
6	Double Flight, 1/2 Pitch, Uncut Cone Screw

Figure 3.2: Screw Profile for Wenger X-20 single screw extruder

Table 3.1: Diet formulation and proximate analysis of extruded wheat and sorghum-based diets.

Ingredient (% , as is)	Whole wheat	Sorghum
Fishmeal	10.00	10.00
Soybean meal	39.00	39.45
¹ CPC	8.00	8.00
Fish oil	2.00	2.00
Soy oil	3.02	2.65
Lecithin	1.00	1.00
² Cholesterol	0.12	0.12
Whole wheat	23.20	0.00
Rice Bran	10.00	10.00
³ Sorghum	0.00	23.12
⁴ Mineral premix	0.07	0.07
⁴ Vitamin premix	0.04	0.04
Choline chloride	0.20	0.20
Stay-C 35% Active	0.10	0.10
CaP-dibasic	1.75	1.75
Bentonite	1.50	1.50

¹Cargill, Inc., Wayzata, MN, USA

²MP Biomedicals Inc., Solon, OH, USA

³Nu-Life Market, Scott City, KS, USA

⁴Ziegler Bros, Inc., Gardners, PA, USA

All other ingredients were sourced through Fairview Mills (Seneca, KS, USA)

Fish oil and cholesterol were applied through liquid coating of the extruded pellets.

Table 3.2: Measured feed rate of different feeds at varying RPM's and hopper fill level

Feed	Hopper Level	RPM		
		12	15	18
Wheat	Low	96.00 ^a	121.64 ^d	149.18 ^g
	Medium	102.82 ^b	131.18 ^e	160.36 ^h
	High	107.18 ^{bc}	138.00 ^f	166.91 ⁱ
Sorghum	Low	94.36 ^a	121.64 ^d	147.27 ^g
	Medium	101.73 ^{bc}	131.18 ^e	159.00 ^h
	High	105.82 ^c	138.55 ^f	165.55 ⁱ
P-value:				
Model	<0.0001			
Feed	0.046			
Hopper Level	<0.0001			
RPM	<0.0001			

Table 3.3: Ro-tap particle size analysis of wheat and sorghum-based diets

Diet	dgw	sgw	dgw (84th Percentile)	dgw (16th Percentile)
Wheat	253	1.79	453	141
Sorghum	276	1.8	497	153

Table 3.4: Percentage of mixed diet that passed through each U.S. sieve during particle size analysis

U.S. Sieve:	#8	#12	#16	#20	#30	#40	#50	#70	#100	#140	#200	#270
Opening (μ):	3327	1851	1168	841	595	420	297	210	149	105	74	53
Wheat	100	100	100	99.81	94.09	74.64	57.65	43.33	23.38	3.47	0.49	0.01
Sorghum	100	100	100	99.75	91.60	69.21	52.21	38.11	17.11	3.16	0.68	0.10

Table 3.5: Screw profile description for Wenger X-20 single screw extruder

Flight Location	Description
1	Single Flight, Full Pitch Screw
2	Small Steam Lock
3	Double Flight, 1/2 Pitch Screw
4	Medium Steam Lock
5	Large Steam Lock
6	Double Flight, 1/2 Pitch, Uncut Cone Screw

Table 3.6: Process energy inputs for diets with different levels of preconditioner steam addition

Diet	STE (kJ/kg)	PC Temp (°C)	SME (kJ/kg)	Total Energy (kJ/kg)	Die Temp (°C)
W60	113.4	58	260.4	373.8	110
W80	171.5	80	226.5	398.1	117
W95	236.8	97	228.0	464.7	127
S60	120.4	60	287.5	407.9	118
S80	177.1	76	257.4	434.5	125
S95	279.8	94	237.1	516.9	131

STE: Specific thermal energy

SME: Specific mechanical energy

Table 3.7: Degree of gelatinization in preconditioned diets with varying amounts of thermal energy

DSC Results for Raw and Preconditioned Diets			
Diet	Peak Gelatinization (°C)	Enthalpy (J/g)	Degree of Gelatinization (%)
Wheat-Raw Mix	70.55	1.94	-
Sorghum-Raw Mix	79.23	1.84	-
W60	70.88	0.98	49.62
W80	70.33	0.98	49.34
W95	72.77	0.98	49.68
S60	81.11	1.29	29.88
S80	79.17	1.20	34.87
S95	81.05	1.13	38.46

Table 3.8: Degree of gelatinization of dried pellets with varying amounts of total energy inputs

Degree of Starch Degradation of Dried Pellets	
Diet	Degree of Gelatinization (%)
W60	94.56 ^{bc}
W80	98.75 ^a
W95	97.47 ^{abc}
S60	83.00 ^d
S80	85.07 ^d
S95	90.49 ^c
Two-way ANOVA	
Model	<.0001
Process Energy	0.0003
Grain	<.0001
Process Energy*Grain	0.0075

Means not sharing the same letter are significantly different by the Tukey's HSD-test (Parametric ANOVA) at the 5% level of significance, with letters representing the interaction between grain source and process energy.

Analysis was done in accordance to AOAC 996.11

Table 3.9: Wet and dry bulk densities of extruded shrimp feed

Bulk Density of Sinking Feeds		
Diet	Wet Bulk Density (g/L)	Dry Bulk Density (g/L)
W60	509.5 ± 0.71 ^{ab}	614.82 ± 1.18 ^b
W80	493.0 ± 2.23 ^b	590.16 ± 0.89 ^d
W95	513.5 ± 2.12 ^{ab}	601.24 ± 3.85 ^c
S60	524.0 ± 2.83 ^a	626.61 ± 3.60 ^a
S80	508.0 ± 9.90 ^{ab}	623.31 ± 1.63 ^{ab}
S95	505.5 ± 6.36 ^{ab}	620.14 ± 0.74 ^{ab}
Two-Way Anova		
Model	0.0135	<0.0001
Grain	0.0528	<0.0001
Thermal Energy	0.0124	0.0004
Grain*Thermal Energy	0.0316	0.0019

Table 3.10: Pellet diameter, length, and expansion ratios of extruded shrimp feed

Expansion Characteristics of Dried Pellets							
Diet	Pellet Diameter (mm)	*Pellet Length (mm)	*Pellet Mass (g)	ER	**Specific Length (mm/g)	***Piece Density (g/cm ³)	Void Fraction %
W60	2.03 ± 0.15ab	1.43 ± 0.15b	0.00355 ± 0.0001b	1.43 ± 0.04b	402.83 ± 12.89Ba	0.7667 ± 0.042B	45.27 ± 0.15b
W80	2.02 ± 0.10abc	1.57 ± 0.19a	0.00398 ± 0.0003ab	1.42 ± 0.01b	393.75 ± 4.33Ba	0.7898 ± 0.014B	43.67 ± 0.05c
W95	2.14 ± 0.13a	1.66 ± 0.16a	0.00455 ± 0.0001a	1.58 ± 0.03a	365.3 ± 7.69Bb	0.7676 ± 0.002B	45.99 ± 0.10a
S60	1.91 ± 0.13c	1.46 ± 0.18b	0.00366 ± 0.0001b	1.26 ± 0.02c	399.94 ± 5.60Aa	0.8761 ± 0.026A	37.12 ± 0.34d
S80	1.99 ± 0.15bc	1.59 ± 0.18a	0.00391 ± 0.0003ab	1.37 ± 0.04bc	407.36 ± 1.79Aa	0.7899 ± 0.021A	43.47 ± 0.08c
S95	1.95 ± 0.15bc	1.59 ± 0.22a	0.00405 ± 0.0001a	1.32 ± 0.03bc	392.03 ± 12.70Ab	0.8529 ± 0.049A	37.53 ± 0.03d
Two-Way Anova							
Model	<0.0001	0.0010	0.0144	0.0004	0.0239	0.0404	<0.0001
Grain	<0.0001	0.8699	0.1904	<0.0001	0.045	0.0088	<0.0001
Process Energy	0.0455	<0.0001	0.0048	0.0054	0.0154	0.3930	<0.0001
Grain*Process Energy	0.0455	0.3584	0.1270	0.0059	0.1248	0.0886	<0.0001

*Lowercase lettering is for differences in process energy

**Uppercase lettering is for differences within grain; lowercase lettering is for differences in process energy

***Uppercase lettering if for differences within grains

Unless specified, all other labeling refers to differences based on the grain*process energy interaction

Table 3.11: Physical Pellet Qualities

Extruded Pellet Qualities			
Diet	% sank after one minute	PDI	Water Stability
W60	81.3 ± 2.31	99.05 ± 0.17	77.0 ± 4.24 ^a
W80	79.3 ± 4.16	99.00 ± 0.01	79.0 ± 1.41 ^a
W95	88.0 ± 5.29	98.9 ± 0.18	80.0 ± 0.00 ^a
S60	84.7 ± 7.02	99.03 ± 0.06	59.0 ± 1.41 ^b
S80	87.3 ± 3.06	99.30 ± 0.13	72.0 ± 2.83 ^a
S95	87.3 ± 1.15	98.83 ± 0.29	77.0 ± 1.41 ^a
Two-Way Anova			
Model	0.1342	0.2252	0.0007
Grain	0.1045	0.4865	0.0004
Thermal Energy	0.1529	0.1234	0.0017
Grain*Thermal Energy	0.2559	0.2972	0.0092

Chapter 4 - Evaluation of different levels of process energy in sorghum and wheat-based diets on the growth performance of pacific white shrimp *Litopenaeus vannamei*

Abstract

This research evaluated the effect of different levels of extrusion process energy during the production of wheat and sorghum-based feeds on the growth performance and digestibility of Pacific white shrimp (*Litopenaeus vannamei*). Process energy consisted of mechanical and thermal components, which were both modulated via varying preconditioner steam addition. Diets were formulated to be isonitrogenous (36% protein) and isolipidic (8% fat), with three levels of thermal energy input during preconditioning (high, medium, and low). All diets showed increased starch gelatinization with increased thermal energy, with the wheat-based diets undergoing a greater degree of gelatinization than the sorghum-based diets. There were no significant differences in final biomass, weight gain, feed conversion ratio (FCR), or survival among the different treatments. However, digestibility results showed that wheat-based diets had significantly higher apparent digestibility coefficients (ADC) for both protein and energy compared to sorghum-based diets. Overall, increased starch gelatinization that correlated with greater digestibility was observed, but this effect was reduced in sorghum-based diets. These findings suggest that sorghum can be used as a viable alternative to wheat in shrimp feed without negatively impacting their growth performance, while also offering potential cost savings to producers.

4.1 Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is one of the top-produced aquaculture species requiring a high-quality, water-stable pellet [15]. Historically, feed has accounted for 50-70% of the total cost of production [38]. Carbohydrates (CHO), such as the starches found in cereal grains, are typically included in formulations even though aquatic animals do not have any specific requirements for carbohydrates [35]. Increasing dietary carbohydrates has been used as a strategy to minimize the use of dietary protein, as carbohydrates can act as an immediate energy source for many crustaceans [44]. Research has shown that carbohydrates have a dietary protein-sparing effect [7][8]. This protein-sparing effect of increasing dietary carbohydrates can help significantly reduce feed costs as carbohydrates are the least expensive nutrient for aquatic animals that are energy-yielding [38]. Carbohydrate sources can also act as a binder that improves various pellet qualities; most notably water stability and pellet durability [39].

Feed manufacturers typically select carbohydrate-rich ingredients based primarily on cost and availability, often without considering the potential effects on shrimp growth. In 1994, Cruz-Suarez et al. [7] tested diets with wheat meal, corn flour, and sorghum flour, and they found that the wheat-based diet resulted in the best growth rate, with corn and sorghum flour being the least efficient. Those authors noted, however, that cheaper carbohydrate sources such as sorghum can overcome the economic impact of slightly higher FCRs in terms of shrimp production cost. Similarly, a literature review by Cuzon et al. [8] found that wheat starch was highly digestible (90%) by *L. vannamei*, further proving its suitability as a source of energy.

Another consideration for the choice of CHO source is its ability to act as a pellet binder; this comes from the native starch present in the grain, and in the case of wheat, the presence of gluten. Feeds formulated with wheat starch showed improved pellet-forming ability, water stability, and lesser amounts of fines during handling [40]. Similarly, vital wheat gluten inclusion was shown to yield a strong pellet with a regular cell structure that yielded a smooth, non-porous surface [12]. The presence of gluten in wheat has led to wheat starch being the preferred CHO in shrimp feed as it improves pellet quality. However, wheat is often more expensive than other cereal grains and requires higher inputs such as irrigation and nitrogen application, increasing its environmental impact.

As water becomes scarcer in high-production regions, it will become necessary for feed producers to reduce their reliance on costly cereal grains such as wheat. Grain sorghum is a non-GMO cereal crop with improved drought tolerance when compared to wheat, which suffers extreme yield loss and crop failure when undergoing periods of drought and water stress [20]. At low water use, sorghum has been shown to have better yields per acre than maize [42]. It is one of the top five cereal crops globally, with the largest producer being the United States (62.3 million tons) in 2020 [46]. Grain sorghum has a high starch content (70-72%), protein (11-12.8%), and total dietary fiber (8.4-10.9%) [22]. This high starch content makes sorghum an attractive choice for extruded feed applications as starch serves as the primary binder in extruded feeds, helping to bind the pellet together and prevent disintegration as it is submerged in water [35].

Extrusion processing has quickly become the primary method for producing feed for aquaculture. Compared to older technologies such as pellet milling, extruded feed

experiences much higher levels of thermal energy, pressure, and moisture levels [34]. This change in processing conditions leads to increased levels of gelatinization in starch, increasing the availability of carbohydrates when consumed. Energy is transferred to raw material through both mechanical energy (SME) and thermal energy (STE). Extrusion processing includes preconditioning, extrusion using mechanical screws, and cutting with a rotational knife [33]. Preconditioning helps ensure diet uniformity through continuous mixing of the raw material with steam and water. This preheats the material and begins the cooking process and starch gelatinization, which is then finalized using mechanical screws to generate mechanical energy [23]. Thermal energy occurs primarily in the preconditioner through liquid water and steam addition [27][30]. This helps ensure improved final product quality; by starting the process of starch gelatinization in the preconditioner, raw material granules are hydrated and softened. This plasticization of the raw diet helps increase extruder stability, which in turn leads to improvements in final product quality [17][19].

An English-based literature review identified few publications connecting processing and shrimp performance and one pertaining to the impact of varying steam addition levels on the growth rates and digestibility of Pacific white shrimp [9]. Given that carbohydrates can act as an energy source for crustaceans and that gelatinization increases availability of carbohydrates when consumed, the hypothesis of this study was that increasing the total amount of process energy via preconditioner steam addition would result in increased digestibility of the feed when consumed. Process energy consists of thermal and mechanical energy, which were both modulated by varying the amounts of preconditioner steam addition. Therefore, the objective of this study was to

evaluate the impact of three levels of process energy in both wheat and sorghum-based diets on the digestibility and growth rates of Pacific white shrimp.

4.2 Materials and Methods

4.2.1 Experimental Design and Diets

A 6-week growth trial was conducted to compare the effects of different process energy inputs (high, medium, and low) during extrusion of sorghum and whole wheat based practical diets for white shrimp. This constituted a 3x2 factorial design with a total of 6 experimental treatments. Total process energy inputs were modulated by varying the preconditioner steam input which impacted both mechanical and thermal energy levels. Diets were formulated to be isonitrogenous (36% protein) and isolipidic (8% lipid). Diet formulations are presented in Table 4.1. Titanium dioxide was included in the dry mix at 0.6% inclusion for the digestibility trial before extrusion of the feeds. The experimental diets were prepared with a pilot-scale single screw extruder (model X20, Wenger Manufacturing, Inc., Sabetha, KS) at Kansas State University (Manhattan, KS) using standard procedures for shrimp feeds. Diets were top coated with 2% fish oil and 0.12% cholesterol prior to feeding. Proximate composition analysis and amino acid (AA) composition were analyzed for all experimental diets at MidWest Laboratories (Omaha, NE, USA) and University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) (Tables 4.2, and 4.3, respectively). Starch gelatinization was analyzed for all diets in triplicate at MidWest Laboratories (Omaha, NE, USA) and is shown in Table 4.4.

4.2.2 Growth trial

A growth trial took place at E.W. Shell Fisheries Center (Auburn University, Auburn, AL, USA). During the growth trial, six replicate groups for each of the six dietary treatments and four replicate groups for the commercial diet were then randomly assigned and shrimp were fed 4 times per day. Fifteen post-larvae shrimp were hand-sorted to uniform size and stocked into each aquarium tank (120 L, 52 x 52 x 49.5cm) in the experimental system. A total of 40 aquarium tanks were used in the study. The total amount of shrimp per tank was counted weekly to adjust the survival rate. The daily feeding ration was calculated using our historical data of estimated weekly weight gain, expected feed conversion ratio (FCR) of 1.6, the survival rate of the corresponding week, and the actual feeding response of the animal [29]. At the end of the growth trial, shrimp were counted, and group weighed to determine mean final biomass, final weight, survival, and feed conversion ratio (FCR) according to standard calculations [28]. Upon termination of the trial, shrimp were weighed and counted and 5 individuals per tank were randomly selected, packed in sealed bags, and stored in a freezer (-20°C) for chemical analysis. Shrimp samples were dried in an oven at 90°C to a constant weight, using the methods described by the A.O.A.C. [3], and then ground in a coffee grinder and stored. Total energy content was determined using a micro-calorimetric adiabatic bomb using benzoic acid as standard (Parr 6725, Moline, IL, USA) [16].

4.2.3 Digestibility trial

The digestibility trial was performed in a recirculating aquaculture system comprised of a battery of 80-L glass tanks connected to a common reservoir, a biological

and a physical filter. Additionally, a charcoal cartridge filter with 10-inch 50 µm wound polypropylene filter cartridge (Pentair plc (PNR), Golden Valley, MN). was supplemented to the system to adsorb any particulates that might contribute to water quality issues. During the entire trial, water quality parameters were maintained within acceptable ranges for shrimp culture [5].

Nine shrimp (average initial individual weight of $30.33 \pm 1.98\text{g}$) were stocked into 36 glass aquaria of the abovementioned system. Six replicate aquaria were offered one of the six experimental diets, and the resulting fecal material from each two tanks was pooled in a total of three replicate fecal samples/diet. The shrimp were acclimated to experimental diets for three days prior to fecal material collections. Feces were collected four times a day for a period of three consecutive days. Shrimp were offered feed in the morning, then after one hour, the first collection of feces was discarded. The subsequent fecal collections were rinsed with distilled water, and oven-dried for 24 hours prior to storage until analyzed. Gross energy of the diets and the fecal material was analyzed using a semi-micro calorimeter (Model 6725, Parr Instrument Co., Moline, IL, USA). Titanium dioxide concentration was determined according to Short et al. [37], in which samples were ashed, then digested using 7.4M sulfuric acid, then adding hydrogen peroxide, resulting in yellow-orange coloration. After 48 hours of incubation, the absorbance of the samples was read using a spectrophotometer at 410nm (Geneysis 20, Thermo Fisher Scientific, Waltham, MA, USA), and titanium dioxide concentration was determined using the equation from the standard curve. Samples were sent for protein and amino acid analysis in the laboratories of the University of Missouri (University of Missouri Agricultural Experiment Station

Chemical Laboratories, Columbia, MO, USA). The apparent digestibility coefficients for protein (APD), and energy (AED) of the diets (D) were calculated according to Cho et al. [6] as follows:

$$\text{ADP and AED (\%)} = 100 - \left[100 \times \left(\frac{\% \text{ TiO}_2 \text{ in feed}}{\% \text{ TiO}_2 \text{ in feces}} \times \frac{\% \text{ nutrients in feces}}{\% \text{ nutrient in feeds}} \right) \right] \quad (4.1)$$

4.2.4 Water quality

Dissolved oxygen (DO) was maintained near saturation using air stones in each culture tank via a common airline connected to a regenerative blower (Model R4P115, Gast Manufacturing, Benton Harbor, MI). Dissolved oxygen, salinity and water temperature were measured twice daily using a YSI-2030 Pro digital oxygen/temperature meter (YSI Corporation, Yellow Springs, Ohio, USA), and total ammonia N (TAN) and nitrite-N were measured twice per week using YSI 9300 photometer (YSI, Yellow Springs, OH). The pH of the water was measured two times per week during the experimental period using a pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA) (Table 4.5).

4.2.5 Statistical analysis

All data were analyzed using SAS (V9.4, SAS Institute, Cary, NC, USA). Growth indices and apparent digestibility coefficients of trout were analyzed using two-way ANOVA to determine significant differences ($P < 0.05$) among treatments followed by Tukey's multiple comparison test to evaluate significant differences between treatment means.

4.3 Results and Discussion

4.3.1 Proximate Analysis

The proximate composition and amino acid profile of the extruded diets are shown in Tables 4.2 and 4.3. Protein and fat contents of the diets ranged from 37.70 to 38.50% and 6.90 to 7.22%, respectively, with formulation called for 36% protein and 8% fat. There was a slight increase in amino acid content in the low thermal energy diets, but their values were minimal.

4.3.2 Starch Gelatinization

Gelatinization data for the extruded diets ranged from 83.00% to 98.75% and are shown in Table 4.4. In both grains, gelatinization increased as more process energy was used in the manufacturing of the diets. These differences were significant; sorghum-based diets were significantly different from all wheat-based diets with the exception of the high-energy sorghum diet (90.49%). All wheat-based diets ranged from 94.56% to 98.75%; there were no significant differences between the high-energy diet and the low-energy diet, indicating that the impact of process energy inputs is less for wheat than it is for sorghum. Part of the change in process energy between treatments was due to an increase in thermal energy contributed by steam during preconditioning. Research on thermal energy in extrusion is limited, but these results were also seen by Pacheco et al [30] in dog food; results there showed that as thermal energy during extrusion increased via steam addition, so did the gelatinization of the pellet. Dietary composition can affect the degree of starch gelatinization due to differences in starch source and also interactions with other dietary components [13][31]. This might have been a factor in the present study as can be seen

from the lower degree of gelatinization in the sorghum-based diets as compared to the wheat-based diets. Temperature and water content are some of the processing factors affecting gelatinization in starch [24]. Although there was no difference between sorghum and wheat-based diets with regard to independently controlled extrusion conditions including moisture, higher process temperatures were observed for the former due to raw material steam absorption differences [18]. However, sorghum-based feeds still had lower overall starch gelatinization than wheat-based feeds. Sorghum starch is more resistant to gelatinization as compared to other grains due to the surrounding protein matrix in the endosperm that acts as a barrier, although the starch granules are similar to those in other cereals such as corn in terms of shape and size [36][43][45]. Wheat starch has a gelatinization temperature range of 58-64°C, while sorghum has a gelatinization temperature range of 68-76°C [41][43], indicating that either more heat or longer exposure to increased temperatures is necessary to achieve full gelatinization in sorghum-based diets. It should be noted however that these gelatinization temperature ranges are based on a calorimetric technique, whereas the gelatinization data reported in this study was based on enzymatic digestion. Similar results were observed in a previous study that reported lower gelatinization (84-86%) for sorghum-based dry extruded cat food as compared to rice and corn-based cat food (89-90%), based on the enzymatic method [25].

4.3.3 Growth Trials

The growth trial was performed without any significant water quality or disease issues. Water quality was within suitable parameters for the culture of Pacific white shrimp as shown in Table 4.5. No significant differences were observed in final biomass, final

mean weight, weight gain, feed conversion ratio (FCR), survival, gross energy, and energy retention when Pacific white shrimps were fed with diets containing different wheat and sorghum process energy input via preconditioner steam addition ($P > 0.05$), as shown in Table 4.6. There were no significant differences between diets based on grain source used and process energy, and there was no interaction between them. Limited studies with sorghum in Pacific white shrimp diets exist; inclusion of sorghum DDGs in both extruded and pelleted diets was studied with results showing successful incorporation into practical diets for Pacific white shrimp at levels up to 40% in a clear water system without negatively affecting growth, survival and feed conversion ratio [2][47]. However, this is not a straight comparison, as sorghum DDGs were used as a protein source and a replacement for soybean meal rather than a primary carbohydrate source. Sorghum DDGs were subjected to processing during the ethanol manufacturing process, with much of the starch being removed, so their final composition had much higher protein, fiber and lipids (34%, 10% and 9%, respectively) and lower starch (24%) than grain sorghum. Other research has, however, shown that omnivorous fish such as tilapia can consume sorghum-based diets with no adverse effects on growth rates. Tilapia fed a diet of 25% grain sorghum or up to 30% sorghum starch had increased growth performance in comparison to tilapia fed corn, wheat, barley and rice [1][4].

4.3.4 Digestibility Trials

Protein and energy digestibility coefficients (APDD and AEDD, respectively) are shown in Table 4.7 and energy digestibility results are also plotted with respect to starch gelatinization in Figures 4.1 and 4.2. Apparent digestibility coefficients of energy ranged

from 74.29 to 82.93%. Overall, there is a strong relationship between digestible energy and degree of gelatinization ($r=0.9197$) indicating that greater degree of starch gelatinization resulted in a greater amount of digestible energy as shown in Figure 4.1. However, when the data was broken down according to grain type, differences were observed between wheat and sorghum-based feeds; starch gelatinization and digestible energy had a stronger correlation in sorghum-based feeds ($r=0.7439$) than in wheat-based feeds ($r=0.5783$) as shown in Figure 4.2. Starch digestibility in sorghum is complex; it can be affected by the starch-protein interaction, anti-nutritional factors such as tannins (although the sorghum used in the current study was tannin-free), and non-starch polysaccharides such as cellulose or hemicellulose [36][45]. Due to these reasons, sorghum starch is not as readily gelatinized as wheat as was stated previously. But increasing amounts of process energy resulted in a significant impact on degree of gelatinization in sorghum-based feeds (increase from 83 to 90.5%) and consequently on digestible energy as well. In the case of wheat-based feeds, gelatinization range was already fairly high (94.5-98.7%) irrespective of the energy input, so the impact on digestible energy was less. Another potential explanation might be the high water stability of the wheat-based pellets irrespective of process energy input resulting in less loss of nutrients before the feed is consumed, while sorghum-based diets had a significant improvement in water stability with higher process energy [18]. The latter was related to the increase in gelatinization and consequently binding ability of starch. Thus, in the case of sorghum, two inter-linked reasons led to a higher correlation between starch gelatinization and energy

digestibility as compared to wheat; one, the degree of starch susceptibility due to gelatinization and two, water stability.

Significant differences for both AEDD and APDD did exist between grain source ($P < 0.001$), and between diets with different levels of process energy ($P = 0.001$), but no interaction between grain source and process energy existed ($P = 0.056$). Significantly higher levels of AEDD were reported in wheat-based diets (79.03 to 82.93%) than in sorghum-based diets (74.29 to 76.79%). ADE values of 77.1% and 76.2% for wet-extruded wheat and sorghum-based diets, respectively, were reported by Davis & Arnold [10]. This is in line with the degree of gelatinization results in this research as well. Wheat-based diets were overall more gelatinized than the sorghum-based diets; this resulted in a higher amount of energy digested during consumption. AEDD values increased in both grain types as thermal energy input increased. These trends held true for the apparent protein digestibility of the diets as well; wheat-based diets had an APDD of 86.99 to 82.59%, and sorghum-based diets had an APDD of 79.81 to 77.29%. In both diets, higher thermal energy input levels result in an increased digestibility coefficient. The lower energy and protein digestibility of sorghum-based diets is likely due to the protein being tightly bound in the endosperm, and the starch being relatively inaccessible because of the surrounding protein matrix acting as a barrier, as was discussed earlier.

Limited research exists in aquaculture regarding the digestibility of sorghum-based diets. A 2006 study with carp reported a protein digestibility value of 71.86% in carp [11]. Conversely, a study with sunshine bass showed lower sorghum protein digestibility values of 60% [32]. It is likely that these differences are likely due to processing methods;

sorghum starch is bound in a protein matrix which will limit the ability of enzymes to access and digest it. Processing methods, such as extrusion, help release the starch from the protein matrix and increase the digestion of sorghum [26]. Studies have shown that extrusion enhances the protein digestibility of sorghum by 18% or a maximum of 30% [14][21]. The amount of process energy appeared to also help with this based on data from the current study; as process energy input levels increased via increased steam addition, the degree of starch gelatinization also increased, indicating that more of the starch was released from the protein matrix, which in turn correlated to a higher AEDD and APDD in both diets.

4.4 Conclusion

The results of this study indicate that grain sorghum can be used in practical diets for Pacific white shrimp as a replacement for whole wheat without any negative effects on growth, survival, and FCR. Results also show that reduced process energy inputs via reduced preconditioner steam addition do not significantly impact growth rates, creating possible cost-saving opportunities for feed producers. Whole wheat-based diets showed higher apparent digestibility values for both protein and energy in comparison to sorghum, especially in high-process energy diets. Based on these observed results, sorghum can be used as a carbohydrate source in shrimp cultivation.

4.5 References

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4.6 Figures and Tables

Figure 4.1: Linear regression of digestibility and starch gelatinization for all treatments

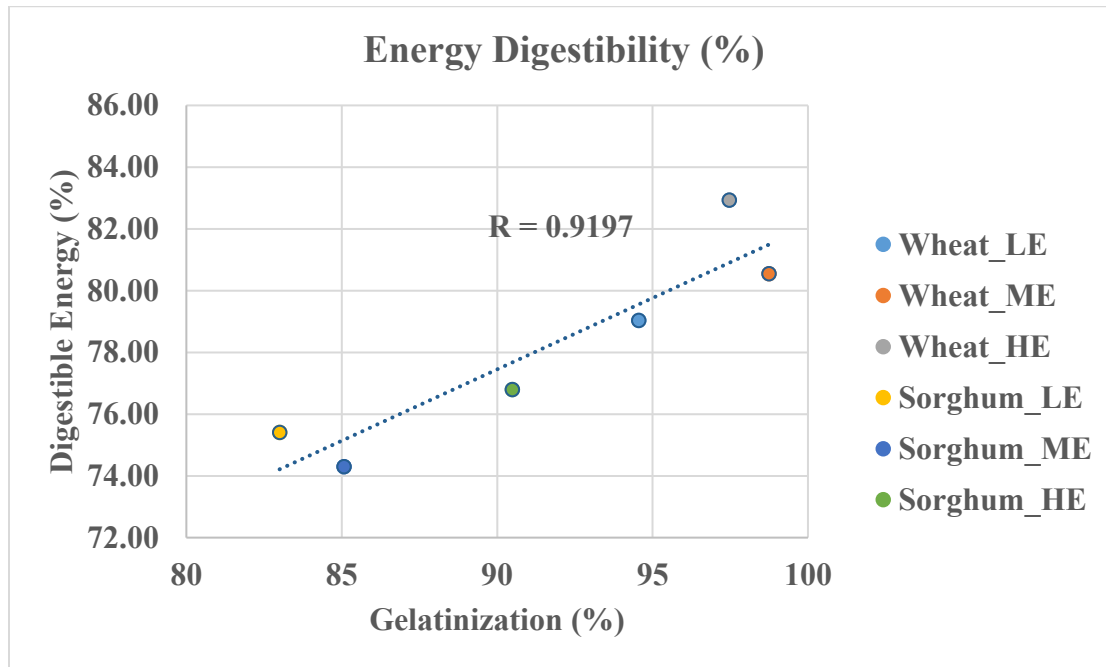


Figure 4.2: Linear regression of digestibility and starch gelatinization based on grain type

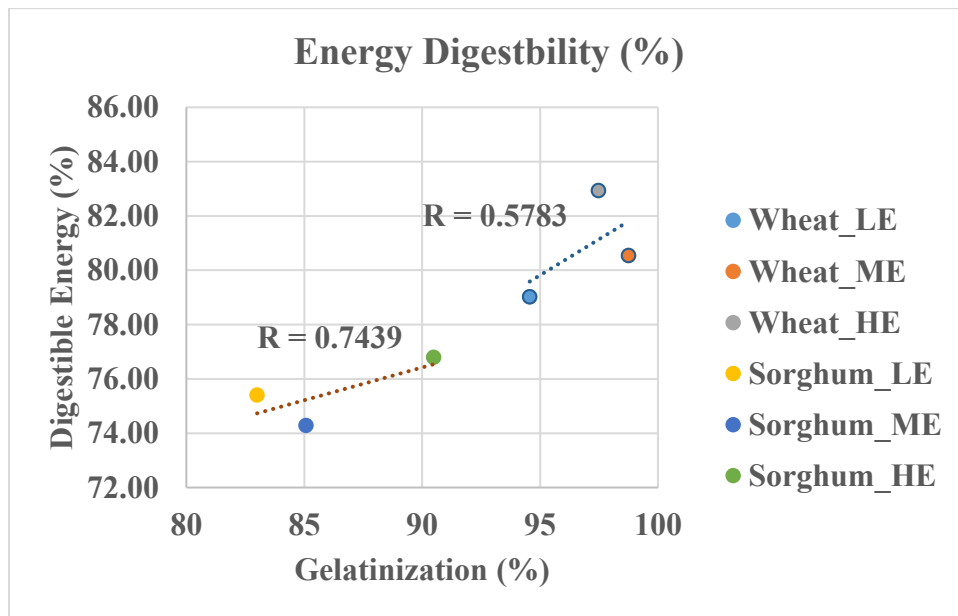


Table 4.1: Diet formulation and proximate analysis of shrimp diets containing different wheat and sorghum process energy input.

Ingredient (% , as is)	Whole Wheat	Sorghum
Fishmeal	10.00	10.00
Soybean meal	39.00	39.45
¹ CPC	8.00	8.00
Fish oil	2.00	2.00
Soy oil	3.02	2.65
Lecithin	1.00	1.00
² Cholesterol	0.12	0.12
Whole wheat	23.20	0.00
Rice Bran	10.00	10.00
³ Sorghum	0.00	23.12
⁴ Mineral premix	0.07	0.07
⁴ Vitamin premix	0.04	0.04
Choline chloride	0.20	0.20
Stay-C 35% Active	0.10	0.10
CaP-dibasic	1.75	1.75
Bentonite	1.50	1.50

¹Cargill, Inc., Wayzata, MN, USA

²MP Biomedicals Inc., Solon, OH, USA

³Nu-Life Market, Scott City, KS, USA

⁴Ziegler Bros, Inc., Gardners, PA, USA

All other ingredients were sourced through Fairview Mills (Seneca, KS, USA)

Table 4.2: Proximate composition of practical shrimp diets with different processing energy of sorghum and wheat. Diets were made at Kansas State University. Values represent means of two replicates. Analysis was performed by Midwest Laboratories, Inc., Omaha, NE

Proximate composition (as is)	Wheat_LE	Wheat_ME	Wheat_HE	Sorghum_LE	Sorghum_ME	Sorghum_HE
Moisture (%) ¹	5.47	6.06	6.47	5.64	6.13	6.12
Crude Protein (%) ²	37.85	37.70	38.10	38.05	38.50	38.05
Crude Fat (%) ³	7.22	7.13	7.16	7.02	6.90	6.98
Fiber (%) ⁴	3.20	2.90	2.85	3.20	2.75	3.05
Ash (%) ⁵	10.35	10.15	10.20	10.40	9.92	9.88
Sulfur (%)	0.39	0.38	0.38	0.39	0.39	0.40
Phosphorus (%)	1.25	1.24	1.24	1.26	1.20	1.26
Potassium (%)	1.31	1.31	1.31	1.33	1.29	1.40
Magnesium (%)	0.29	0.29	0.28	0.30	0.28	0.31
Calcium (%)	1.27	1.28	1.27	1.28	1.23	1.27
Sodium (%)	0.10	0.09	0.09	0.09	0.09	0.10
Iron (ppm)	217.50	220.50	223.50	234.00	237.00	227.50
Manganese (ppm)	85.80	84.05	90.15	81.50	76.85	80.20
Copper (ppm)	38.90	39.55	44.05	41.00	39.15	41.35
Zinc (ppm)	79.80	78.50	86.95	77.10	75.75	79.95

¹AOAC 930.15; ²AOAC 990.03; ³AOAC 954.02 (via acid hydrolysis); ⁴AOCS Ba-6a-05; ⁵AOAC 942.05; All minerals were tested using AOAC 981.01;

HE: high process energy

ME: medium process energy

LE: low process energy

Table 4.3: Amino acid profile of practical white shrimp diets with different thermal energy input of sorghum and wheat. Diets were made at Kansas State University. Results are expressed as g/100 g as is. Values represent means of two replicates. Analysis was performed by University of Missouri

	Wheat_LE	Wheat_ME	Wheat_HE	Sorghum_LE	Sorghum_ME	Sorghum_HE
Essential amino acids (EAA)						
Arginine	2.21	2.21	2.18	2.17	2.16	2.22
Histidine	0.90	0.90	0.90	0.91	0.90	0.91
Isoleucine	1.67	1.65	1.66	1.72	1.68	1.68
Leucine	3.31	3.27	3.25	3.54	3.55	3.56
Lysine	2.00	1.99	1.99	1.99	1.97	2.00
Methionine	0.68	0.67	0.66	0.68	0.67	0.68
Phenylalanine	1.83	1.81	1.80	1.87	1.87	1.88
Threonine	1.33	1.31	1.32	1.35	1.35	1.36
Tryptophan	0.41	0.39	0.33	0.39	0.38	0.37
Valine	1.79	1.78	1.78	1.81	1.80	1.82
Non-essential amino acids (NEAA)						
Alanine	2.03	2.00	2.00	2.20	2.20	2.21
Aspartic Acid	3.33	3.28	3.31	3.41	3.37	3.41
Cysteine	0.55	0.55	0.54	0.54	0.54	0.53
Glutamic Acid	7.15	7.08	7.09	7.03	7.00	7.07
Glycine	1.73	1.71	1.70	1.70	1.69	1.69
Hydroxylysine	0.04	0.04	0.03	0.03	0.04	0.04
Hydroxyproline	0.18	0.16	0.16	0.17	0.16	0.17
Ornithine	0.03	0.03	0.03	0.03	0.03	0.03
Proline	2.06	2.02	2.03	2.06	2.04	2.05
Serine	1.54	1.51	1.51	1.57	1.58	1.60
Taurine	0.20	0.19	0.18	0.19	0.20	0.20
Tyrosine	1.27	1.30	1.20	1.29	1.31	1.38
Sum AA	36.24	35.85	35.65	36.65	36.49	36.86

Analysis was performed according to the AOAC Official Method 982.30 E(a,b,c) chp. 45.3.05, 2006

Tryptophan was analyzed by alkaline hydrolysis – AOAC Official Method 988.15, chp. 45.4.04, 2006

Table 4.4: Degree of gelatinization by the enzymatic method. Values represent the means of three replicates. Analysis was performed by Midwest Laboratories, Inc., Omaha, NE, USA.

Diet	Degree of Gelatinization (%)
Wheat_LE	94.56 ^{bc}
Wheat_ME	98.75 ^a
Wheat_HE	97.47 ^{ab}
Sorghum_LE	83.00 ^d
Sorghum_ME	85.07 ^d
Sorghum_HE	90.49 ^c
Two-way ANOVA	
Model	<.0001
Process Energy	0.0003
Grain	<.0001
Process	
Energy*Grain	0.0075

Means not sharing the same letter are significantly different by the Tukey's HSD-test (Parametric ANOVA) at the 5% level of significance, with letters representing the interaction between grain source and process energy.

Analysis was done in accordance to AOAC 996.11 [3]

Table 4.5: Water quality parameters of the experimental systems used for the growth and the digestibility trials.

Parameters	Growth Trial	Digestibility Trial
Dissolved oxygen (mg L ⁻¹)	6.97 ± 0.33	7.09 ± 0.23
Temperature (°C)	28.08 ± 1.31	27.51 ± 0.71
Salinity (g L ⁻¹)	7.27 ± 0.15	9.52 ± 0.67
Total ammonia nitrogen (mg L ⁻¹)	0.04 ± 0.08	0.06 ± 0.04
Nitrite nitrogen (mg L ⁻¹)	0.10 ± 0.05	0.10 ± 0.07
pH	8.10 ± 0.07	7.8 ± 0.00

Table 4.6: Response of white shrimp (mean initial weight $0.82\text{g} \pm 0.05$) fed diets containing different process energy input of sorghum and wheat within a 6-week period. Values represent means of six replicates for experimental diets.

Diets	Survival (%)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	TGC	FCR	Gross Energy (cal/g)	Energy Retention (%)
Wheat_LE	84.44	9.02	8.20	999	0.23	1.47	4537	22.25
Wheat_ME	86.67	8.94	8.13	995	0.23	1.46	4407	21.62
Wheat_HE	81.11	9.10	8.27	1008	0.23	1.52	4403	21.49
p-value	0.71	0.86	0.88	0.98	0.81	0.80	0.64	0.78
PSE	4.77	0.19	0.20	42.56	0.01	0.07	111.64	0.81
Sorghum_LE	84.44	8.79	7.96	963	0.23	1.49	4400	20.84
Sorghum_ME	83.33	8.84	8.02	976	0.23	1.51	4434	21.65
Sorghum_HE	80.00	9.26	8.44	1036	0.24	1.44	4434	21.76
p-value	0.54	0.40	0.39	0.42	0.36	0.57	0.92	0.66
PSE	2.88	0.26	0.26	40.51	0.01	0.05	67.09	0.77
Two-way ANOVA								
Model	0.86	0.73	0.73	0.86	0.67	0.93	0.90	0.89
Ingredient	0.65	0.75	0.76	0.79	1.00	0.96	0.73	0.57
Process energy	0.48	0.39	0.39	0.57	0.35	1.00	0.83	0.99
Ingredient*Process energy	0.91	0.70	0.68	0.73	0.60	0.52	0.59	0.52

Table 4.7: Apparent digestibility coefficients of protein (APD), energy (AED) of the diet (D) offered to Pacific white shrimp, *Litopenaeus vannamei*.

Diet	AEDD	APDD
Wheat_LE	79.03 ± 1.23 ^{Ab}	82.59 ± 1.07 ^{Ac}
Wheat_ME	80.55 ± 0.38 ^{Aab}	84.73 ± 0.65 ^{Ab}
Wheat_HE	82.93 ± 1.34 ^{Aa}	86.99 ± 0.52 ^{Aa}
p-value	0.01	0.001
PSE	0.62	0.45
Sorghum_LE	75.41 ± 0.52 ^{Bab}	78.28 ± 0.56 ^B
Sorghum_ME	74.29 ± 0.79 ^{Bb}	77.79 ± 0.97 ^B
Sorghum_HE	76.79 ± 1.00 ^{Ba}	79.81 ± 1.75 ^B
p-value	0.02	0.18
PSE	0.46	0.69
Two-way ANOVA		
Model	<.0001	<.0001
Ingredient	<.0001	<.0001
Process energy	0.001	0.001
Ingredient*Process energy	0.056	0.056

The values from each diet are means and SD of triplicate tanks. Means within the same column not sharing any letter are significantly different by the Tukey's HSD-test (Parametric ANOVA) at the 5% level of significance, with uppercase and lowercase letters representing ingredient and process energy, respectively.

Chapter 5 - Effect of amylose content and grain sorghum on the extruded feed characteristics and growth performance of rainbow trout *Oncorhynchus mykiss*

Abstract

This study evaluated the feed quality and growth performance of rainbow trout fed extruded diets formulated with various sorghum grains and wheat. Four practical-type diets were formulated to be isonitrogenous and isolipidic at 45% protein and 18% lipid using wheat (W18), non-waxy red sorghum (S24), waxy red sorghum (S4), or white sorghum (S19) as the primary carbohydrate source. Diets were extruded using a Wenger X-20 single screw extruder and fed to rainbow trout (mean initial weight $\pm$ SD, 48.3 $\pm$ 0.70 g; 15 fish per tank) over a 14-week growth trial in a recirculating system at 15C with five replicate 400L tanks per diet. Feed manufacturing energy consumption varied due to grain choice, with W18 requiring the highest total energy (532kJ/kg) and S24 the lowest (442 kJ/kg). Water stability and pellet durability (PDI) were significantly altered by grain choice; water stability was lowest in S4 (86.6%) and highest in W18 (87.8%). PDI was highest in S4 (93.4%) and lowest in W18 (86.0%). W18 and S24 showed superior floating ability (100%), with S24 having the lowest (53.5%). Feed conversion ratios (FCR) were significantly affected by grain choice; S4-fed fish had the highest feed intake (155.8%bw/d) and FCR (0.96). W18 and S24-fed fish exhibited lower feed intake (~140%bw/d) and improved FCR values (0.86 and 0.88, respectively). Visceral index and filet ratio also showed significant differences

suggesting the need for further investigation to define the physiological implications of dietary grain type on nutrient utilization by rainbow trout. Overall, the results suggest that sorghum grains, both waxy and non-waxy, offer promising alternatives to wheat in aquafeeds, supporting rainbow trout growth with improved levels of pellet durability and acceptable water stability.

5.1 Introduction

The increased demand for high quality protein as the global population grows has led to the annual consumption of fish increasing at a rate of 3% every year between 1961 and 2019 (FAO, 2022), a rate that is almost double the annual population growth rate. Aquatic species, both farmed and captured, account for approximately 17% of global animal protein intake, and approximately 3 billion people receive at least 20% of their daily protein intake from fish (FAO, 2022). Globally, aquaculture production produced 88 million tons of seafood, approximately 50% of all seafood consumed (FAO, 2020), and the proportion of seafood that comes from aquaculture continues to increase as concerns and fears over depleted ocean stocks continue to build. Due to this, aquaculture production has increased significantly in recent years, growing at an average rate of 5.3% from 2001-2018 (FAO, 2020).

Currently, 70% of aquaculture farms rely on commercially produced compound feeds; from 2021 to 2022, the aquafeed industry grew 2.72%. Fish feed is estimated to account for 50-70% of the total farm production cost (FAO, 2010). The handling and transport of aquatic feeds places significant stress on the pellets and as such, there are high expectations and demands on the quality of the pellets to reduce breakage and fines.

Poor mechanical durability impacts not only the economics of the farm, but excess organic material can cause severe stress in aquatic environments. In salmonid aquaculture, a single percentage of lost feed will cost up to USD\$30 million annually, with discharges of up to 30,000 tons of organic matter into the water (Haubjerg, 2016).

The production of aquatic feed, due to these quality concerns, has become increasingly done through extrusion. This shift was also driven by the growing importance of floating feed requirements, which allows producers to monitor consumption to minimize water pollution. Extrusion works by combining raw ingredients with heat, water, steam, pressure, and mechanical force. This transforms ingredients into a viscous dough which is then forced through small, shaped openings, known as die holes, resulting in the formation of fully cooked pellets. The speed of this cooking process and energy transfer is relatively fast, distinguishing extrusion from more traditional pellet mill production methods (Riaz, 2020).

Energy comes in two forms during extrusion: specific mechanical energy (SME) and specific thermal energy (STE). Measuring these forms of energy is crucial for understanding energy consumption per kilogram of feed produced, and they are the driving forces behind the extrusion cooking process (Monti et al., 2016). As material is conveyed to the die, the frictional rubbing action of the screw against the viscous dough creates mechanical energy and shear. STE, on the other hand, takes place primarily in the preconditioner as steam is injected to heat the raw material within its barrel (Pacheco et al., 2018; Chen et al., 2025)).

Carbohydrates (CHO), such as the starches found in cereal grains, are generally included in feed formulations despite aquatic animals not having specific requirements for

carbohydrates (Romano & Kumar, 2019). Multiple studies have shown that CHO inclusion can have a protein-sparing effect upon feeding (Kaushik & Kim, 1992; Skiba-Cassy et al, 2024). This can substantially reduce feed costs since carbohydrates are the least expensive energy-yielding nutrient available for aquatic species (Singha et al, 2024). Not all aquatic species can readily digest carbohydrates, however. Rainbow trout have a limited capacity to digest and utilize dietary carbohydrates; this is influenced by starch processing, diet composition, and their environmental conditions. Short-term feeding of a high-carbohydrate diet was found to increase the specific growth rate in rainbow trout, which indicates some ability to utilize dietary carbohydrates for growth (Wang et al, 2024). The same study, however, found that high-carbohydrate diets can lead to increased fat accumulation in the liver, suggesting that rainbow trout have difficulty efficiently processing large amounts of carbohydrates. Research has also shown that the efficiency of digestible carbohydrate utilization for growth in rainbow trout is lower than it is for fat and protein. The net energy efficiency of digestible carbohydrates is estimated to be around 55-73%, with larger trout showing an improved better utilization of energy (Geurden et al., 2018; Lyons et al., 2024).

In addition to their nutritional role, CHO sources contribute functional benefits to feed processing (Stone et al, 2003). The native starch in cereal grains acts as a natural binder, enhancing pellet quality attributes such as water stability and pellet durability. Feeds formulated with wheat starch showed improved pellet-forming ability, water stability, and lesser amounts of fines during handling (Tiarniyu & Solomon, 2012). The gluten naturally present in wheat further enhances binding capacity. Vital wheat gluten has

been shown to produce strong pellets with a consistent cell structure and a smooth, non-porous surface (Draganovic et al., 2013). Due to these advantages, wheat is often the preferred grain in aquatic feed manufacturing. However, wheat production is resource-intensive, requiring high levels of irrigation and nitrogen inputs, which increases both its cost and environmental footprint. As water scarcity intensifies in key agricultural regions, the aquafeed industry may need to shift away from water-dependent grains like wheat.

Grain sorghum presents a promising alternative. As a non-GMO cereal crop with notable drought tolerance, sorghum outperforms wheat under water-stressed conditions, which often result in severe yield losses and crop failure for wheat (Hadebe et al., 2017). At lower water inputs, sorghum has demonstrated higher yields per acre than maize (van Oosterom et al., 2021). Globally, it ranks among the top five cereal crops, with the United States being the largest producer at 62.3 million tons in 2020 (Zarei et al., 2022). Sorghum is rich in starch (70–72%), contains a moderate protein level (11–12.8%), and offers a significant amount of dietary fiber (8.4–10.9%) (Zarei et al., 2022). Sorghum's high starch content makes it particularly attractive for extruded aquafeeds, where starch functions as the primary binder to maintain pellet integrity during water immersion (Romano & Kumar, 2019). There is limited research available on sorghum use in aquatic feeds. A literature review (Zarei et al., 2022) found promising but limited research indicating that sorghum use would be suitable in feeds for omnivorous fish such as carp and tilapia. A previous study by our group at Kansas State University (in partnership with Auburn University) found that sorghum served as an acceptable replacement for whole wheat in extruded diets for

pacific white shrimp, with no negative impact on pellet quality or growth rates when suitably processed (Graff et al., 2024).

Starch content of the cereal grain can also play a role in digestibility; waxy grains have high amounts of amylopectin and little to no amylose. The digestibility of starch found in sorghum can be relatively lower than other cereals such as corn due to the starch-protein matrix, but that can be mitigated by releasing the starch granules using various processing methods (Zarei et al., 2022). Starch content and type has been shown to have an impact on digestibility, with high-amylopectin levels (i.e., waxy sorghum) having a positive impact on digestibility (Dreher et al., 1984; Rooney & Plugfelder, 1986). Research on waxy sorghum use in aquatic feed is very limited, but research in other areas does exist. Sang et al. reported that waxy sorghum had a higher *in vitro* digestibility than normal sorghum. Results in the poultry and swine industry are inconsistent and are mostly focused on waxy maize. A study by Collins, Moran & Stilborn (2003) found that broiler feed based on waxy maize increased body weight gains and improved chilled carcass weight and meat yield after deboning in comparison to normal yellow dent corn. However, a study by Li et al. in 2017 found that waxy maize starch (in comparison to normal maize starch) had no impact on average daily gain or feed to gain ratios when fed to finishing pigs. Currently, research on amylose and amylopectin levels in starch indicate that amylose inhibits starch digestion, with waxy sorghum having more complete starch digestion and therefore performing better in diets (Liu et al., 2015). Furthermore, poultry and swine feed are primarily produced by pellet-milling, whereas aquatic feed is produced through extrusion. The high levels of mechanical shear and energy during processing will, in theory,

increase the digestibility of the feed. The objectives of this study were to determine the impact of grain amylose percentage on raw material functionalities, extrusion processing energy use (specific thermal energy and specific mechanical energy), as well as steam loss during preconditioning, and how those functionalities and processing results impact key pellet qualities such as bulk density and pellet expansion. A secondary objective of this study was to compare wheat-based diets to sorghum-based diets in terms of pellet quality and animal performance.

5.2 Materials and Methods

5.2.1 Experimental Design

Four treatment diets were formulated to assess the impact of different carbohydrate sources on energy use during extrusion processing, extrudate properties, and growth performance of rainbow trout. Diets were formulated using ground wheat (W18), normal red sorghum (S24), waxy red sorghum (S4), and white sorghum (S19), with the W/S referring to wheat/sorghum and the number referring to the percentage of amylose in the diet. Diets were formulated to have a grain inclusion level of 22.5%. Diets were formulated to have approximately 45% crude protein and 18% crude lipids. Diet formulation is shown in Table 5.1. A primary objective of this study was to determine the effect of replacing wheat (W18) with normal red sorghum (S24). Another objective of this study was to compare the effect of starch type and content in sorghum grains by comparing three sorghum grains with different amylose contents: normal red sorghum (S24), white sorghum (S19), and waxy red sorghum (S4).

5.2.2 Ingredients

Ingredients were procured through a local feed mill (Fairview Mills, Seneca, KS, USA). All sorghum varieties were sourced through a local farmer with the assistance of the Sorghum Checkoff program. The Emphyreal 75 – Corn Protein Concentrate was procured through Cargill, Inc (Wayzata, MN, USA). The feather meal was sourced through Scoular (Omaha, NE, USA). The taurine was sourced through Optimal Aquafeed (Omaha, NE, USA). Raw material protein (AOAC 990.03, nitrogen combustion method), fat (AOAC 920.39, ether extract method), ash (AOAC 942.05, combustion method), and total dietary fiber (AACC method 32-07, enzymatic-gravimetric method) were analyzed through proximate analysis conducted by Midwest Laboratories (Omaha, NE, USA).

5.2.3 Single Kernel Characterization System (SKCS)

Hardness index, mean kernel weight and mean kernel diameter of the whole grains were analyzed using a SKCS 4100 (Perten Instruments, Inc., Springfield, IL) that was connected to Windows software SK4100 version 2.1.0.1 (Bean et al. 2006). True density was measured with an Automatic Density Analyzer, Ultrapyc 1200e (Quantachrome Instruments, FL).

5.2.4 Amylose Content

5.2.4.1 Iodine Staining

Two methods of analysis were performed to identify the presence and amount of amylose and amylopectin in the whole wheat and sorghum grains, iodine-staining and colorimetric analyses. For the iodine-staining test, 80 grams of grain were ground in a Waring blender with approximately 160 ml of water. The ground mixture was then filtered

through a No. 200 wire sieve (75 μ m) from Fisher Scientific (Fair Lawn, NJ, USA) to remove bran and fine fiber. The slurry was centrifuged once at 3000 $\times$ g for 15 min (Beckman, Model J2-21 Centrifuge). Sorghum starch was suspended in 0.5 mL of distilled water and mixed with 20 μ L of 0.1N iodine solution. 20 μ L of the stained suspension was transferred onto a microscope slide, covered with a coverslip, and immediately observed under a light microscope (Olympus BX51, Olympus America, Inc., Melville, NY) using a 40X objectives lens.

5.2.4.2 Colorimetric Analysis

For the colorimetric method, weights were recorded the reference starch (20 mg), and the ground grain samples (35-40 mg). These weights were used to estimate the concentration of amylose in the dissolved samples after completion of the test. 0.1 mL of 80% ethanol was added to the sample before it was vortexed for nine seconds, then 1 mL of 90% DMSO:0.6M urea was added while vortexing the test tubes. The mixture was then heated in a 100°C hot block to dissolve the starch before another five mL of UDMSO was added. The samples were then heated at 100°C for 30 minutes, being vortexed every five minutes. After cooling, final weights were recorded and 0.1 mL of the dissolved starch solution was transferred to a plastic test tube with five mL of 0.5% TCA and vortexed briefly. 100 μ L of 0.01 N I₂-KI was added and immediately vortexed to mix. The samples were allowed to sit for 30 minutes at room temperature, and then read at 620 nm against a blank consisting of 5 mL of 0.5% TCA and 100 μ L of 0.01 N I₂-KI solution. A calibration curve was created using the standard amylose/amylopectin sample.

5.2.5 Total Starch

Colorimetric analysis was performed to measure total starch content of the whole grains. Total starch was determined by weighing 100 mg of the sample into a glass tube (Pyrex 9826). 0.2 mL of 80% ethanol was added with a 1 mL repeater pipette and vortexed for 10 seconds. A stir bar was then added with 2 mL of cold 1.7M sodium hydroxide with a 25 mL repeater pipette and vortexed for 15 seconds. Samples were then placed into an ice bath on a magnetic heating block and stirred for 15 minutes. A vortex was performed for 15 seconds every five minutes. Samples were removed from the ice bath, and 8 mL of 600 mM sodium acetate buffer with a pH of 3.8 was added and vortexed for 10 seconds. 0.1 mL of alpha-amylase was then added, as well as 0.1 mL of amylogucosidase using 1 mL repeater pipettes. Tubes were then capped and vortexed for 3 seconds. Samples were then incubated in a water bath at 50°C for 30 minutes before being removed and allowed to cool to room temperature. 2 mL aliquots of each sample were then transferred into 2 mL centrifuge tubes and centrifuged at 13,000 rpm for 5 mins. 1 mL of each centrifuged sample was then diluted to 9 mL of 100 mM sodium acetate buffer with a pH of 5.0, before transferring 0.1 mL of the sample aliquots into glass test tubes. A 50 mL repeater pipette was used to add 3 mL of GOPOD to the samples, glucose standards, and blank (100 mM sodium acetate buffer) before being vortexed for 3 seconds. Samples were incubated in a water bath, uncovered, for 20 minutes at 50°C. After removal from the water bath, adsorption was read at 510 nm using a spectrophotometer (Cary 60, Agilent Technologies).

5.2.6 Grinding, Energy Collection, and Mixing

The whole grains were ground using a pilot-scale hammer mill (Model D Comminuter, Fitzpatrick, Westwood, MA, USA). Grain grinding was done using a screen with a 1.65 mm hole size. Energy use during grinding was measured using a three-phase power quality logger (Fluke Corporation, Everett, WA, USA). The dry ingredients were mixed in a double-ribbon blender (Wenger Manufacturing, Sabetha, KS, USA). Diets were mixed for 3 minutes for uniformity. Post mixing, all diets were then ground through the same hammermill with a 1.02 mm screen to provide a uniform particle size within each diet. Based on the energy usage rate and throughput, energy use in kJ/kg was calculated for both pre- and post-batch grinding.

5.2.7 Particle Size Analysis

Particle size analysis was performed using a rotating-tapping sieve stack (Ro-Tap, Tyler Co., Mentor, OH, USA) according to the ASAE S319.2 standard method (ASAE, 1995) to determine the fineness of feed materials through sieving. Flow agent and sieve agitators (brushes and rubber balls) were used. The Ro-Tap test utilizes a sieve stack with decreasing screen sizes; it begins with a #6 screen and ends with a #270 screen. One hundred grams of material is placed in the top sieve. The shaker is then turned on for ten minutes. The remaining material on each sieve is measured and the geometric mean diameter (dgw) and the geometric standard deviation (sgw), as well as the percentage of particles that pass through each sieve, are calculated. The dgw is calculated in microns, while the sgw is unitless.

5.2.8 Rapid visco analysis

The pasting properties of formulations were studied using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Waltham, MA, USA) by measuring viscosity changes in slurry form during a controlled heating and cooling process with the application of moderate shear. AACC standard method 76-21.02 was followed for the test (AACC Method 76-21.02 STD1). Briefly, ~3g of each sample was combined with ~25 ml deionized water to create a 14% (w/v basis) suspension and placed in the RVA. Stirring was carried out throughout the test as specified in the standard. The temperature cycle involved holding at 50°C for 1 min, heating 95°C at 12.2°C per min, followed by holding again at 95°C for 2.5 min, and cooling to 50°C at 11.8°C per min before a final holding period for 2 min. Parameters such as pasting temperature and peak viscosity were recorded using the pasting profiles that were generated from each test. RVA analyses were conducted in duplicate for each formulation.

5.2.9 Extrusion Processing

Prior to extrusion, flow rate of each formulation delivered by the volumetric feeding system of the extruder was calibrated at three feeder screw speeds, 8, 11, and 14 rpm. Measurements were done in duplicate and averaged over both high and low fill levels of the live-bottom feed hopper. Formulations were processed using a 37.3 kW (50 hp) pilot-scale single screw extruder (X-20, Wenger Manufacturing, Sabetha, KS, USA) with a screw diameter of 82.6 mm (3.25 in) and L/D ratio of 10:1. The extruder barrel consisted of 6 heads divided into three heating zones, with the inlet head having a smooth liner and rest of the heads having spiral liners. The screw profile (Figure 5.1) had single-flighted full pitch

screws in the feed intake section, transitioned to double-flighted half pitch screws for creating compression, and ended with a cone screw at the discharge for directing flow into the die opening. Two small, one medium, and one large steam locks were interspersed between the screw elements to provide resistance to flow, increase material fill and lead to desired mechanical energy input.

During the extrusion experiments raw material mash in the feed hopper was maintained at medium fill level. The feeder screw speed was set at 18 rpm, which corresponded to dry mash feed rate of 148.30-160.73 kg/h at medium hopper fill as obtained from the calibration for each formulation. The mash was conditioned to 94°C in a Wenger model 2 DDC pre-conditioner, with water and steam injection at 7.82-8.46 kg/h and 13.45-13.54 kg/h, respectively. Water was injected into the extruder barrel at an average rate of 18.5 kg/h, to achieve an in-barrel moisture of 28.5% wb. The extruder barrel temperatures were set at 40, 70 and 90°C from inlet to discharge, and the screw speed at 400 rpm. A die with five-2.50 mm openings was used to shape the product exiting the extruder, which was cut at a speed of 1219 rpm using a rotary knife system with 6 hard blades. A thermocouple probe was inserted into the material stream immediately downstream of the discharge screw to measure the die temperature. Each formulation was extruded for 50 min. Process data were recorded using a computerized data acquisition system, with the exception of die temperature and extruder motor power consumption data that were recorded using a video camera and transcribed every two minutes. The extruded pellets were conveyed using a negative pressure pneumatic system to a pilot-scale Wenger series 4800 two-pass gas-fired continuous dryer and single-pass

cooling system. Drying temperature and time were 104.4°C (220°F) and 17.5 min, while cooling was done with room temperature air at for 4.5 min.

5.2.10 Specific Mechanical Energy and Thermal Energy

Specific mechanical energy (SME) transferred from the screw to the material during the extrusion process was calculated as described below from the power consumed by the extruder motor measured using a wattmeter (Flory et al., 2023).

$$SME \left(\frac{kJ}{kg} \right) = \frac{W - W_0}{m_f} \quad (5.5)$$

where W is the power consumed during processing (kW), W_0 is power consumed at no load with the screw running and no material flowing through (kW) and m_f is the dry feed rate or rate of material delivered by the feeder screw in kg/sec as determined using volumetric feed delivery calibration.

Specific thermal energy (STE) input during the extrusion process was calculated for selected treatments from net heat transferred to the material due to steam absorbed during preconditioning, as follows (Chen et al., 2025).

$$STE \left(\frac{kJ}{kg} \right) = \frac{Q_{thermal}}{m_f} \quad (5.6)$$

where $Q_{thermal}$ is the net rate of thermal energy or heat absorbed from steam (kJ/s), which was calculated using the below equation from the enthalpies of steam injected and unabsorbed steam lost to atmosphere at the preconditioner discharge.

$$Q_{thermal} \left(\frac{kJ}{s} \right) = (m_s \cdot h_s - m_s \cdot 4.18 \cdot T_r) - (m_{sl} \cdot h_{sl} - m_{sl} \cdot 4.18 \cdot T_r) \quad (5.7)$$

where h_s and h_{sl} are enthalpies of steam injected and steam lost to atmosphere (kJ/kg), respectively, as obtained from the steam tables, m_s and m_{sl} are rates of steam injection

and loss (kg/s), respectively, the constant 4.18 is the specific heat of water at room temperature in kJ/kg°C and T_r is room temperature (°C). The rate of steam loss from the preconditioner was calculated using mass balance as described below.

$$m_{sl} \left(\frac{kg}{s} \right) = (m_f + m_s + m_w) - m_f \left(\frac{1-X_{wf}}{1-X_{wpc}} \right) \quad (5.8)$$

where m_w is the water injection rate into the preconditioner (kg/s) and the second term on the right of the equation is the rate of conditioned mass exiting the preconditioner with X_{wf} and X_{wpc} representing the fractional wet basis (or as is) moisture contents of the feed material and the conditioned mass, respectively, measured using oven drying method (135°C, 2 h).

5.2.11 Bulk Density

Extruded product bulk density (g/L) was measured by recording the mass of pellets filling a 1-liter cup to the brim. Two replicate measurements were made for both wet product directly off the extruder discharge and dry product after the dryer.

5.2.12 Extrudate Characterization

Twenty replicates were measured from the first and last bag of each treatment, for a total of forty replicates. Individual mass, diameter and length of each pellet were recorded. These data were used for calculating sectional expansion ratio (ER), specific length (L_{sp}) and piece density (P_d) as described below (Karkle et al., 2012).

Expansion ratio (ER) is measured by taking the square of the extrudate diameter and dividing that by the square of the die diameter. Where D_e is the extrudate diameter, and

D_d is the diameter of the die.

$$ER = \frac{D_e^2}{D_d^2} \quad (5.9)$$

Specific length is the length of one pellet divided by the weight of the same pellet.

This is recorded in millimeters per gram (mm/g).

$$L_{sp}(\frac{mm}{g}) = \frac{L_e}{m_e} \quad (5.10)$$

Where L_e is the measured length of the extrudate, and m_e is the mass of the extrudate.

Piece density of the dried pellets was calculated from the measurements collected on the individual pellets. Each pellet had its diameter (cm) and length (cm) measured (at the widest point) using digital calipers. The weight (g) was also measured. Piece density was calculated through the following equation.

$$Piece\ Density\ (\frac{g}{cm^3}) = \frac{m_e}{V_e} \quad (5.11)$$

Where m_e is the mass of the pellet and V_e is the volume of that same pellet. The volume is calculated by dividing the width in half to get the radius (r), then multiplying r^2 by π and the pellet length.

5.2.13 Floatability

Floating/sinking of the extruded pellets was determined by randomly selecting fifty pellets from the first and the last bag of each treatment and immersing them in water. The number of pellets that sank was counted every five minutes for thirty minutes. Data was collected in duplicate per bag, for a total of four reps per treatment.

5.2.14 Pellet Durability Index

Pellet durability index (PDI) is the standard for measuring the number of broken pellets, dust, and fines that are generated through repeated handling. PDI was tested using a Holmen NPH100 pneumatic tester. Pellets were first sieved using a #10 screen to remove broken pellets and fines. One hundred grams of sieved pellets were tested. Samples were placed in the chamber and then air-agitated for 60 seconds using 70 mbar of pressure. Upon test completion, the fines were automatically sieved from the tested sample. PDI is defined as the percentage of pellets remaining after the conclusion of the test. Two replicates were measured from both the first and last bag of each treatment, resulting in four replicates per treatment.

5.2.15 Water Stability

Water stability of the extruded pre-test pellets was measured using a modified method of the version described by Adediji, et al. (2017). Five grams of feed were weighed and then placed in a mesh tea ball before being placed in a beaker of distilled water at room temperature. The samples were soaked for 15, 30, and 60 minutes, removed from the container, and then dried in a convection oven for 24 hours at 90°C. Sample weight was collected post-drying, with the difference in mass between pre and post-soak weights after drying being defined as ‘water stability’, as shown in the following equation after having a moisture content correction factor applied to it:

$$\text{Water Stability} = \frac{\text{Post soak weight} \times (1 - X_{w\text{post}})}{\text{Pre soak weight} \times (1 - X_{w\text{pre}})} \times 100$$

(5.12)

Where the initial MC% (X_{wpre}) is the moisture content of the dried pellet before the water stability test, and the final MC% (X_{wpost}) is the moisture content of the dried pellets that underwent water stability testing after drying. This moisture content was determined using the standard AACC 44-19.01 method of two hours drying at 135° C (AOAC, 1999). The corrected water stability was calculated by taking the original water stability value and multiplying it by the moisture correction factor for each test.

5.2.16 Growth and Digestibility

A growth and digestibility study was performed at the Bozeman Fish Technology Center (Bozeman, MT). Diets were fed to rainbow trout for 14 weeks with an average start weight of 48.3 ± 0.70 grams, in a recirculating system at 15C with five replicate 400L tanks per diet. There were 5 tanks per diet with 15 fish per tank. Tank weights were collected every three weeks and feeding took place twice per day until satiation. Feed conversion ratio (FCR) was calculated using the following equation:

$$FCR = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}} \quad (5.13)$$

There was a 1-week acclimation period for digestibility at the conclusion of the growth study. The feed had 0.1% Yttrium Oxide included in the formulation during feed processing as a digestibility marker. Feces were collected manually by placing pressure on the fish abdomen and collecting samples directly into a plastic collection pan. Apparent digestibility coefficients were calculated using the following equation:

$$ADP \text{ and } AED (\%) = 100 - \left[100 \times \left(\frac{\% \text{ Y2O3 in feed}}{\% \text{ Y2O3 in feces}} \times \frac{\% \text{ nutrients in feces}}{\% \text{ nutrient in feeds}} \right) \right] \quad (5.14)$$

Upon the conclusion of the study, three fish were randomly sampled. Liver, viscera and filet, as well as whole body weight, were collected and recorded in order to determine the hepatosomatic index, filet ratio, and visceral index using the following equations:

$$\text{Hepatosomatic Index (HSI)} = \frac{\text{Liver weight}}{\text{Fish weight}} \times 100 \quad (5.15)$$

$$\text{Filet Ratio} = \frac{\text{Filet weight} \times 2}{\text{Fish weight}} \times 100 \quad (5.16)$$

$$\text{Visceral Index} = \frac{\text{Visera weight}}{\text{Fish weight}} \times 100 \quad (5.17)$$

5.2.17 Statistical Analysis

Statistical analyses were performed on SAS software (SAS Institute, Inc, Cary, NC, USA). One-way ANOVA ($P < 0.05$) using the general linear model was employed to determine statistical difference between treatment means with the fixed effect being grain. Tukey's post-hoc test ($P < 0.05$) was performed for pairwise comparison between means after a significant ANOVA result. Correlation analysis was done using Excel 2021 (Microsoft, Inc., Redmond, WA, USA).

5.3 Results and Discussion

5.3.1 Grain Characteristics and Grinding

Total starch values ranged from 56.1 to 64.4% with S24 having the greatest amount of starch. Apparent amylose testing resulted in values of 18.4 and 24.2% for W18 and S24, respectively. S4 and S19 resulted in 4.8 and 19.3%, respectively. Higher amylose content tends to be associated with denser, more compact endosperms and starch granules, while

waxy grains have a softer, more floury endosperm (Yang et al., 2024). Total starch content also plays a role in grain density, and as expressed earlier, those values in the tested samples ranged from 56.1 to 64.4%, also impacting the density of the grains. Wheat generally contains 60-75% total starch, with amylose content ranging from 22-30% (Liu et al., 2023). In this study, the tested wheat had reported values of 58.88% total starch, and 18.37% amylose content on a dry basis. Red sorghum typically ranges from 64-74% for total starch content, with 24-34% amylose content (Yang et al., 2024). S24, the normal red sorghum variety used in this study, had 64.44% total starch and 24.2% amylose content. Figures 5.6 through 5.9 show the iodine stained flour; a dark purple stain represents the presence of amylose, and as shown in Figure 5.8, only S4 presented with amylose free granules. Kernel hardness, diameter, weight, and true density are shown in Table 5.3. Both the initial grain grinding and post-batch grind energy use has a high correlation with the true density of the grain ($R=0.908$ and 0.956 , respectively). As grain density increases, it requires more energy for the hammermill to reduce the particle size as the tightly packed nature of the grain is more resistant to particle size reduction, thereby increasing the time it takes to reduce the grain to a small enough particle size where it can pass through the screen.. Energy use varied during grain grinding, with W18 requiring the most energy (75.31 kJ/kg) and S24 requiring the least (33.91 kJ/kg). All sorghum grains used similar amounts of energy. Throughput followed similar trends as energy use, but inversed; W18 had the lowest throughput during grinding at 410.91 kg/hr, while S24 had the highest at 766.2 kg/hr. These values are shown in Table 5.5, while energy use and throughput during the post-mix

grind are shown in Table 5.6. Post-batch grinding is used to create a uniform particle size for extrusion processing, with the distribution being shown in Figure 5.

Particle size analysis of the ground grains ranged from an average particle size (dgw) of 285 to 375.5 microns. Particle size of the grain appears to be driven by kernel hardness ($R=0.976$), with grains that had a higher hardness index having a larger final particle size. S4 and S19 had hardness index values of 75.03 and 80.03, respectively, and resulted in average particle sizes of 367.5 and 375.5 microns, respectively. Conversely, W18 and S24 were 66.5/59.88 for hardness index, and 331.5/285.0 microns for particle size analysis, respectively. This is potentially due to how the grain is ground; hammermilling is an impact driven process. As harder kernels are more resistant to fracturing, it is possible that they are fracturing into larger particles during processing as opposed to shattering into many finer particles. Ground grain particle size is shown in Table 5.7, while the particle size of the final diet is shown in Table 5.8.

5.3.2 Extrusion Processing

Energy inputs during single-screw extrusion processing comes primarily in two forms; thermal energy (STE) during preconditioning, and mechanical energy (SME) which is generated by the frictional rubbing action of the screws as it conveys the raw material towards the die (Kantrong et al., 2018; Monti et al., 2016). Preconditioner steam loss (%), STE, SME, and Die temperatures are shown in Table 5.11. Preconditioning is used to transfer heat and moisture to the raw diet by way of steam and water injection; this energy transfer serves to help soften and plasticize the diet before it is discharged into the extruder barrel and screws (Riaz, 2000). As all diets were processed similarly in terms of

how much steam and water is injected, any differences in steam absorption are due to the inherent properties of the raw material, which needs more investigation. RVA profiles (figure 5.2) provide insight into the thermal behavior of carbohydrates in each grain. W18 and S24 display the highest peak viscosities, indicating a high degree of starch gelatinization potential and strong water-binding during thermal processing. The lower pasting temperatures (70° and 78°C, respectively) as shown in Figure 5.3 suggest that starch granules in these grains begin to swell and gelatinize earlier in the cooking process, leading to higher viscosity in the barrel during extrusion processing. In sorghum grains, peak viscosity increases as amylose content increases (445 to 1479.7 cp), while pasting temperature decreases with S4 having a pasting temperature of 81°C and S24 having a pasting temperature of 78°C. S4 had the least amount of steam loss (1.6%), which led to the highest STE value (228.5 kJ/kg); S24 had the highest steam loss and the lowest STE (10% and 185 kJ/kg, respectively). Very few papers go into steam loss during preconditioning; previously reported values in pet food ranged from 50.2-54.1% (Chen et al., 2025) which are much higher than values reported here. STE in previous studies ranged from 161 to 430 kJ/kg (Maichel, 2021; Pacheco et al., 2018; Monti et al., 2016). All diets had a preconditioning temperature discharge temp of approximately 94°C, despite the differences in STE. As the product is preconditioned, the starch present in the product begins to be gelatinized. This in turn reduces the mechanical energy that is required to fully cook the product (Riaz, 2000). Another effect of STE and preconditioning is the previously mentioned softening and plasticization of the diet; as the diet becomes more thermally cooked, this softening effect will reduce the mechanical energy required to convey the

material (Levine, 2014; Pacheco et al., 2018). Those trends broadly exist in our data; as STE decreases, we see a subsequent increase in SME. The only exception to this trend is the wheat-based diet with the second highest STE value and the highest SME value. This is consistent with higher SME values W18 (20.17 kJ/kg) and S24 (189.99 kJ/kg) diets, as well as elevated die temperatures (126 and 125°C, respectively) in comparison to the S19 (178.2 kJ/kg and 123°C) and S4 (160.69 kJ/kg and 119°C) diets.

5.3.3 Expansion Characteristics

Expansion characteristics of diets are shown in Table 5.12. Wet bulk densities (off the extruder) ranged from 372 to 431.5. It was previously mentioned that as STE decreased, SME tended to increase, which resulted in a greater amount of heat and energy at the die, which presented an increased die temperature. Total energy transfer from the extruder to the molten mash is what drives expansion, conveyed in the form of mechanical and thermal energy (Monti et al, 2016). This increase of energy transfer in the barrel, and at the die, resulted in increased expansion of the extruded pellets in the immediate wet bulk density. Die temperature was highest for the W18 and S24 treatment (126 and 125°C), and decreased as amylose content of the grain decreased in S19 and S4 (123 and 119°C). This trend is also represented by other measures of expansion. Radial expansion measured by SEI showed W18 having the greatest expansion (1.68) and S4 the least (1.30), which matches the existing trends in pasting temperature during RVA analysis. Specific length, a measure of longitudinal expansion, was greatest in the W18 and S24 diet, although statistical analysis failed to show any significant differences. Piece density (g/cm³) also had the same trend, with W18 being the least dense (0.4723) and S4 the most (0.6308).

RVA curves support this expansion trend. As stated, W18 and S24 had the greatest peak viscosities, indicating strong starch swelling capacity and water uptake, as well as the lowest pasting temperatures, which in turn would help drive the expansion observed after extrusion. In contrast, the lower amylose content in sorghum grains developed much less viscosity and required a higher temperature to do so, which is reflected in the reduced SEI and increased bulk density.

5.3.4 Floating Ability

There were no differences between the W18 and S24 diets with regard to floatability of the extruded pellet as shown in Table 5.13. Over 30 minutes, both maintained approximately 100% floating pellets. S4 started with 59.5% pellets floating, and over thirty minutes, slowly decreased to approximately 53%. S19 started with 74.5% floating, and increased over 30 minutes to 88.5% floating. These differences indicate that there are internal structural differences in how the pellet bound together during processing.

5.3.5 Pellet Durability Index

Reduced levels of SME led to a reduction in expansion and more compact, dense pellet, which in turn led to a higher pellet durability index. The pellet durability index of the diets ranged from 86.03 to 93.41% with W18 having the lowest PDI and the S4 diet having the highest PDI (Table 5.14). These differences can largely be attributed to the internal structure of the pellet; the W18 and S24 pellets were more expanded in size than the S19 and S4. This created weaker points where parts of the pellet could fracture and chip away during handling. Friesen et al. (2024) found that different varieties of sorghum showed significant differences in pellet durability when processed through a pellet mill. Waxy

sorghum produced pellets that exhibited higher durability when compared to corn and non-waxy sorghum, even when ground to the same particle size (Friesen et al., 2024).

5.3.6 Water Stability

At 15 minutes, water stability ranged from 91.85-90.84 and at 60 minutes, values ranged from 84.76-81.63 (Table 5.15). W18 tended to have the highest water stability and S4 had the lowest. While the differences were minor between most of the grains, there was a consistent trend with W18 having the highest water stability as opposed to the sorghum grain-based feeds. The starch granules in sorghum are trapped in a protein-based matrix (Zarei et al., 2022). It is likely that more energy is needed to fully process and gelatinize sorghum-based feeds in order to improve the water stability.

5.3.7 Trout Feeding Trial Results

Proximate analysis of the finished product (Table 5.16) shows that gross energy, dry matter, protein, ash, and fiber content were generally consistent across all extruded and coated diets. Gross energy values ranged narrowly from 5.087 to 5.148 kcal/kg, with S24 diets displaying the highest value. Crude protein content ranged from 45.2% in S19 to 47.3% in W18 diets. However, the most notable variation was observed in crude fat content, ranging from 9.87% in S4 to 11.2% in W18. This variation is likely due to differences in oil absorption during coating, which is affected by pellet expansion. W18 diets had the highest expansion, facilitating greater oil penetration. In contrast, S4 pellets, with the lowest expansion, exhibited the lowest fat levels. Crude fiber content was highest in S4 (1.58%) and S19 (1.52%), indicating that these grain types contributed more fibrous material to the final product.

5.3.7.1 Growth Performance and Nutrient Retention

Performance data (Table 5.17) show that feed intake on a %bodyweight per day was significantly higher in tanks fed the S4 (155.8) and S19 (151.8) diets compared to W18 (139.7) and S24 (141.8). However, these higher intakes did not translate to greater mass gain, which remained statistically similar across treatments ($p = 0.6779$). As a result, the feed conversion ratio (FCR) was most efficient in W18 (0.86) and S24 (0.88) diets, and significantly poorer in S4 (0.96) and S19 (0.94) treatments ($p = 0.0009$).

Crude protein intake (g/fish) did not differ significantly among treatments ($P=0.8178$) with S19 (159.0) and S24 (159.1) having the highest numerical intake. Similarly, protein retention showed no significant differences ($P=0.7241$). Protein retention was highest in S19 (43.3%) and lowest in S4 (41.4%). Gross energy intake per fish was likewise not significantly different among treatments ($P=0.6475$); S19 had the highest numerical intake (1779.1 kcal/fish). Energy retention did not differ significantly among treatments ($P=0.2119$), with S24 having the highest numerical retention at 47.7%, and S4 having the lowest at 38.8%.

There are relatively few studies evaluating the use of sorghum in diets for aquaculture. However, research examining the inclusion of sorghum distillers dried grains (DDGs) in both extruded and pelleted shrimp feeds has shown that sorghum DDGs can be successfully incorporated at levels up to 40% in clear water systems without negatively impacting growth, survival, or feed conversion ratio (Adedeji et al., 2017; Zhou, 2014). It's important to note, though, that these studies used sorghum DDGs primarily as a protein source to replace soybean meal, rather than as a carbohydrate source. Due to the ethanol

production process, sorghum DDGs have a different nutritional profile than whole grain sorghum, containing higher levels of protein (34%), fiber (10%), and lipids (9%), and significantly less starch (24%). In contrast, other studies have shown that omnivorous species like tilapia tolerate sorghum-based diets well. In fact, tilapia fed diets containing 25% grain sorghum or up to 30% sorghum starch demonstrated improved growth performance compared to those fed diets based on corn, wheat, barley, or rice (Abdel et al, 2016; Al-Ogaily et al, 1996). Finally, our previous research has shown that sorghum could be incorporated in pacific white shrimp with no impact on FCR or growth rates when compared to wheat-based diets (Graff et al., 2024).

5.3.7.2 Whole-Body Composition

Whole-body composition of fish (Table 5.17) did not differ significantly among treatments. Moisture, protein, fat, ash, and energy content remained statistically stable across all diets. Moisture levels ranged from 67.1% to 68.1%, crude protein from 17.64% to 17.94%, and crude fat from 12.72% to 13.04%, suggesting that dietary carbohydrate source had minimal impact on body composition under the conditions tested.

5.3.7.3 Apparent Digestibility Coefficients and Tissue Deposition

Apparent digestibility of nutrients (Table 5.20) remained consistent for dry matter, fat, protein, and gross energy across all diets. Dietary carbohydrate source did, however, influence visceral fat deposition and carcass characteristics (Table 5.19). Values represent the percentage of total weight of the whole body fish. The visceral somatic index (VSI) was highest in fish fed S4 (11.22%), significantly different from S19 (9.62%) and lower than W18 (9.98%) and S24 (10.75%) ($p = 0.0102$). Filet ratios followed an inverse trend, with S4 and

S24 diets resulting in the lowest yield (~57.4%), compared to 59.34% in W18. Though differences in filet ratio were significant ($p = 0.0336$), post-hoc comparisons did not differentiate treatments. These findings have economic implications, as filet yield is critical to marketable output. The lower protein retention in S4, coupled with higher VSI and unchanged energy retention, suggests that retained energy may have been stored as visceral fat rather than lean tissue.

5.4 Conclusion

This study demonstrates that grain sorghum, particularly sorghum with a high amylose content, is a suitable alternative to wheat in aquafeeds, offering comparable digestibility and performance with good pellet quality. Sorghum with lower amounts of amylose content than is found in normal US grain sorghum also support similar growth and digestibility, though efficiency was reduced due to increased feed intake and shifts in tissue partitioning. With processing refinement, lower amylose sorghum types may be optimized to match or exceed wheat's efficiency while leveraging their mineral digestibility and sustainability advantages.

5.5 References

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5.6 Figures and Tables

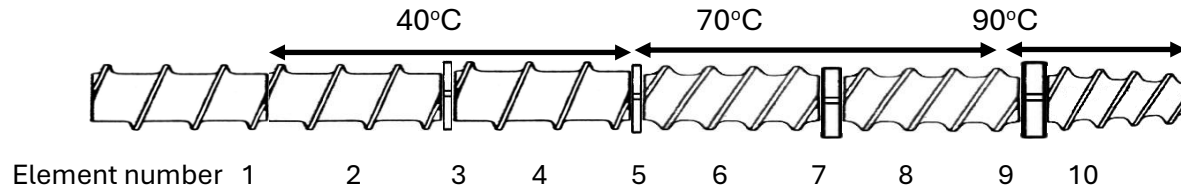


Figure 5.1: Pilot-scale single screw extruder screw profile and set barrel temperatures

1= inlet screw; 2 =single flight full pitch screw; 3=small steam lock; 4=single flight full pitch screw; 5=small steam lock; 6=double flight half pitch screw; 7=medium steam lock; 8=double flight half pitch screw; 9=large steam lock; 10=double flight half pitch cone screw.

Figure 5.2: RVA Curve for Ground Grains

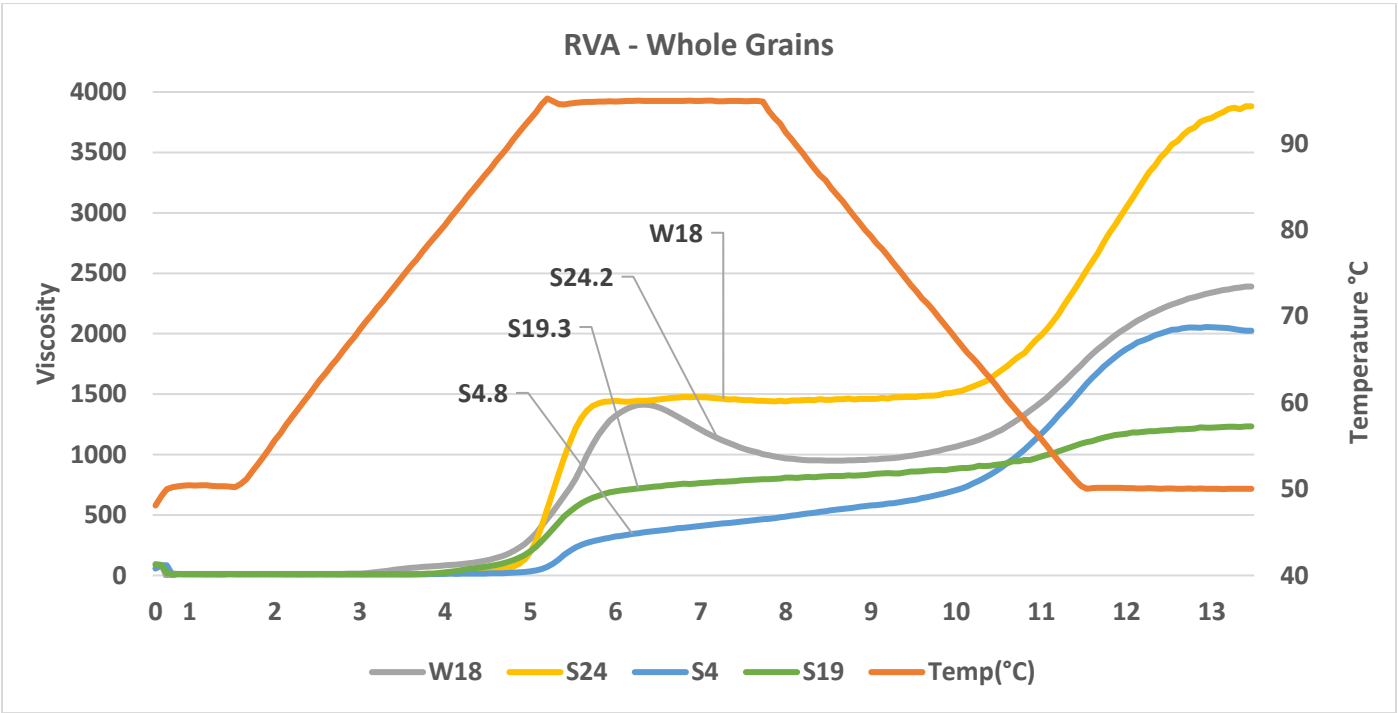


Figure 5.3: Peak Viscosity and Pasting Temp for Ground Grains

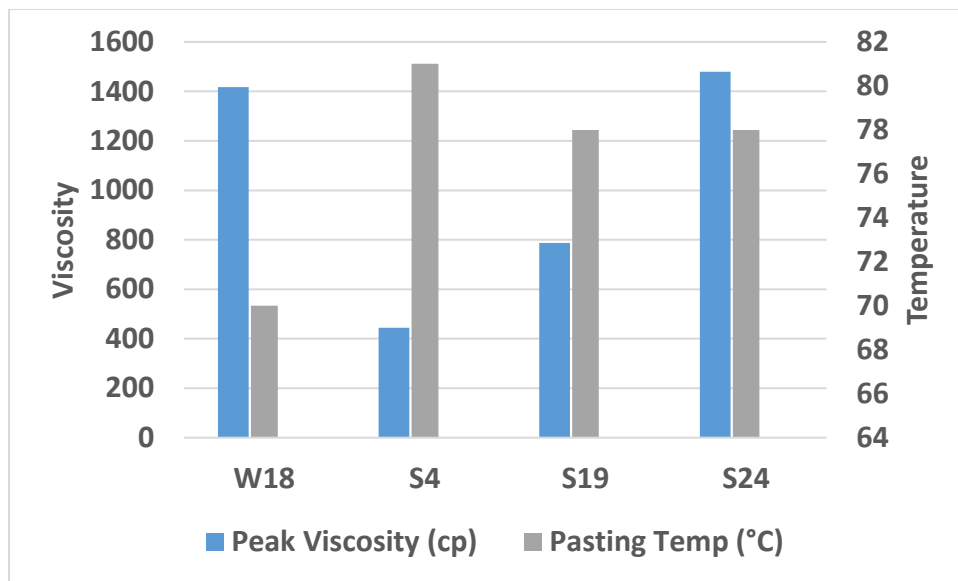


Figure 5.4: Cumulative Particle Size for Ground Grains

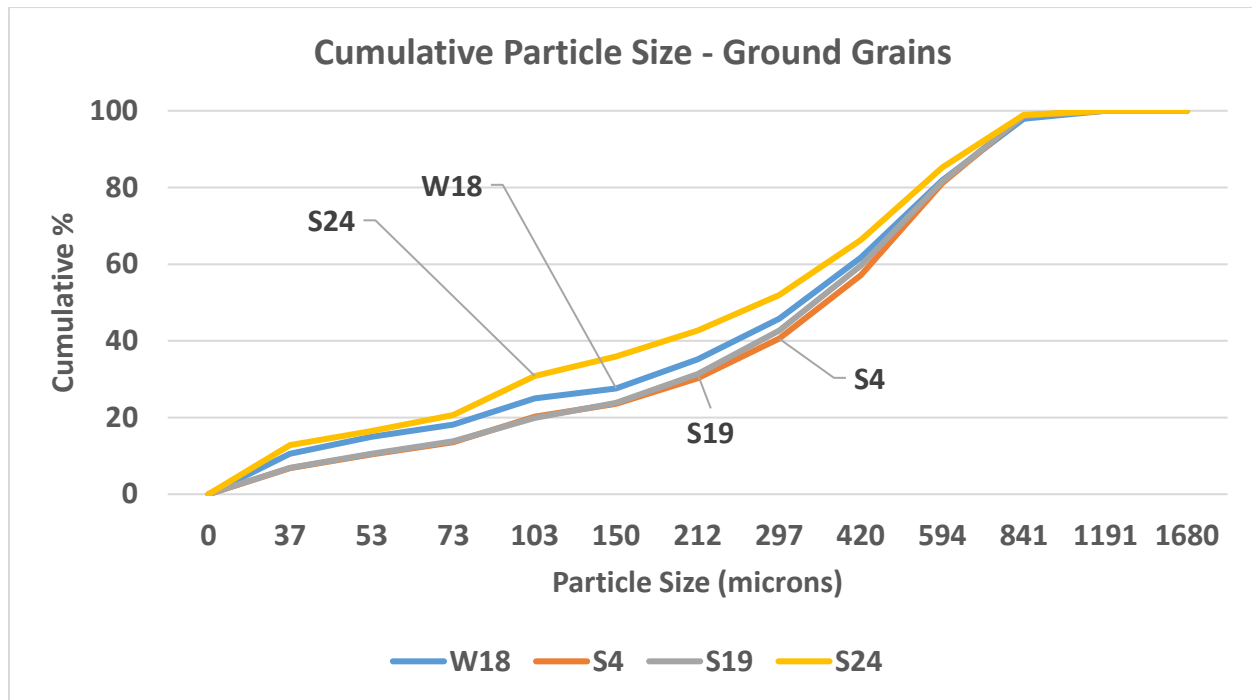


Figure 5.5: Cumulative Particle Size for Mixed Diets

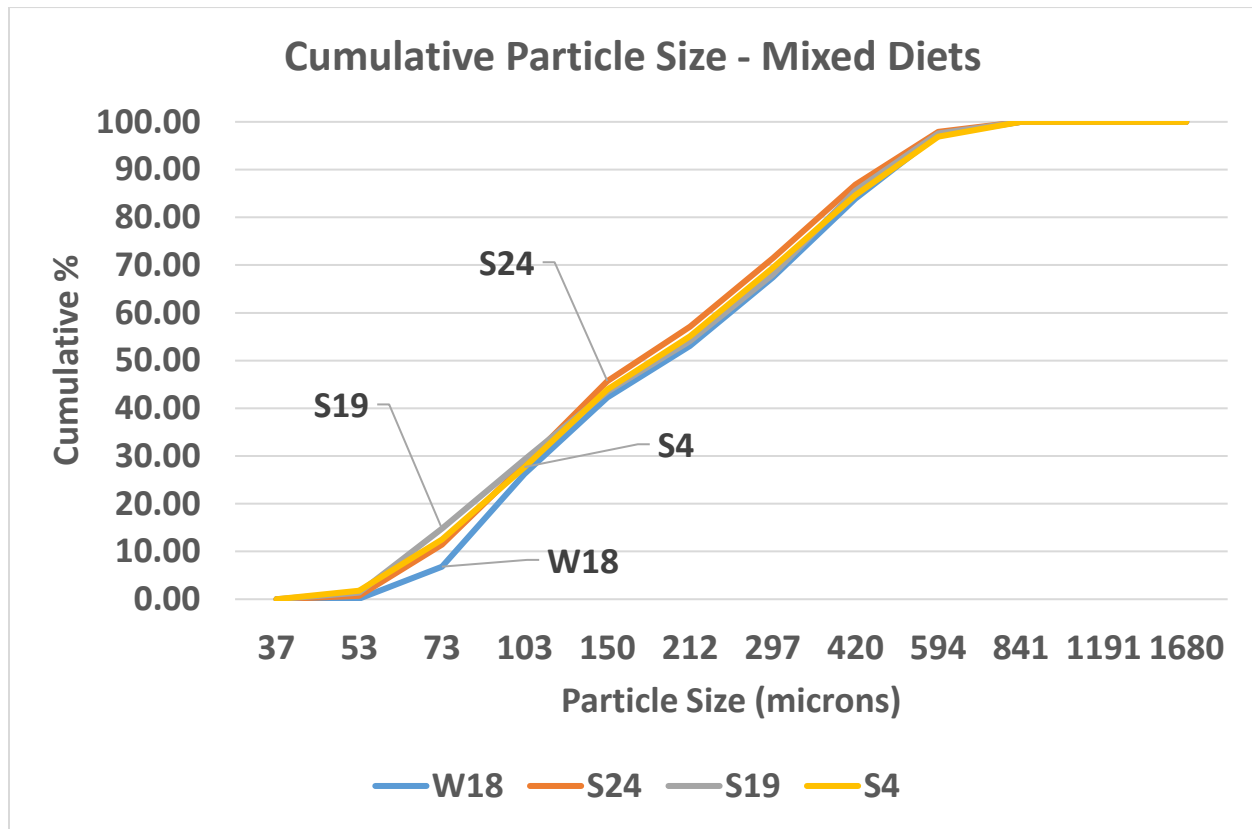


Figure 5.6: Iodine Stain for W18

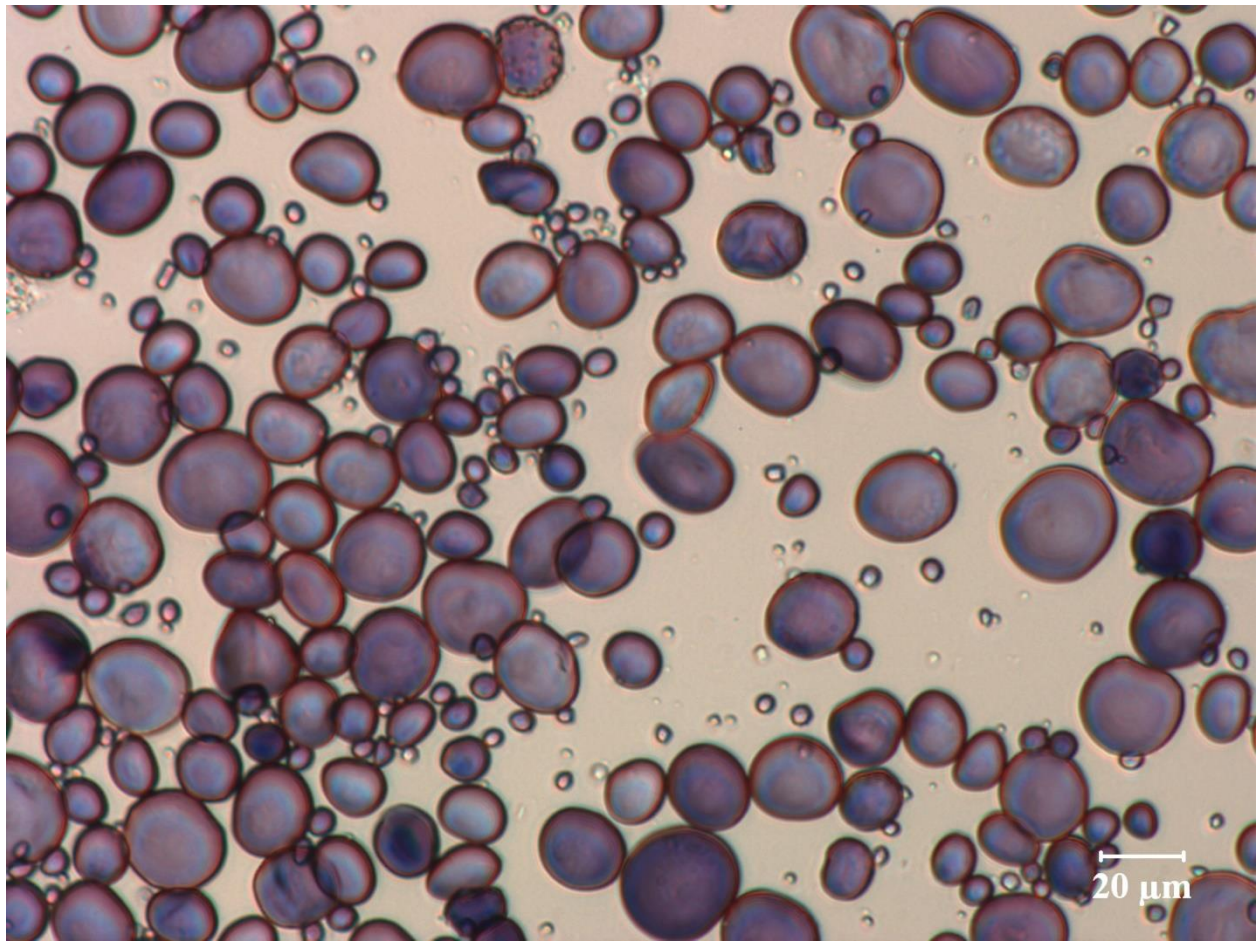


Figure 5.7: Iodine Stain for S24

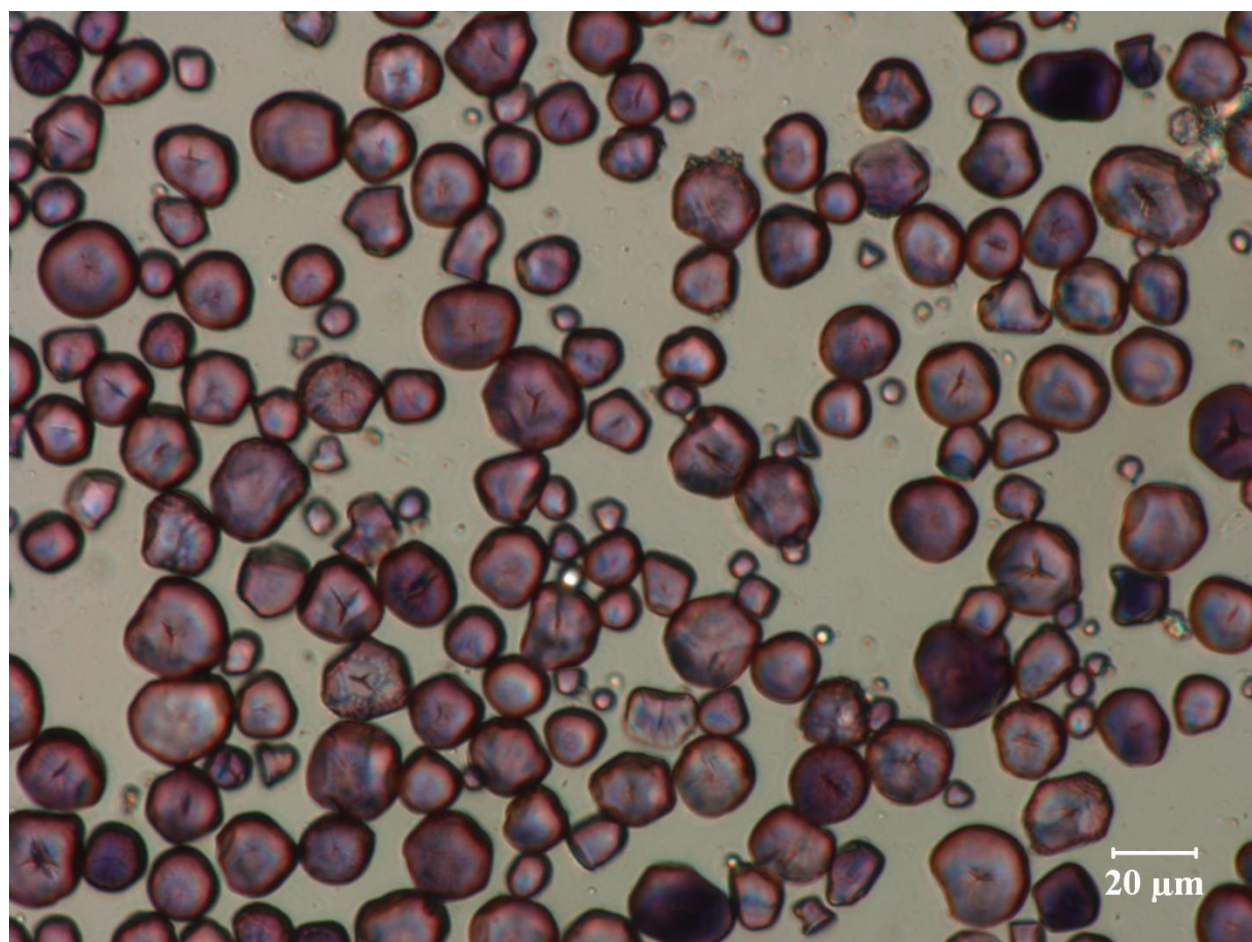


Figure 5.8: Iodine Stain for S4

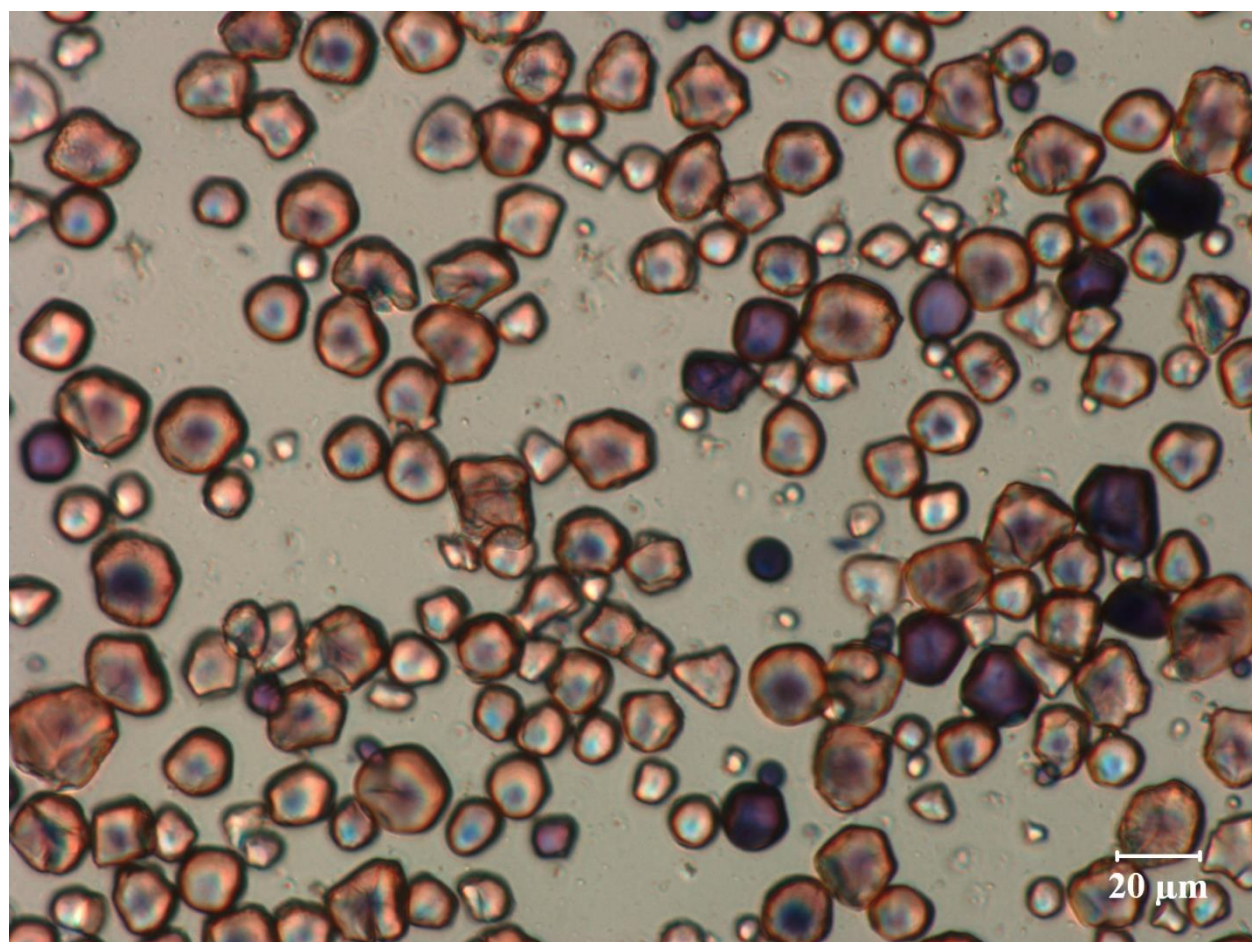


Figure 5.9: Iodine Stain for S19

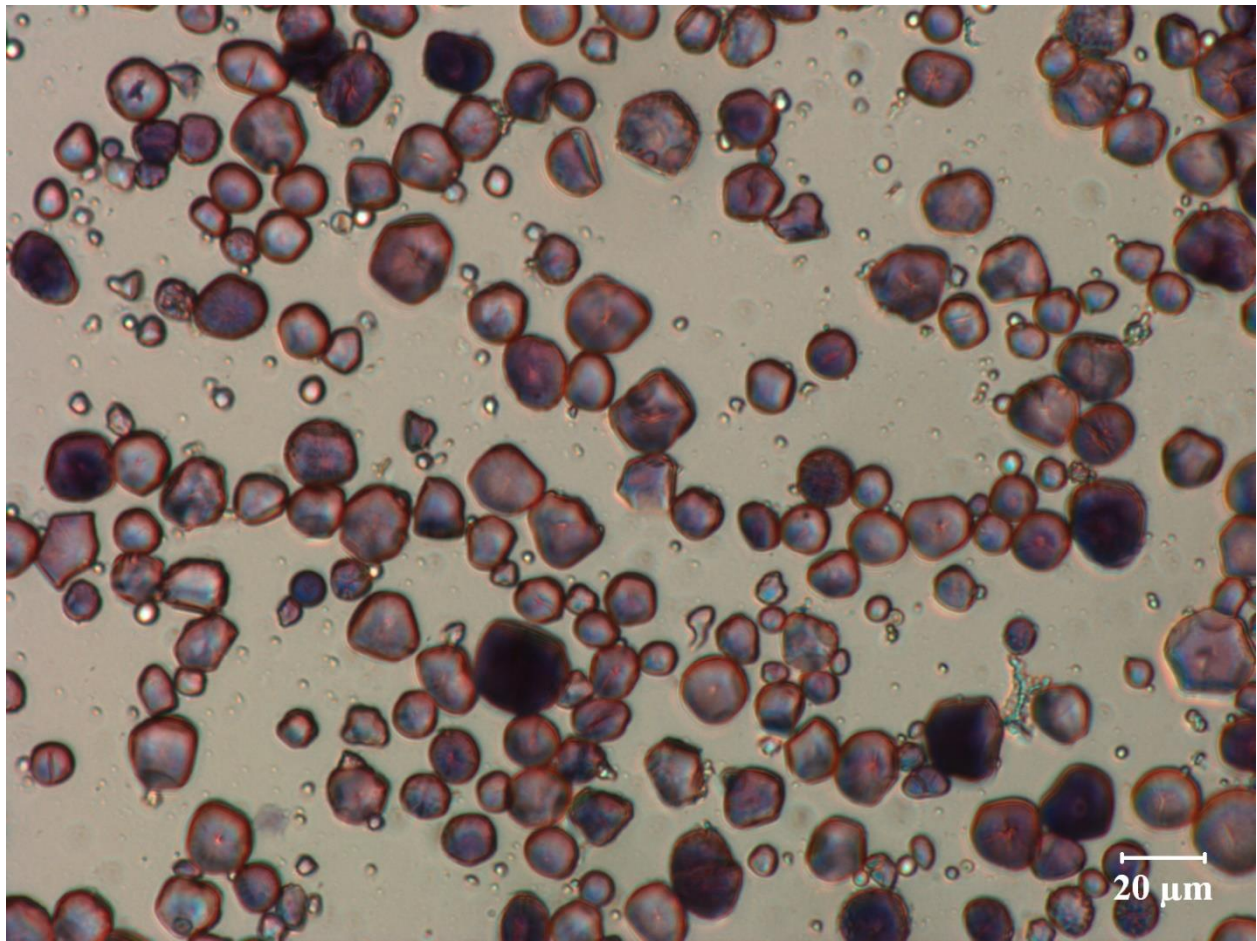


Table 5.1: Dietary Formulation for Rainbow Trout

Ingredient	Inclusion Percentage
Menhaden Fishmeal	13.55
Poultry by-product	13.6
Corn Protein	
Concentrate	5
Blood Meal	5
Feather Meal	5
Soybean Meal	12
Ground Grain	22.52
Monocalcium	
Phosphate	2.3
Lysine HCl	2.18
DL-Methionine	0.59
Threonine	0.63
Soy Lecithin	1.5
Stay-C 35	0.2
Vitamin Premix	1
Choline Chloride 50%	1
Yttrium Oxide	0.1
TM ARS 1440	0.1
Astaxanthin	0.08
Taurine	0.5
Calcium Propionate	0.15
Menhaden Fish Oil	6.8
Poultry Fat	6.2

Table 5.2: Proximate Analysis of Major Ingredients

	Feather Meal	S4	S19	S24	W18	Fishmeal	Soybean meal	Corn Protein Concentrate	Blood meal	Poultry meal
Moisture %	7.21	11.76	11.54	11.34	10.28	8.61	11.09	8.48	11.12	5.70
Dry Matter %	92.79	88.24	88.46	88.66	89.73	91.40	88.91	91.53	88.89	94.31
Crude Protein %	85.35	11.8	11.55	9.94	14.25	63.55	43.05	75.9	92.2	65.3
Crude Fat%	6.21	3.52	2.94	2.85	1.77	9.15	2.56	2.71	0.31	11.6
Crude Fiber%	0.8	2.05	2.6	2.15	2.65	0.4	2.9	0.8	0.25	0.85
Ash%	2.37	1.68	1.55	1.51	1.81	20	7.97	0.95	0.89	15.25

Table 5.3: Single Kernel Characterization of Grains

Diet	Kernel Characterization			
	Hardness Index	Kernel Diameter (mm)	Kernel Weight (mg)	True Density (g/cc)
W18	66.54 ± 1.36 ^c	2.59 ± 0.01 ^c	28.45 ± 0.13 ^b	1.388 ± 0.003 ^a
S4	75.03 ± 5.32 ^b	2.78 ± 0.03 ^a	34.11 ± 0.49 ^a	1.357 ± 0.001 ^c
S19	80.03 ± 1.75 ^a	2.39 ± 0.02 ^d	22.85 ± 0.26 ^c	1.369 ± 0.001 ^b
S24	59.88 ± 4.52 ^d	2.64 ± 0.02 ^b	27.79 ± 0.40 ^b	1.345 ± 0.002 ^d
SEM	0.87	0.01	0.19	0.001
P-Value	<.0001	<.0001	<.0001	<.0001

Table 5.4: Amylose/Amylopectin Analysis of the Grain

Starch Analysis of the Grain		
	Total Starch (d.b.)	Apparent Amylose (d.b.)
W18	58.88 ± 1.07 ^{ab}	18.37 ± 0.71 ^b
S4	61.39 ± 1.92 ^{ab}	4.83 ± 0.56 ^c
S19	56.06 ± 3.99 ^b	19.30 ± 2.32 ^b
S24	64.44 ± 2.12 ^a	24.20 ± 0.45 ^a
P- Value	0.0186	<.0001

Table 5.5: Energy Use during Grinding of Whole Grains

Grain*	Energy use (kJ/kg)	Throughput (kg/hr)
W18	75.31 ± 5.95 ^a	410.91 ± 15.82 ^c
S4	41.45 ± 0.09 ^b	651.1 ± 2.82 ^b
S19	42.86 ± 0.18 ^b	619.17 ± 3.38 ^b
S24	33.91 ± 0.55 ^b	766.2 ± 13.62 ^a
P-Value	<.0001	<.0001

*Grain grinding was done with a 1.65 mm screen

W18 and S19 had 3 replicates

S24 and S4 had 2 replicates

Table 5.6: Energy Use during Grinding of Mixed Diets

Mixed Diet*	Energy use (kJ/kg)	Throughput (kg/hr)
W18	43.33 ± 0.77 ^a	467.17 ± 6.91 ^c
S4	34.76 ± 1.07 ^c	567.26 ± 1.75 ^a
S19	36.95 ± 1.63 ^b	514.78 ± 11.48 ^b
S24	32.97 ± 0.37 ^c	545.44 ± 11.59 ^a
P-Value	<.0001	<.0001

*Post-batch grinding was done with a 1.02 mm screen

Table 5.7: Particle Size Analysis

Ro-Tap Particle Size Analysis for Ground Grains		
Diet	dgw	sgw
W18	331.5 ^b	2.82 ^a
S4	367.5 ^a	2.59 ^b
S19	375.5 ^a	2.60 ^b
S24	285.0 ^c	2.88 ^a
SEM	2.17	0.01
P-Value	<0.0001	0.0002

Table 5.8: Particle Size Analysis of Mixed Diets

Ro-Tap Particle Size Analysis for Mixed Diets		
Diet	dgw	sgw
W18	190.5	2
S4	180	2.085
S19	181.5	2.07
S24	177	2.01
SEM	3.1	0.027
P-Value	0.1285	0.1931

Table 5.9: Processing Conditions for rainbow trout feed

Process Setting	Unit	W18	S4	S19	S24
<i>Raw Materials</i>					
Feeder Screw	RPM	17.6	17.6	17.6	17.6
Feed Rate	kg/hr	152.73	151.32	148.3	160.73
<i>Preconditioning</i>					
Steam Flow	kg/hr	13.54	13.45	13.49	13.48
Water Flow	kg/hr	8.13	8.46	8.1	7.82
Discharge Temp	°C	94.17	93.96	93.34	94
<i>Extrusion</i>					
Shaft Speed	RPM	400	400	400	400
Motor Load	%	50.79	47.27	49.1	50.18
Motor Power	kW	9.97	8.52	9.3	9.68
Steam Flow	kg/hr	0	0	0	0
Water Flow	kg/hr	17.92	18.28	18.73	19.29
In-barrel Moisture	MC%	28.66	28.84	28.84	28.26
Knife Speed	RPM	1215	1215	1215	1215
<i>Barrel Temps</i>					
Zone 1 (Set/Actual)	°C	40/52	40/54	40/55	40/55
Zone 2 (Set/Actual)	°C	70/73	70/72	70/73	70/73
Zone 3 (Set/Actual)	°C	90/131	90/130	90/130	90/131
Die Temp	°C	125.9	118.6	122.8	124.8
Cone Head Pressure	psi	300	300	300	300

Table 5.10: Extrusion Processing Metrics

Diet	Steam Loss (%)	STE (kJ/kg)	SME (kJ/kg)	Die Temp (°C)
W18	6.50%	212.05	201.72	126
S4	1.70%	228.48	160.69	119
S19	8.40%	191.66	178.2	123
S24	10.00%	184.99	189.99	125

Table 5.11: Expansion Characteristics of Extrudates

Diet	Wet Bulk Density (g/L)	Dry Bulk Density (g/L)	SEI	Specific Length (mm/g)	Piece Density (g/cm ³)
W18	372 ± 8.49 ^b	412.75 ± 7.42 ^c	1.68 ± 0.14 ^a	258.73 ± 19.07	0.4723 ± 0.0372 ^c
S4	431.5 ± 14.85 ^a	461.25 ± 3.18 ^a	1.30 ± 0.19 ^c	254.41 ± 26.40	0.6308 ± 0.0678 ^a
S19	422.5 ± 3.54 ^a	444.75 ± 0.35 ^{ab}	1.33 ± 0.15 ^c	253.68 ± 24.65	0.6104 ± 0.0464 ^a
S24	382 ± 5.66 ^b	426.75 ± 6.01 ^{bc}	1.54 ± 0.15 ^b	257.4 ± 29.67	0.5207 ± 0.0379 ^b
SEM	6.49	3.56	0.025	3.99	0.008
P- Value	0.0068	0.0025	<0.0001	0.7805	<0.0001

Table 5.12: Floatability of Extruded Pellets

Minutes:	% Floating					
	5	10	15	20	25	30
W18	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a	100 ± 0.0 ^a
S4	59.5 ± 6.4 ^b	56.5 ± 0.7 ^b	57.5 ± 0.7 ^b	57.5 ± 2.1 ^b	53 ± 4.2 ^b	53.5 ± 3.5 ^b
S19	74.5 ± 3.5 ^{ab}	78.5 ± 2.1 ^{ab}	82 ± 2.8 ^{ab}	85.5 ± 4.9 ^{ab}	87.5 ± 3.5 ^a	88.5 ± 3.5 ^a
S24	99 ± 1.4 ^a	99 ± 1.4 ^a	99 ± 1.4 ^a	99.50.7 ^a	99.5 ± 0.7 ^a	99.5 ± 0.7 ^a
SEM	6.93	7.19	7.12	6.72	7.1	7.22
P-Value	0.0032	0.0031	0.0037	0.0024	0.0016	0.002

Table 5.13: Pellet Durability Index

Diet ¹	PDI, %
W18	86.03 ± 0.16 ^d
S4	93.41 ± 0.35 ^a
S19	89.74 ± 0.22 ^b
S24	87.45 ± 0.11 ^c
PSE	0.11
P-Value	<.0001

Holmen 100, 60 sec

¹Samples were sifted before and after as specified by Feed Manufacturing Technology

V

Table 5.14: Water Stability of Extruded Pellets

Minutes:	15	30	60
W18	91.85 ± 0.20 ^a	87.82 ± 0.18 ^a	84.64 ± 0.27 ^a
S4	90.84 ± 0.43 ^b	86.60 ± 0.74 ^b	81.63 ± 0.90 ^b
S19	91.17 ± 0.06 ^b	87.81 ± 0.08 ^a	84.76 ± 0.18 ^a
S24	91.22 ± 0.30 ^b	87.30 ± 0.57 ^{ab}	84.32 ± 0.12 ^a
SEM	0.14	0.24	0.24
P-Value	0.0023	0.0108	<.0001

Table 5.15: Proximate Analysis of Extruded Diets

Item	W18	S4	S19	S24
Gross energy, kcal/g	5.1121	5.125	5.087	5.148
Dry Matter, %	94.75	94.21	94.36	94.89
Crude Protein, %	47.3	46.6	45.2	45.8
Crude Fat, %	11.2	9.87	10.8	10.6
Ash, %	9.6	9.5	9.63	9.47
Crude Fiber, %	1.05	1.58	1.52	1.27

Table 5.16: Protein and Energy Retention for Rainbow Trout fed four experimental diets

Item	W18	S4	S19	S24	SEM	P-value
Feed Intake, %bodyweight/day	139.7 ± 4.3 ^b	155.8 ± 5.0 ^a	151.8 ± 5.0 ^a	141.8 ± 4.7 ^b	1.88	<.0001
Mass Gained, g/fish	372.4 ± 21.6	383 ±27.6	369.2 ±18.1	367.9 ±16.8	9.58	0.6779
Feed Conversion Ratio	0.86 ± 0.03 ^b	0.96 ± 0.04 ^a	0.94 ± 0.04 ^a	0.88 ± 0.02 ^b	0.015	0.0009
CP intake, g/fish	153.5 ±12.7	154.8 ±9.0	159 ±13.3	159.1 ±10.3	5.13	0.8178
CP gain, g/fish	64.6 ±6.2	64 ±2.00	68.6 ±2.6	68.3 ±5.9	2.04	0.2746
Protein Retention, %	42.1 ±3.7	41.4 ±2.3	43.3 ±2.7	42.9 ±2.3	1.26	0.7241
GE Intake, kcal/fish	1698 ±141.3	1705.9 ±100.6	1779.1 ±131.5	1752.3 ±98.7	53.44	0.6475
GE gain, kcal/fish	775.9 ±136.1	659.1 ±84.5	756.3 ±136.1	836.8 ±83.2	50.55	0.1368
Energy Retention, %	45.8 ±7.7	38.8 ±5.8	42.8 ±9.0	47.7 ±2.3	3	0.2119

Table 5.17: Whole body analysis of rainbow trout fed experimental diets

Item	W18	S4	S19	S24	SEM	P-value
Moisture %	67.4 ± 0.6	68.1 ± 1.2	67.4 ± 1.5	67.1 ± 1.7	0.5853	0.7
Crude Protein %	17.9 ± 0.5	17.7 ± 0.9	17.9 ± 1.3	17.6 ± 0.8	0.4086	0.95
Crude Fat %	12.7 ± 0.2	12.88 ± 0.7	12.9 ± 0.7	13.0 ± 0.7	0.2692	0.836
Ash %	1.8 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	1.9 ± 0.2	0.0662	0.289
Energy kcal/g	2.0 ± 0.3	2.1 ± 0.3	2.0 ± 0.4	2.1 ± 0.2	0.1485	0.912

Table 5.18: Body condition indices of rainbow trout

Diet	Visceral Index	Filet Ratio	Hepatosomatic Index
W18	9.9 ± 0.6^{ab}	59.34 ± 1.73^a	1.22 ± 0.04^a
S4	11.2 ± 0.8^a	57.38 ± 1.11^a	1.30 ± 0.13^a
S19	9.6 ± 0.7^b	58.88 ± 0.85^a	1.20 ± 0.11^a
S24	10.7 ± 0.6^{ab}	57.37 ± 0.82^a	1.33 ± 0.21^a
SEM	0.315	1.403	0.018
P-Value	0.0102	0.0336	0.3871

Table 5.19: Apparent Digestibility of Nutrients Coefficients for rainbow trout

Item	W18	S24	S19	S4	SEM	<i>P</i> - value
ADC of DM, %	66.61 ± 1.61	66.95 ± 1.49	67.63 ± 0.94	68.96 ± 1.04	0.5818	0.052
ADC of Fat, %	94.96 ± 2.40	94.01 ± 1.52	93.38 ± 1.44	92.20 ± 1.71	0.809	0.597
ADC of Protein, %	80.16 ± 2.25	79.33 ± 1.64	79.97 ± 0.71	79.20 ± 0.74	0.6627	0.684
ADC of GE, %	77.54 ± 1.30	78.37 ± 1.16	78.09 ± 0.71	77.82 ± 0.95	0.5118	0.69
ADC of Phosphorus, %	42.43 ± 2.54 ^b	46.27 ± 4.34 ^b	46.17 ± 1.61 ^b	60.57 ± 3.08 ^a	1.3673	<.0001

Chapter 6 - Energy balance and efficiency during the drying and cooling of extruded sorghum and wheat-based feeds under variable processing conditions

Abstract

A comprehensive energy balance was conducted to evaluate how extrusion and drying parameters influence energy use and pellet quality in aquatic feeds formulated with sorghum- and wheat-based diets. Sorghum-based diets were extruded at three feed rates (10, 12, and 14 RPM) to examine the effects of bed depth, while wheat-based diets were extruded at a constant feed rate and dried at three dryer temperatures (200, 220, and 240°F) to investigate thermal efficiency. For sorghum diets, increasing RPM raised the rate of moisture removal but began to compromise efficiency and product uniformity at higher feed rates due to increased bed depth. Dryer efficiency improved slightly with higher RPMs but remained low overall due to heat loss through exhaust air. In wheat-based treatments, increased drying temperature improved moisture removal but decreased dryer efficiency, largely due to elevated gas usage and energy loss through the exhaust. Pellet quality indicators such as bulk density and durability were impacted by both drying conditions and product bed depth, with signs that excessive heat or overexposure during drying reduced product durability and altered bulk density due to the competing effects of moisture loss and pellet shrinkage. The results highlight critical trade-offs between throughput, drying efficiency, and pellet integrity in the thermal processing of aquatic feeds.

6.1 Introduction

As the global demand for high-quality protein rises, fish consumption has increased by 3% annually from 1961 to 2019; this is nearly double the population growth rate (FAO, 2022). Aquatic species now provide approximately 17% of the world's animal protein, with around 3 billion people getting at least 20% of their daily protein from fish (FAO, 2022). Aquaculture supplies about 50% of the global supply of seafood (88 million tons as of 2020), with that number continuing to grow each year due to concerns over ocean stock depletion (FAO, 2020). From 2001 to 2018 specifically, aquaculture production expanded at an average annual rate of 5.3% (FAO, 2020).

Approximately 70% of aquaculture farms use commercially produced feeds; from 2021 to 2022, the aquafeed industry grew by 2.72%, outperforming most other animal feed sectors (Global Feed Survey Archives, 2023). Fish feed represents a large percentage of the total cost of production, typically accounting for 50-70% of the total cost (FAO, 2010). In order to produce a pellet that is highly durable and water stable, extrusion has become the preferred processing method. Extrusion processing blends ingredients with heat, steam, water and mechanical energy to create cooked pellets that are formed by being forced through a die hole (Riaz, 2020). Extrusion is efficient and sustainable, requiring little water and offering enhanced energy use (Stojceska et al., 2009; Lazou, 2022). Several studies have looked at the energy efficiency and use during extrusion processing of various products (Pacheco et al., 2018; Baller et al, 2021; Maichel, 2021; Cheng et al., 2025). Pellets are typically conveyed from the extruder by either a conveyor belt or pneumatically; due to airflow during conveying, there could be a moisture reduction of 2-3% before drying

itself (Haubjerg, 2016). The post-extrusion drying phase is a critical operation in the production of modern aquafeed. The primary objective of this stage is to reduce the moisture content of the wet extrudate, which exits the extruder at approximately 25-30% moisture (Ali et al., 2021). This dehydration is fundamental for inhibiting microbial growth and ensuring the product's longevity and safety during transport and storage. A second, equally vital objective is the solidification of the pellet's internal structure. Drying creates a porous matrix that is essential for subsequent oil absorption (Haubjerg et al., 2012) and for establishing the final technical quality attributes of the feed, such as its mechanical strength and hydrodynamic properties (Haubjerg et al., 2012; Haubjerg, 2016). However, the drying process presents a significant engineering challenge, defined by an inherent trade-off between maximizing energy efficiency and preserving the final technical quality of the product (Haubjerg, 2016). There is a well-documented reluctance within the aquafeed industry to implement aggressive energy-saving strategies, stemming from the considerable risk of compromising critical quality parameters, most notably the mechanical durability of the pellets (Haubjerg, 2016). This highlights that effective drying is not merely a process of water removal but a sophisticated control problem that requires a nuanced understanding of material science, thermodynamics, and process engineering.

The drying stage is not an isolated operation but the final step that "sets" the structural properties initiated during extrusion. The extrusion process itself utilizes high temperature, pressure, and mechanical shear to transform raw ingredients into a cooked, plasticized, and molten dough, where starches are gelatinized and proteins are denatured (Riaz, 2000). As this molten mass exits the extruder die, it expands, forming the pellet's initial porous

structure. At this point, the pellet is in a hot, moist, and malleable "rubbery" state. The subsequent drying process is responsible for removing water and heat, causing the material to transition from this rubbery state to a rigid, solid, "glassy" state (Haubjerg, 2016). The specific conditions under which this transition occurs; namely the temperature, humidity, and velocity of the drying air, dictate the final microstructure, internal stresses, and surface characteristics of the pellet (Haubjerg, 2016). Consequently, a poorly controlled drying process can introduce micro-fractures or create a brittle structure, negating the positive transformations achieved in the extruder and resulting in a final product of inferior quality (Haubjerg, 2016).

Drying is one of the most energy-intensive operations in food and feed processing, creating a strong incentive for optimization (Mouhoubi et al, 2019; Akgun & Kontas, 2024). However, this optimization is constrained by the delicate balance between energy use and final product quality (Haubjerg, 2016). The energy consumed by any given dryer is a direct function of its operating parameters. These parameters include air temperature, air velocity, and drying time (Nwakuba et al., 2016). A study published in 2018 found that it was possible to optimize mechanical durability of the pellets, reducing the number of fines after testing, by optimizing the drying conditions. Pellets that were dried to a lower end moisture content resulted in a higher number of fines after testing durability (3-5%), while pellets that were dried less severely had a lower number of fines after testing (2-3%). They found that drying with humid air and higher temperatures resulted in less fines than drying

with dry air and lower temperatures (Haubjerg & Veje, 2018). Some research is regarding drying after extrusion processing, specifically in pasta. Drying extruded products is a complex process; a glass transition hypothesis has been used to attempt to link drying processing with product quality (Li et al., 2018; Kasapis, 2005). There has been some research on drying and its relationship to product quality in several types of products; rice, noodles, pasta, and apricot (Cnossen et al., 2001; Dai et al., 2015; Mercier 2019). It is believed that higher temperature and high humidity in the initial drying stage will help reduce crust formation on the product and improve product quality (Dai et al., 2015). This research aimed to develop a preliminary energy balance and look at how different drying conditions impacted energy use, energy loss, and drying efficiency. There are some limitations; the drying system used in this study is not able to fully reach saturation due to its design. Because of this, as temperatures rise and fall, humidity cannot be controlled. It has been shown that both humidity of the air and temperature of the air during drying can impact product quality, with hotter, more humid air resulting in a decreased number of fines. With that limitation in mind, it was hypothesized that reducing the feed rate in sorghum-based feeds would result in more moisture loss during drying, improved drying efficiency, and reduced mechanical durability due to excessive heat stress causing fracture points in the pellet. Conversely, drying wheat-based feeds at different temperatures would similarly impact product properties. It was hypothesized that hotter drying temperatures would also reduce mechanical durability due to the inability to control humidity in the drying system used when compared to lower drying temperatures, exposing

the product to higher temperatures with the same humidity level, resulting in increased fines after testing durability.

6.2 Materials and Methods

6.2.1 Diets and Formulations

Two treatment diets were formulated to assess the impact of different carbohydrate sources on energy use during extrusion processing and dryer energy use. Diets were formulated using ground wheat (GW) and normal red sorghum (NRS). Diets were formulated to have a grain inclusion level of 22.52%. Diets were formulated to have approximately 45% crude protein and 18% crude lipids. Diet formulation is shown in Table 6.1.

Ingredients were procured through a local feed mill (Fairview Mills, Seneca, KS, USA). The normal red sorghum was sourced through a local farmer with the assistance of the Sorghum Checkoff program. The Empyreal 75 – Corn Protein Concentrate was procured through Cargill, Inc (Wayzata, MN, USA). The feather meal was sourced through Scoular (Omaha, NE, USA). The taurine was sourced through Optimal Aquafeed (Omaha, NE, USA). Crude protein (AOAC 990.03, nitrogen combustion method), crude fat (AOAC 920.39, ether extract method), ash (AOAC 942.05, combustion method), and total dietary fiber (AACC method 32-07, enzymatic-gravimetric method) were analyzed through proximate analysis conducted by Midwest Laboratories (Omaha, NE, USA).

6.2.2 Experimental Design

Two experimental designs were utilized for this study. For the sorghum-based diets, pellets were extruded at three different feeder screw rpm's in order to change the level of

feed on the dryer belt, or bed depth, to determine the impact of dryer belt bed depth on product moisture removal, pellet qualities and energy use. For the wheat-based diets, the same feed rate was used for all treatments, but three different drying temperatures were used to determine the effect of dryer temperature on pellet qualities, moisture removal, and dryer energy use. The sorghum-based feeds were set at 10, 12 and 14 rpm, which corresponds to a feed rate of 81.98, 108.22, and 121.26 kg/h, respectively. The mash was conditioned to 94-99°C in a Wenger model 2 DDC pre-conditioner, with water and steam injection at 4.8-7.1 kg/h and 13.5 kg/h, respectively. Water was injected into the extruder barrel at rate of 4.0-9.7 kg/h, to achieve an in-barrel moisture of 28-31% wb. Drying temperature and time were 104.4°C (220°F) and 17.5 min, while cooling was done with room temperature air at for 4.5 min for the sorghum based feeds. For the wheat-based feeds, the feeder screw speed was set at 10 rpm for the wheat-based treatments, which corresponded to dry mash feed rate of 79.00 kg/h as obtained from the calibration. Drying time was the same, with three different temperatures being used: 93.3°, 104.4°, and 115.5°C (200, 220 and 240°F).

6.2.3 Grinding and Mixing

The whole grains were ground using a pilot-scale hammer mill (Model D Comminuter, Fitzpatrick, Westwood, MA, USA). Grain grinding was done using a screen with a 1.65 mm hole size. The dry ingredients were mixed in a double-ribbon blender (Wenger Manufacturing, Sabetha, KS, USA). Diets were mixed for 3 minutes for uniformity.

Post mixing, all diets were then ground through the same hammermill with a 1.02 mm screen to provide a uniform particle size within each diet.

6.2.4 Feed Rate Calibration

Prior to extrusion, flow rate of each formulation delivered by the volumetric feeding system of the extruder was calibrated at three feeder screw speeds, 8, 11 and 14 rpm. Measurements were done in duplicate and averaged over both high and low fill levels of the live-bottom feed hopper. Formulations were processed using a 37.3 kW (50 hp) pilot-scale single screw extruder (X-20, Wenger Manufacturing, Sabetha, KS, USA) with a screw diameter of 82.6 mm (3.25 in) and L/D ratio of 10:1. The extruder barrel consisted of 6 heads divided into three heating zones, with the inlet head having a smooth liner and rest of the heads having spiral liners. The screw profile (Figure 6.1) had single-flighted full pitch screws in the feed intake section, transitioned to double-flighted half pitch screws for creating compression, and ended with a cone screw at the discharge for directing flow into the die opening. Four steam locks were interspersed between the screw elements to provide resistance to flow, increase material fill and lead to desired mechanical energy input. Calibrated feed rates are shown in Table 6.2.

6.2.5 Extrusion Processing

During the extrusion experiments raw material mash in the feed hopper was maintained at medium fill level. The extruder barrel temperatures were set at 40, 70 and 90°C from inlet to discharge, and the screw speed at 400 rpm. A die with five-2.50 mm openings was used to shape the product exiting the extruder, which was cut at a speed of 1219 rpm using a rotary knife system with 6 hard blades. A thermocouple probe was

inserted into the material stream immediately downstream of the discharge screw to measure the die temperature. Each formulation was extruded for 30 min. Process data were recorded using a computerized data acquisition system, with the exception of die temperature and extruder motor power consumption data that were recorded using a video camera and transcribed every two minutes. The extruded pellets were conveyed using a negative pressure pneumatic system to a pilot-scale Wenger series 4800 two-pass gas-fired continuous dryer and single-pass cooling system.

6.2.7 Extrudate Drying and Cooling

Product was dried using a Wenger 4800 two-pass gas-fired continuous dryer with one heating zone and single-pass cooling system. Product is conveyed using a negative pressure pneumatic conveying system and discharged through a sloped oscillating spout spreader. Product is carried through the dryer and cooler on a stainless-steel woven screen belt conveyor. Ambient air and dryer exhaust air temperature and humidity were collected using an Amprobe THWD-5 Relative Humidity Temperature Probe (Amprobe, Everett, WA, USA). Ambient air data was collected at the dryer intake below the feed spreader, and exhaust air data was collected by inserting a probe through a hole in the dryer exhaust. Volumetric flow of the exhaust air was collected at the same location. Readings were taken at the beginning and end of each collection period. Product was collected off the extruder, off the spreader, and off the cooler for moisture content analysis. Gas usage (cubic feet) was determined by visual observation of the gas meter at the beginning and end of each collection.

The total mass of material entering a system must equal the total mass exiting the system. Across all treatments, the sole input streams are the raw material, steam, and water; the sole exit streams are raw material, steam, and in the case of drying, moisture loss. Mass balancing was performed as described by Riaz (2000) and Monti, et al. (2016). The mass flow rate of the dryer is calculated through the following equation:

$$md = m_{ex}((1 - xw_{ex})/(1 - xw_d)) \quad (6.1)$$

Where md is the mass of raw material exiting the drying system, m_{ex} is the extruded material flow rate entering the dryer in kg/hr, xw_{ex} is the moisture content of the extruded product, and xw_d is the dried product moisture content. This equation was used to find the moisture loss during every step in the process; from the extruder to off the spreader conveyor and from product entering the dryer off the spreader to product exiting the dryer.

As total energy into a system must equal the total energy out, energy analysis was performed according to the following equation:

$$Q_g(\frac{kJ}{h}) + Q_{dia}(\frac{kJ}{h}) + Q_{cia}(\frac{kJ}{h}) + Q_{wprod}(\frac{kJ}{h}) = Q_{dprod}(\frac{kJ}{h}) + Q_{dea}(\frac{kJ}{h}) + Q_{cea}(\frac{kJ}{h}) + Q_{loss}(\frac{kJ}{h}) \quad (6.2)$$

Where Q_g is the energy supplied by natural gas in the dryer; Q_{dia} is the inlet air of the dryer before being heated; Q_{cia} is the inlet air of the cooler; Q_{wprod} is the extruded product entering the dryer; Q_{dprod} is the dried product exiting the cooler; Q_{dea} is the air exiting the dryer exhaust; Q_{cea} is the cooler air exiting the exhaust; Q_{loss} is the overall energy lost during the process due to air leakage and heat loss through the dryer walls.. After finding the kJ/hr values for each of the above variables, variables were then converted to a kJ/kg basis by dividing by the dry material feed rate.

Q_g was solved by the following equation, using a standard rate of 1055 kJ/cubic foot of natural gas and multiplying that by the amount of gas used in one hour.

$$Q_g \left(\frac{kJ}{h} \right) = Cubic \frac{ft}{hr} \times \frac{1055 \text{ kJ}}{1 \text{ cubic ft}} \quad (6.3)$$

Q_{wprod} and Q_{dprod} was solved using the following equation:

$$Q_{wprod} \left(\frac{kJ}{h} \right) = msf * Cp * ^\circ C \quad (6.4)$$

Where msf is the flow rate (kg/hr) of product entering/exiting the dryer; Cp is the specific heat of the product (found by multiplying 4.18 times the moisture content and adding 1.5 times the dry solids of the product); and $^\circ C$ is the temperature of product entering/exiting the dryer (assumed to be 40°C/26.7°C for all treatments).

Q_{dia} , Q_{cia} , Q_{dea} and Q_{cea} were found through the following equation:

$$Q_{dia} \left(\frac{kJ}{h} \right) = mf * \Delta H \quad (6.5)$$

Where mf is the mass feed rate of air into the system (kg/hr) and ΔH is the enthalpy of air in kJ/kg. The enthalpy of air was determined by using the measured temperature and humidity of the ambient air and the utilization of a psychometric calculator (Psychometric Calculator).

The mass feed rate of air (mf) was determined by taking the measured volumetric air flow in CFM and using the following equations:

$$Volume \text{ flow } \left(\frac{m^3}{hr} \right) = CFM * .0283 \left(\frac{m^3}{min} \right) \quad (6.6)$$

$$Mass \text{ flow } \left(\frac{kg}{hr} \right) = Volume \text{ Flow } * Density \left(\frac{kg}{m^3} \right) \quad (6.7)$$

The ambient air density was found by using the following equation:

$$Density \left(\frac{kg}{m^3} \right) = \frac{Pd * 1000}{287.05 * Tk} + \frac{Pv * 1000}{461.50 * Tk} \quad (6.8)$$

Where Pd is the partial pressure of dry air in kPa; Pv is the actual vapor pressure (kPa) and Tk is the temperature of the ambient air in Kelvin.

Pv is found through the following equation:

$$Pv = RH * \left[0.61078 * \exp \left(\frac{17.27 * Tc}{Tc + 237.3} \right) \right] \quad (6.9)$$

Where RH is the relative humidity of the ambient air which is multiplied by the Tetens equation for saturation vapor pressure in brackets (Tc is the temperature in Celsius). Pd is then found by the following equation:

$$Pd = Patm - Pv \quad (6.10)$$

Where Patm is the atmospheric pressure (assumed to be 101.325 kPa).

After solving for each variable, Qloss can be determined through the following equation:

$$Q_{loss} = (Qg + Qdia + Qcia + Qwprod) - (Qdprod + Qdea + Qcea) \quad (6.11)$$

While the dryer efficiency can be determined through the following equation:

$$Efficiency(\%) = \frac{Qdprod + Qdea + Qcea}{Qg + Qdia + Qcia + Qwprod} * 100 \quad (6.12)$$

Specific Energy Consumption (SEC) was calculated through the following equation:

$$SEC \left(\frac{MJ}{kgH2O} \right) = \left(\frac{Qg \left(\frac{kJ}{kg} \right)}{Xr_{kg} \left(\frac{kg}{kg} \right)} \right) \div 1000 \quad (6.13)$$

Where Qg is the energy used by natural gas per kg of material, and Xr_{kg} is kilograms of moisture removed per kilogram of material.

Efficiency was also calculated using the latent heat of evaporation method through the following equation:

$$Efficiency (Latent Heat)(\%) = \left(\frac{Xr_{kg} \left(\frac{kg}{kg} \right) * 2600 \left(\frac{kJ}{kg} \right)}{Qg \left(\frac{kJ}{kg} \right)} \right) \quad (6.14)$$

Finally, efficiency was calculated again through an energy balance using the following equations:

$$M_1 Cp_1 T_1 - M_2 Cp_2 T_2 + \Delta E_g = M_{ac2} h_{ac2} + M_{ad2} h_{ad2} - M_{ac1} h_{ac1} - M_{ad1} h_{ad1} \quad (6.15)$$

Where M_1 is the mass of material entering the dryer, Cp_1 is the specific heat, and T_1 is the temperature of the product entering the dryer. $M_2 Cp_2 T_2$ is the mass, specific heat, and temperature of product exiting the dryer. ΔE_g is the energy contribution from gas. $M_{ad1} h_{ad1}$ is the mass and energy from air entering the dryer, with $M_{ac1} h_{ac1}$ being the mass and energy of air entering the cooler. Variables with a subscript of 2 are product/air/energy exiting the dryer. Using enthalpy values from a psychometric chart, the feed rate where the equation balanced itself to have zero losses or inefficiencies was found. The actual feed rate was then divided by the calculated feed rate to determine the overall efficiency of the drying system.

6.2.9 Product Quality Measurements

Extruded product bulk density (g/L) was measured by recording the mass of pellets filling a 1-liter cup to the brim. Two replicate measurements were made for both wet product directly off the extruder discharge and dry product after the dryer.

Pellet durability index (PDI) is the standard for measuring the number of broken pellets, dust, and fines that are generated through repeated handling. PDI was tested using a Holmen NPH100 pneumatic tester (Tekpro, Norfolk, VA, USA). Pellets were first sieved using a #10 screen to remove broken pellets and fines. One hundred grams of sieved pellets were tested. Samples were placed in the chamber and then air-agitated for 60 seconds using 70 mbar of pressure. Upon test completion, the fines were automatically

sieved from the tested sample. PDI is defined as the percentage of pellets remaining after the conclusion of the test. Three replicates were measured for each treatment.

6.2.10 Statistical Analysis

Statistical analyses were performed on SAS software (SAS Institute, Inc, Cary, NC, USA). One-way ANOVA ($P < 0.05$) using the general linear model was employed to determine statistical difference between treatment means. Tukey's post-hoc test ($P < 0.05$) was performed for pairwise comparison between means after a significant ANOVA result.

6.3 Results and Discussion

6.3.1 Impact of Feed Rate

Incoming energy for the sorghum-based diets are shown in Table 6.3, and outgoing energy is shown in Table 6.4. Qwprod values increased from 90.86 to 96.48 kJ/kg as RPM increased from 10 to 14. Correspondingly, total moisture loss rose from 16.07 kg/hr at 10 RPM to 25.15 kg/hr at 14 RPM. When looked at on the basis of kg of water/kg of material (Table 6.6), these numbers are less differentiated; 10 RPM resulted in 0.20 kg water/kg raw material being removed, while 14 RPM resulted in 0.21 kg/kg being removed. Final moisture content remained low across all RPMs; both 10 and 12 RPM treatments resulted in approximately 4.0% final moisture, while the 14 RPM condition showed a slightly higher final moisture of 4.98%. This 1% increase may suggest that deeper bed depth at higher feed rates began to limit moisture removal. However, it's also noteworthy that the 14 RPM treatment started with a higher off-extruder moisture content (23.24%) compared to the others (21.66–22.26%), which likely contributed to the higher final moisture.

Gas usage per kg of feed (Q_{gas}) decreased notably from 27,735 kJ/kg at 10 RPM to 20,964 kJ/kg at 14 RPM, owing to the increased mass of feed moving through the dryer system. Despite these changes, dryer efficiency remained relatively stable: 29.52% at 10 RPM, 29.48% at 12 RPM, and 29.05% at 14 RPM (Table 6.5). As observed in all sorghum-based treatments, a substantial portion of incoming energy was lost, primarily through exhaust air and system inefficiencies, with Q_{loss} (kJ/kg) being approximately 70% of the energy flow out of the system. The experimental dryer was not designed to replicate commercial-scale operations, and its airflow pattern (in which air is immediately exhausted after heating) limits energy transfer to the product, resulting in expected low efficiency, as opposed to a drying system that removes air from the system once it has reached its saturation point

6.3.2 Impact of Drying Temperature

In contrast, wheat-based feeds were processed at a single feed rate but subjected to drying temperatures of 93, 104, and 115°C. Incoming energy, outgoing energy, and efficiency are shown in Tables 6.7, 6.8 and 6.9. Energy input via gas combustion (Q_{gas}) remained constant between 93 and 104°C treatments (both 24,623.57 kJ/kg). At 115°C, however, gas consumption increased significantly to 32,644.10 kJ/kg, representing a ~30% increase. Both the dryer and cooler exit air had increased energy values (kJ/kg) as dryer temperature increased.

Final product moisture content (Table 6.10) followed an expected trend with decreasing values as drying temperature increased. Off-cooler moisture decreased from

4.99% at 93°C to 3.49% at 115°C, which aligns with the total moisture loss data: 0.201 kg/kg at 93°C, 0.205 kg/kg at 104°C, and 0.217 kg/kg at 115°C. Dryer efficiency peaked at 33.69% for the 104°C treatment, slightly higher than 32.35% at 93°C, before declining to 27.99% at 115°C. This decline in efficiency is primarily attributed to the increase in gas input, without a proportional increase in energy transferred to the product. Enhancing efficiency at higher temperatures might be possible by increasing the product mass flow by raising the feed rate off the extruder provided that final moisture targets are still achieved.

As drying temperature increased, more energy escaped through exhaust air (Q_{dea}), rising from 4,895.72 kJ/kg at 93°C to 5,554.52 kJ/kg at 115°C, reinforcing the observation that higher drying temperatures led to more energy being carried away rather than absorbed by the product.

6.3.3 Impact on Pellet Qualities

When comparing the sorghum-based feed processed at 10 RPM and the wheat-based feed dried at 104°C, several key differences emerge that may be attributed to the intrinsic properties of the grains. Both treatments had similar feed rates, sorghum at 81.98 kg/hr and wheat at 79.00 kg/hr, and identical drying temperatures (approximately 104°C), providing a reasonable basis for comparison. Despite these similarities, the energy input and moisture removal characteristics differed notably.

The sorghum feed at 10 RPM required significantly more energy per unit mass, with a total incoming energy of 34,516.93 kJ/kg, compared to 31,808.32 kJ/kg for the wheat

feed. Additionally, sorghum's gas usage per kg of feed was much higher (27,735.25 kJ/kg) than wheat's (24,623.57 kJ/kg), indicating that sorghum may be more energy-intensive to process under similar conditions. This could be due to differences in grain structure, or how water is bound within the matrix, making moisture removal more challenging for sorghum.

Moisture loss also followed this trend: the wheat-based feed lost slightly more water overall (16.21 kg/hr) compared to the sorghum feed (16.07 kg/hr), and achieved a lower final moisture content of 3.91% versus 4.06% for sorghum. This suggests that wheat may allow for more efficient water removal despite similar processing conditions. This is further supported by the dryer efficiency values: wheat at 104°C achieved a higher dryer efficiency of 33.69%, while the sorghum treatment lagged behind at 29.52%. Collectively, these differences point toward wheat being more thermally responsive and drying more efficiently, potentially due to its more porous structure, which may facilitate faster moisture diffusion and evaporation during drying.

Pellet quality was impacted by drying parameters, and how it was impacted was determined by the change in drying settings. In sorghum-based feeds, a lower feed rate (10 vs 14 RPM) turns into a thinner bed depth of product on the dryer belt. This exposes the extruded product to more heat and air during drying processing. This was reflected in the amount of moisture that was lost in each product when compared to the moisture content of the product entering the dryer. For sorghum-based feeds, at 10, 12 and 14 RPM, moisture content entering the dryer was 21.18, 21.22 and 22.07%, respectively. Product

exiting the cooler had a moisture content of 4.06, 3.94 and 4.98%, in the same order. This represents a reduction in moisture content by 80.83, 81.4, and 77.44%, respectively. There was minimal difference between the amount of moisture reduced at 10 and 12 RPM, but the amount of moisture removed as a percentage of the incoming moisture content decreased at 14 RPM due to the additional amount of product on the belt. It is likely that some pellets received inadequate drying due to being buried under other pellets, preventing hot air from adequately reaching it. For wheat-based feeds dried at 93, 104 and 115°C, starting moisture contents were 21.65, 21.21 and 21.77%, while ending moisture contents were 4.99, 3.91 and 3.49%; a reduction in moisture content by 76.95, 81.57, and 83.97%, respectively. As the bed depth remained unchanged through all wheat-based treatments, the only driving factor for the additional proportion of moisture removed was the additional heat input into the system through higher temperatures. Bulk density was also measured off the extruder (OE) and off the dryer (OD); these values are shown in Table 6.11. For sorghum-based feeds, OE bulk densities ranged from 434-453 g/L, while OD bulk densities ranged from 432-462 g/L. OD bulk densities were significantly different from each other, with the highest feed rate (and bed depth) treatment having the lowest bulk density at 432 g/L, and the thinnest bed depth having the highest bulk density at approximately 462 g/L. For all three treatments (10, 12 and 14 RPM), bulk density changed by 6.43, -0.14, and -1.05%, with negative number representing a lower bulk density off the dryer when compared to the extruded bulk density. Bulk density of the product changes during drying due to two opposite phenomena; the first is moisture loss. As the product is exposed to hot air during drying, moisture is removed from the product and transferred to the air, resulting

in a lower bulk density. The other phenomena is product shrinkage; depending on which of these is predominant, the bulk density will either increase due to product shrinkage or decrease due to moisture loss. For sorghum-based feeds, it is clear that in the 12 and 14 RPM treatments, moisture loss was the dominant phenomena during drying, as shown by the reduction in bulk density at the dryer when compared to the extruder. For 10 RPM, however, the bulk density suddenly increases by approximately 6.5%, representing a high degree of product shrinkage in comparison to moisture loss. There is a clear trend in the three treatments; at 14 RPM, there was a -1.05% change caused by more moisture loss than product shrinkage; at 12 RPM there was a -0.14% change showing that moisture loss and product shrinkage offset each other; and at 10 RPM there was a 6.43% change showing that product shrinkage had become the primary driver of bulk density changes. For wheat-based feeds, this trend becomes even clearer. As the temperature increased from 93, to 104, and finally 115°C, we see a bulk density change of 1.09, 2.79, and 9.26%. While the additional heat did remove a larger proportion of moisture from the product as discussed above, this additional heat also resulted in a greater amount of pellet shrinkage, resulting in increased bulk densities in the final, dried product. These trends are also shown in Figure 6.2. Pellet durability values (Table 6.12) for sorghum-based feeds ranged from 82.8 to 80%, with the 10RPM treatment being significantly different from 12 and 14 RPM. Wheat-based feed ranged from 83.55 to 84.32%, with no significant differences. Haubjerg & Veje, 2018, reported that drying with hotter, more humid air resulted in decreased fines when compared to lower temperature dry air. As humidity in the dryer was unable to be controlled in this study, these results cannot be directly confirmed but some observations

can be made. There is a trend in wheat-based feeds, although not statistically significant, where hotter air with the same amount of humidity led to reduced pellet durability.

Similarly, a thinner bed depth in sorghum-based feeds led to more exposure to hot air at the same humidity when compared to thicker bed depths, and did lead to a significantly reduced pellet durability index. It is logical to conclude that humidity during drying is indeed a primary driver affecting pellet durability post-drying, but when humidity is equal across all treatments, hotter air tends to reduce pellet durability due to excessive drying and product shrinkage.

6.3.4 Dryer Efficiency Calculations

Efficiency was calculated (Table 6.13) using four different equations. When analyzing the sorghum-based diets, energy efficiency based on heat loss remained relatively stable but showed a slight decline from 29.52% at 10RPM to 29.05% at 14 RPM. In contrast, the other two methods indicated improved efficiency with higher throughput. The latent heat of evaporation method showed that efficiency increased from 1.60% at 10 RPM to 2.24% at 14 RPM. Similarly, the efficiency based on the ratio of real to calculated feed rate where losses would be zero saw the largest jump, rising from 2.50% at 10 RPM to 4.00% at 14 RPM. Specific energy consumption mirrored these trends, with 141 MJ per kg of water removed needed for drying at the lowest feed rate, 102.50 MJ/kg at 12 RPM, and 101.07 MJ/kg needed at 14RPM.

For the wheat-based diets, all efficiency calculations showed that 104°C was the most efficient drying setting. Efficiency calculated via heat loss peaked at 33.69% at 104°C,

compared to 32.30% and 27.99% at 93 and 115°C, respectively. This trend was mirrored by the latent heat method, which also peaked at 104°C (1.88%), before falling to 1.51% at 115°C. Likewise, the real vs calculated feed rate method was optimal at 104°C (2.96%) and lowest at 115°C (2.40%). These trends are mirrored in the specific energy consumption; energy needed in terms of MJ per kg of water removed peaked at 115°C (150.10 MJ/kg), and were lowest at 104°C (120.00 MJ/kg). This indicates that increasing the drying temperature, while holding the feed rate constant, led to diminishing returns where the additional energy input was lost to the drying system rather than being effectively used for moisture removal.

A direct comparison of the sorghum diet at 10 RPM and the wheat diet at 104°C, treatments which utilized very similar feed rates (81.98 kg/hr and 79.00 kg/hr, respectively) and identical drying temperatures (104.4°C) reveals that the wheat diet was processed more efficiently across all metrics. The wheat diet achieved a "Heat Loss" efficiency of 33.69%, whereas the sorghum diet was lower at 29.52%. This advantage for wheat was consistent in the other methods as well: "Latent Heat" efficiency was 1.88% for wheat versus 1.60% for sorghum, and the "Real/Calculated Feed Rate" efficiency was 2.96% for wheat compared to 2.50% for sorghum. As noted in section 6.3.3, this suggests that under near-identical operating conditions, the wheat-based feed was more thermally responsive, allowing for more efficient moisture removal than sorghum-based feeds.

These data are similar with results from existing literature. A 2014 study that compared energy parameters in various dryers reported SEC (MJ/kg water) values that ranged from 100 to 200 MJ/kg water (Motevali et al., 2014), similar to the results from this study. Dryer

efficiency values from the same study ranged from 2-6.5%. As dryer energy temperatures increased from 40° to 60°C, reported SEC values decreased, while efficiency increased, contrasting the results of this study, which experienced higher SEC values and decreased efficiency at 115°C. Another study in 2016 reported the energy requirement for drying 1 kg of product at different temperatures using a convective parallel airflow dryer. It was found that SEC values decreased as temperature increased (50-70°C), with values ranging from 54 to 144 MJ/kg water (Nwakuba et al., 2016). Notably, both previously mentioned studies looked at dryers in the range of 40-70°C, with the temperatures in this study ranging from 90-115°C. As these results appear to contradict each other, these results suggest that dryer efficiency improves with a temperature increase up to a certain threshold where any further heat increase instead becomes waste, driving up SEC values and lowering dryer efficiency.

6.4 Conclusion

This energy balance reveals that optimizing drying and cooling for aquatic feeds involves managing competing priorities, maximizing moisture removal while preserving product quality and minimizing energy loss. In sorghum-based diets, increasing feed rate (and therefore bed depth) improved throughput and dryer efficiency modestly, but final product moisture content and bulk density data suggest diminishing returns due to bed depth interfering with moisture removal. Wheat-based diets showed that while higher drying temperatures improved moisture removal, they also caused a notable drop in efficiency and negatively impacted pellet durability, likely due to increased shrinkage and over-drying. The study confirms that product exposure time, dryer configuration, and drying

air conditions significantly influence both energy balance and pellet quality. The overall trends emphasize the need to balance process efficiency with final product characteristics. Future work should consider more controlled air humidity conditions and more precise instrumentation to fine-tune the drying process for aquatic feed manufacturing.

6.5 References

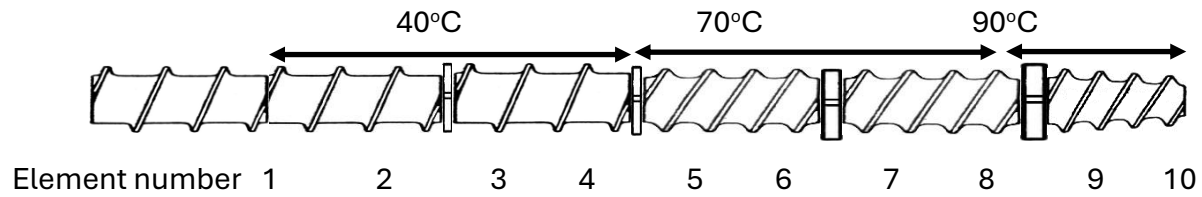
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6.6 Figures and Tables

Figure 6.1: Pilot-scale single screw extruder profile and set barrel temperatures



1= inlet screw; 2 =single flight full pitch screw; 3=small steam lock; 4=single flight full pitch screw; 5=small steam lock; 6=double flight half pitch screw; 7=medium steam lock; 8=double flight half pitch screw; 9=large steam lock; 10=double flight half pitch cone screw.

Figure 6.2: Bulk Density Changes during Drying

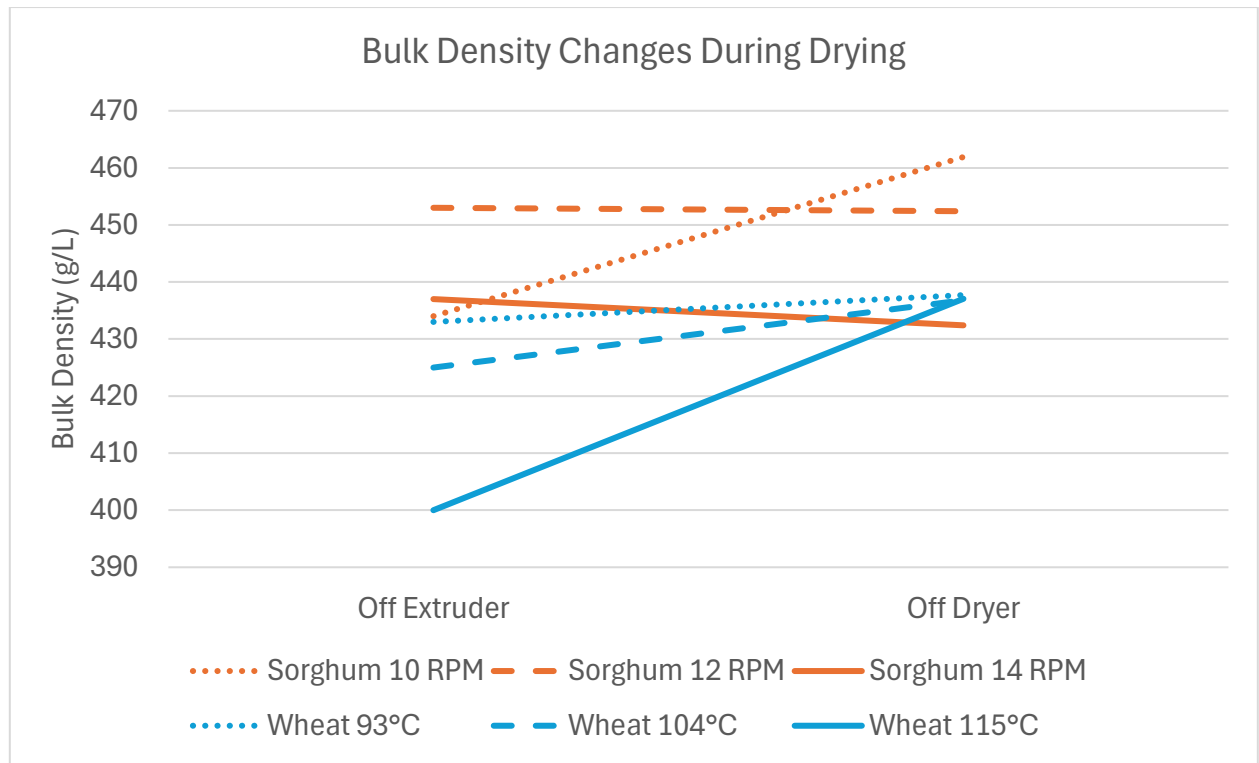


Table 6.1: Dietary formulation for rainbow trout

Ingredient	Inclusion Percentage
Menhaden Fishmeal	13.55
Poultry by-product	13.6
Corn Protein Concentrate	5
Blood Meal	5
Feather Meal	5
Soybean Meal	12
Ground Grain	22.52
Monocalcium Phosphate	2.3
Lysine HCl	2.18
DL-Methionine	0.59
Threonine	0.63
Soy Lecithin	1.5
Stay-C 35	0.2
Vitamin Premix	1
Choline Chloride 50%	1
Yttrium Oxide	0.1
TM ARS 1440	0.1
Astaxanthin	0.08
Taurine	0.5
Calcium Propionate	0.15
Menhaden Fish Oil	6.8
Poultry Fat	6.2

Table 6.2: Feed rate calibration of mixed diets

	RPM	Feed Rate (kg/hr)
Wheat	10	79.00
Sorghum	10	81.98
	12	108.22
	14	121.26

Table 6.3: Incoming Energy for Sorghum-based feeds dried at different feed rates (kJ/kg)

	Feeder Screw RPM	<i>Q_{gas}</i>	<i>Q_{dia}</i>	<i>Q_{cia}</i>	<i>Q_{wprod}</i>
Sorghum	10	28785.25	2509.06	4181.76	90.86
	12	21033.71	1795.52	2992.53	94.40
	14	20964.19	1761.11	2935.18	96.48

Table 6.4: Outgoing Energy for Sorghum-based feeds dried at different feed rates (kJ/kg)

	Feeder Screw RPM	<i>Qdprod</i>	<i>Qdea</i>	<i>Qcea</i>
Sorghum	10	38.77	5026.14	5123.22
	12	40.11	3845.40	3754.42
	14	41.25	3743.92	3696.91

Table 6.5: Energy Balance for Sorghum-based feeds dried at different feed rates (kJ/kg)

	Feeder Screw RPM	<i>Incoming</i>	<i>Outgoing</i>	<i>Qloss</i>	<i>Dryer Efficiency (%)</i>
Sorghum	10	34516.93	10188.13	24328.80	29.52
	12	25916.14	7639.93	18276.22	29.48
	14	23564.56	7482.08	16082.48	29.05

Table 6.6: Moisture content of sorghum-based feeds during processing

	Feeder Screw RPM	<i>Off-Extruder</i> <i>MC%</i>	<i>Off-Spreader</i> <i>MC%</i>	<i>Off-Cooler</i> <i>MC%</i>	<i>Total</i> <i>Moisture</i> <i>Loss (kg/hr)</i>	<i>Total</i> <i>Moisture</i> <i>Loss (kg/kg)</i>
Sorghum	10	22.26	21.18	4.06	16.07	0.203
	12	21.66	21.22	3.94	22.21	0.205
	14	23.24	22.07	4.98	25.15	0.207

Table 6.7: Incoming Energy for Wheat-based feeds dried at different temperatures (kJ/kg)

	°C	<i>Q_{gas}</i>	<i>Q_{dia}</i>	<i>Q_{cia}</i>	<i>Q_{wprod}</i>
Wheat	93	24623.57	2534.02	4223.37	7575.75
	104	24623.57	2658.92	4431.54	7449.48
	115	32644.10	2697.85	4496.41	7559.16

Table 6.8: Outgoing Energy for Wheat-based feeds dried at different temperatures (kJ/kg)

	°C	<i>Qdprod</i>	<i>Qdea</i>	<i>Qcea</i>
Wheat	93	63.32	4895.72	5223.95
	104	40.04	5267.71	5408.93
	115	39.60	5554.52	5585.35

Table 6.9: Energy Balance for Wheat-based feeds dried at different temperatures (kJ/kg)

	°C	<i>Incoming</i>	<i>Outgoing</i>	<i>Qloss</i>	<i>Dryer Efficiency (%)</i>
Wheat	93	31476.86	10182.99	21293.87	32.35
	104	31808.32	10716.69	21091.63	33.69
	115	39934.04	11179.46	28754.58	27.99

Table 6.10: Moisture content of wheat-based feeds during processing

	°C	<i>Off- Extruder MC%</i>	<i>Off- Spreader MC%</i>	<i>Off- Cooler MC%</i>	<i>Total Moisture Loss (kg/hr)</i>	<i>Total Moisture Loss (kg/kg)</i>
Wheat	93	23.94	21.65	4.99	15.88	0.201
	104	22.52	21.21	3.91	16.21	0.205
	115	23.59	21.77	3.49	17.18	0.217

Table 6.11: Bulk density changes in wheat and sorghum-based feeds dried at different parameters

Diet	Drying Setting	Bulk Density (g/L)		% Change
		Off Extruder	Off Dryer	
Sorghum	10 RPM	434 ± 6.36	461.9 ± 1.98a	6.43
	12 RPM	453 ± 4.24	452.4 ± 0.78a	-0.14
	14 RPM	437 ± 2.83	432.4 ± 4.53b	-1.05
PSE		3.33	2.04	
P-value		0.051	0.0044	
Wheat	93°C	433 ± 5.66	437.7 ± 0.28	1.09
	104°C	425 ± 7.07	436.85 ± 4.31	2.79
	115°C	400 ± 14.14	437.05 ± 2.62	9.26
PSE		6.86	2.06	
P-value		0.0843	0.9553	

Table 6.12: Pellet durability index of pellets processed under different drying conditions

Diet ¹	Drying Setting	PDI%
Sorghum	10RPM	82.80 ± .1.18 ^a
	12RPM	87.84 ± 0.18 ^b
	14RPM	88.00 ± 0.66 ^b
PSE		0.4529
P-Value		0.0003
Wheat	93°C	84.32 ± 0.04
	104°C	83.68 ± 0.49
	115°C	83.55 ± 0.38
PSE		0.2083
P-Value		0.0811

Holmen 100, 60 sec

¹Samples were sifted before and after testing
(Schoefield & American Feed Industry
Association, 2005)

Table 6.13: Comparison of different methods for calculating dryer efficiency

Diet	Drying Setting	SEC (MJ/kg water)	Efficiency % (Latent Heat)	Efficiency% (Heat Loss)	Efficiency% (Real/Calculated Feed Rate)
Sorghum	10 RPM	141.48	1.60	29.52	2.50
	12 RPM	102.50	2.20	29.48	3.93
	14 RPM	101.07	2.24	29.05	4.00
Wheat	93°C	122.51	1.84	32.30	2.88
	104°C	120.00	1.88	33.69	2.96
	115°C	150.10	1.51	27.99	2.40

Chapter 7 - A cradle to gate comparative Life Cycle Assessment of wheat and sorghum-based extruded feeds for aquatic species

Abstract

Aquaculture is expanding rapidly to meet the global demand for sustainable protein. However, the environmental impact of feed production remains a critical concern. This study examines the sustainability of extruded feeds for three aquaculture species: rainbow trout, Nile tilapia, and Pacific white shrimp, using wheat and sorghum as primary grain ingredients. A life cycle assessment (LCA) was performed from field to feed gate, assessing impact categories such as global warming potential (GWP), freshwater ecotoxicity (FEW), and smog formation. Processing data, including particle size reduction and thermal energy use during extrusion, were collected at Kansas State University. Results revealed that sorghum-based feeds consistently had lower environmental impacts compared to wheat-based feeds across all species. For trout feed, GWP values were 66.56 and 86.40 kg CO₂ eq for sorghum and wheat, respectively, with corresponding FWE values of 9.85 and 21.79 CTUeco. Tilapia feed exhibited higher FWE values overall due to soybean inclusion, but sorghum still outperformed wheat under all grinding scenarios. For shrimp feed, reducing steam injection improved energy efficiency, with sorghum-based diets achieving the lowest GWP values (99.55-105.88 kg CO₂ eq) compared to wheat (130.28-136.31 kg CO₂ eq). Across all scenarios, key drivers of sorghum's lower impacts included reduced nitrogen fertilizer use, lower diesel consumption, and decreased energy

requirements during extrusion. Sensitivity analysis was performed in three scenarios to determine overall impacts on emission factors.

The extrusion process was also evaluated for its efficiency, with specific thermal energy (STE) calculations showing higher steam loss in sorghum-based feeds compared to wheat. Despite this, sorghum's lower environmental impacts highlight its promise as a sustainable aquafeed ingredient. By combining grain production and feed processing data, this study underscores the potential of grain sorghum to reduce the environmental footprint of aquaculture feed production. These findings provide actionable insights for feed producers and aquaculture stakeholders, encouraging the adoption of sustainable ingredient alternatives. Future research should incorporate real-world growing data, drying processes, and expanded feed formulations to refine these results further.

7.1 Introduction

The increased demand for high quality protein as the global population grows has driven a 3% annual increase in fish consumption every year between 1961 and 2019 (FAO, 2022), a rate that is almost double the annual population growth rate. Aquatic species, both farmed and captured, account for approximately 17% of global animal protein intake, and approximately 3 billion people receive at least 20% of their daily protein intake from fish (FAO, 2022). Globally, aquaculture production produced 88 million tons of seafood, approximately 50% of all seafood consumed (FAO, 2020), and the proportion of seafood that comes from aquaculture continues to increase as concerns and fears over depleted ocean stocks continue to build. Due to this, aquaculture production has sped up significantly in recent years, growing at an average rate of 5.3% from 2001-2018 (FAO, 2020).

Currently, 70% of aquaculture farms rely on commercially produced compound feeds; from 2021 to 2022, the aquafeed industry grew 2.72%. Apart from poultry (1.27%) and layers, (0.31%), all other food animal feed production experienced negative growth (Global Feed Survey Archives, 2023). In 2010, fish feed accounted for 50-70% of the total farm production cost (FAO, 2010). The handling and transport of aquatic feeds is typically pneumatic and places significant stress on the pellets. However, there are high expectations and demands on the quality of the pellets to reduce breakage and fines. Poor mechanical durability impacts not only the economics of the farm, but excess organic material can negatively impact water quality and ecosystem health in aquatic environments. For example, in salmonid aquaculture, a single percentage of lost feed

costs up to USD\$30 million annually, and results in discharges of up to 30,000 tons of organic matter into the water (Haubjerg, 2016).

Due to these quality concerns, the production of aquatic feed, is increasingly done through extrusion. This shift was also driven by the growing importance of floating feed requirements, which allows producers to monitor consumption to minimize water pollution. Extrusion works by combining raw ingredients with heat, water, steam, pressure, and mechanical force. This transforms ingredients into a viscous dough which is then forced through small openings, known as die holes, resulting in the formation of fully cooked pellets. The speed of this cooking process and energy transfer is relatively fast, distinguishing extrusion from more traditional feed production methods like a pellet mill (Riaz, 2020).

Extrusion processing is a sustainable method for producing food and feed. It is an energy-efficient and versatile process that can enhance the nutritional and functional properties of various ingredients. Water consumption is low in comparison to other processing methods as processing can be performed with minimal water addition, as shown in the production of various food products (Stojeceska et al, 2009). It is also very energy efficient, allowing for high efficiency cooking with reduced energy waste (Lazou, 2022). Energy comes in two forms during extrusion: specific mechanical energy (SME) and specific thermal energy (STE) and they are the driving forces behind the extrusion cooking process. Measuring these forms of energy is crucial for understanding energy consumption per kilogram of feed produced (Monti et al., 2016). As material is conveyed to the die, the frictional rubbing action of the screw against the viscous dough creates mechanical energy

and shear (Kantong et al., 2018; Maichel, 2021). STE, on the other hand, takes place primarily in the preconditioner as steam is injected to heat the raw material within its barrel (Pacheco et al., 2018). Research has shown that ingredient composition can impact the degree of steam absorption, and therefore STE, during preconditioning, which has ramifications on extruder flow and product quality (Chen, et al., 2025). There is a need to meet the increasing demand for high-quality aquaculture feed while minimizing environmental impacts.

7.1.1 Goal and Scope

In this study, a life cycle assessment from field to animal feed gate was performed to assess the environmental impacts and footprints of feed production for several species: trout (*Oncorhynchus mykiss*), tilapia (*Oreochromis niloticus*), and shrimp (*Litopenaeus vannamei*). Additionally, several key metrics in the feed processing stage were evaluated to determine environmental impacts, including the amount of energy used during particle size reduction of the raw diet and thermal energy use (through preconditioner steam injection).. Inventories for grain production used in fish diets remained the same across all scenarios, but processing data collected for each processing treatment were incorporated in the inventory. Extrusion processing of the feed was conducted at Kansas State University; energy and water use during processing were collected. Analyzed impact categories include the global warming potential (GWP), eutrophication potential, acidification, smog formation, ozone depletion, and freshwater ecotoxicity. This study aims to analyze the environmental impacts of extruded feeds (wheat-based and sorghum-based) for aquaculture to help producers make informed decisions when considering how

to reduce their environmental footprint and is intended for public release. Environmental impacts of sorghum and wheat-based diets in combination with feed processing scenarios were compared for three aquaculture case study systems, the scope of which is described in the following sections. For the purposes of this study, the functional unit in all aquaculture systems was defined as the amount of feed needed to induce one metric ton of aquatic animal weight gain.

7.1.2 Floating feed for Rainbow trout (*Oncorhynchus mykiss*)

Two identical diets were formulated with the exception of the two primary grains used: hard red winter wheat or red grain sorghum. Processing data collected included grain and post-batch grinding using a pilot-scale hammermill (Model D Comminutor, Fitzpatrick, Westwood, MA, USA), as well as water and energy usage during extrusion processing. The primary goal of this study was to determine the differences between two diets based on the choice of cereal grains. As such, other ingredients were excluded from the scope of this study as their contribution to overall environmental impact of the feed was assumed is identical between the two diets.

7.1.3 Floating feed for Nile tilapia (*Oreochromis niloticus*)

Two diets were formulated using wheat and sorghum-based feeds to meet nutritional needs of Nile tilapia. Contrary to the previous trout scenario, there were slight differences in soybean meal and soy oil usage; as such, the differences between wheat and sorghum-based diets for these ingredients were included in the study using datasets publicly available in U.S. LCA Data Commons. As particle size reduction is an energy intensive process during feed production, three scenarios for each diet were considered.

Energy data were collected during hammermill grinding using three different screen sizes to examine the impact of coarse (1.27 mm), medium (1.02 mm), and fine grinding (0.61 mm) on energy use during processing and the chosen impact categories.

7.1.4 Sinking feed for Pacific white shrimp (*Litopenaeus vannamei*)

Wheat and sorghum-based diets were also formulated for sinking aquatic feed. Similar to the Nile tilapia scenario, there were slight differences in soybean meal and oil usage which were included in the LCA. This scenario focused on different levels of thermal energy (driven by steam injection created by a boiler). Three different levels of steam injection during preconditioning were quantified and examined.

The system boundary for all scenarios is presented in Figure 7.1. The data used for this life cycle assessment were collated from several sources. The cereal grain production data were gathered from the literature published by Kansas State University (CITE). The extruded feed production data was collected during the dissertation work of the author.

Grain sorghum and wheat production were assumed to take place in western Kansas. Transportation costs from the field to the feed mill were excluded due to similarities between the grains. On the feed production side, the boundary ends when the feed is extruded – drying is not included in this study. Drying costs are significant, both in economic terms and environmental impacts, but as the choice of grain does not affect drying parameters (CITE), it was assumed to have negligible impact on differences between the diets.

7.2 Materials and Methods

Dietary formulations for trout, tilapia, and shrimp are shown in Tables 7.1, 7.2 and 7.3, respectively. Ingredients that are included in this life cycle assessment are bolded. Diets were designed to be as identical as possible, with some minor variations in tilapia and shrimp to account for protein content.

7.2.1 Thermal Energy Efficiency of Aquatic Feed

Processing parameters such as screw speed, barrel temperatures, water and steam addition were kept unchanged across treatments, with some natural variability occurring. Process data were collected at the beginning and end of each collection period. Feed rate calibration was performed for each diet for each species. Processing parameters and extruder screw and die information are available in Chapters 2, 4, and 5 of this dissertation.

7.2.2 Mass and Energy Balance

Specific thermal energy (STE) input during the extrusion process was calculated for selected treatments from net heat transferred to the material due to steam absorbed during preconditioning, as follows.

$$STE \left(\frac{kJ}{kg} \right) = \frac{Q_{thermal}}{m_f} \quad (7.1)$$

Where $Q_{thermal}$ is the net rate of thermal energy or heat absorbed from steam (kJ/s), which was calculated using the equation below from the enthalpies of steam injected and unabsorbed steam lost to atmosphere at the preconditioner discharge.

$$Q_{thermal} \left(\frac{kJ}{s} \right) = (m_s \cdot h_s - m_s \cdot 4.18 \cdot T_r) - (m_{sl} \cdot h_{sl} - m_{sl} \cdot 4.18 \cdot T_r) \quad (7.2)$$

where h_s and h_{sl} are enthalpies of steam injected and steam lost to atmosphere (kJ/kg), respectively, as obtained from the steam tables; m_s and m_{sl} are rates of steam injection and loss (kg/s), respectively; the constant 4.18 is the specific heat of water at room temperature in kJ/kg°C; and T_r is room temperature (°C). The rate of steam loss from the preconditioner was calculated using mass balance, as described below.

$$m_{sl} \left(\frac{kg}{s} \right) = (m_f + m_s + m_w) - m_f \left(\frac{1 - X_{wf}}{1 - X_{wpc}} \right) \quad (7.3)$$

where m_w is the water injection rate into the preconditioner (kg/s), and the second term on the right of the equation is the rate of conditioned mass exiting the preconditioner, with X_{wf} and X_{wpc} representing the fractional wet basis (or as is) moisture contents of the feed material and the conditioned mass, respectively, measured using the oven drying method (135 °C, 2 h).

7.2.3 Pellet Qualities

Pellet durability index (PDI) is the standard for measuring the number of broken pellets, dust, and fines that are generated through repeated handling. Partial pellets and fines are assumed to be lost during transportation and handling; thus, the PDI was used to inform the functional unit. PDI was tested using a Holmen NPH100 pneumatic tester. One hundred grams of sieved pellets (U.S. No. 12, 1.7mm) were tested. Samples were placed in the chamber and then air-agitated for 60 seconds. Upon test completion, the fines were

automatically sieved from the tested sample. PDI is defined as the percentage of pellets remaining after the conclusion of the test.

Water stability of the extruded pellets was measured using a modified method of the version described by Adedeji, et al. (2017). Five grams of feed were weighed and then placed in a mesh tea ball before being placed in a beaker of distilled water at room temperature. The samples were soaked for a set period of time (15 mins), removed from the container, and then dried in a convection oven for 24 hours at 90°C. Sample weight was collected post-drying, with the difference in mass between pre and post-soak weights being defined as ‘water stability’, as shown in the following equation:

$$\text{Water Stability} = \frac{\text{Post soak weight}}{\text{Pre soak weight}} \times 100 \quad (7.4)$$

7.2.4 Inventory Data – Cereal Grain Production

Inventory inputs for cereal crop production came from the 2024 Farm Management dataset produced by the department of Agricultural Economics at Kansas State University (Ibendahl & O’Brien, 2023). Outputs to the environment – specifically surface runoff, groundwater leaching, and air pollution – were not included in this dataset and so were estimated for this LCA. It was assumed output emissions to air and water would occur at the same rate from both wheat and sorghum systems, with air emissions equal to 10% or the applied pesticide input and emissions to water at 5%. The relatively low assume rate of chemical export to water reflects semi-arid climate that characterizes dryland cropping system in SW Kansas. Nitrogen fertilizer emissions to air were assumed to volatilize as NH₃ and NO_x at a fixed rate loss of 10%, and N₂O emissions were calculated using the IPCC default value of 1.25% (IPCC, 2006). Data quality is thus uncertain- crop production input

data rates very high on both the qualitative and quantitative scale, but the emissions for agrochemical use and fertilizer application are more uncertain as they are controlled by a variety of interacting environmental variables. The farm management datasets are prepared annually for each region of Kansas by agronomists and economists at Kansas State University to reflect recommended, crop-specific agronomic inputs that accounts for regional variations in average yields, soil quality, rainfall amounts, and input costs. What is less certain is the emissions from these cropping systems; little data exists when it comes to the quantity of chemical and fertilizer runoff, and it can vary widely between farms based on their general practices, physical locations, environmental conditions and methods of application. Crop production inventory is shown in Table 7.4. As these farm management guides are designed for specific regions, it was assumed that both wheat and sorghum were produced in no-till farm ground with no straw harvesting. Since straw was assumed to remain in place to provide biomass and ground cover in a no-till cropping system, allocation was not performed regarding these inputs and outputs. Differences between formulations within each species regarding soybean meal and oil are also included in this assessment. All life cycle inventory data associated with various chemical inputs and agriculture operations were obtained from datasets compiled in the U.S. LCA data commons as indicated in Table 7.4.

7.2.5 Inventory Data – Extruded Feed Production

Inventory inputs and outputs for extrusion processing were collected by the extrusion lab at Kansas State University during production of these feeds. Energy use during hammermill grinding was collected through a three-phase power quality logger

(Fluke Corporation, Everett, WA, USA). Water use (kg/hr) and mechanical energy use (kJ/hr) were collected by a data acquisition system (DAQ) that logged inputs every second; energy use during grinding was collected through an energy logger; boiler fuel use for steam generation was determined through the steam input rates and an estimated boiler efficiency of 80%. Required BTU/hr from the boiler in steam injection was estimated by multiplying the steam enthalpy by the mass flow of steam (kg/hr) and dividing by the estimated efficiency (80%).

$$\text{Boiler Fuel BTU} \left(\frac{\text{kJ}}{\text{kg}} \right) = \frac{\text{Enthalpy} \times \text{Mass Flow} \left(\frac{\text{kg}}{\text{hr}} \right)}{80\%} \quad (7.5)$$

This was then converted to natural gas (m³/hr) and finally divided by the total mass flow rate of the extruder (kg/hr) to convert it to a per kg basis. These data could be highly accurate, but situational – they were collected by a data logger every second for the duration of the project, so data such as the energy use (kJ/hr) is very accurate for that run. However, not every company uses the same machine and not every machine has the same efficiencies; however, while the overall impact might change based on the equipment used for processing, the overall relationship between the wheat-based feed and the sorghum-based feed should remain the same.

Extruder electricity use was calculated through the following equation: Specific mechanical energy (SME) transferred from the screw to the material during the extrusion process was calculated as described below from the power consumed by the extruder motor measured using a wattmeter (Flory et al., 2023).

$$SME \left(\frac{kJ}{kg} \right) = \frac{kW - kW_0}{mr} \quad (7.6)$$

Where W is the power consumed during processing (kW), W0 is power consumed at no load with the screw running and no material flowing through (kW) and mf is the dry feed rate or rate of material delivered by the feeder screw in kg/sec as determined using volumetric feed delivery calibration.

Inventory data for extruded trout feed are shown in Table 7.5. As stated earlier, tilapia feed was ground through three different screen sizes before extrusion; these data (coarse, medium, and fine grind) are shown in Tables 7.6 and 7.7. Similarly, shrimp feed was produced using three different levels of preconditioning (PC) intensity (high, medium, and low PC) as shown in Tables 7.8 and 7.9.

7.2.6 Assessment Phase

The impact categories assessed were global warming potential (kg CO₂ eq), eutrophication (kg N eq), acidification (kg SO₂ eq), smog formation (kg O₃ eq), ozone depletion (kg CFC-11 eq), and freshwater ecotoxicity (CTUeco). The impacts chosen are all midpoint indicators. These impacts were assessed using the TRACI 2.1 impact assessment method. All analysis was calculated in openLCA 2.1.0 using the US LCA Commons database as the primary source of inventory data. Results were generated for one top of feed. These results were scaled to one metric ton (MT) of animal growth by applying species-specific feed conversion ratio (FCR; Chapters 2, 4 and 5) and the particle durability index (PDI) as follows:

$$\text{Feed Needed for 1 MT of growth} = \frac{1 \text{ MT} \times \text{FCR}(\text{from feeding trials})}{\text{PDI}\%} \quad (7.7)$$

7.2.7 Net Soil Organic Carbon Emissions

The inclusion of net soil carbon sequestration or losses in this LCA was intended to provide a more complete perspective and comparison between wheat and sorghum-based feeds. Soil organic carbon (SOC) was estimated using the Tier 1 IPCC method which calculates SOC changes over a 25-year period by considering land use, tillage, and input levels (IPCC, 2003). For this analysis, a reference SOC value (SOC_{ref}) representing cropland in Kansas classified as having High Activity Clay (HAC) soil (38 tons C/hectare). The SOC, under both wheat and sorghum crops, was calculated using the following equation:

$$\text{SOC} = \text{SOC}_{\text{ref}} \times \text{Flu} \times \text{Fmg} \times \text{FI} \quad (7.8)$$

SOC_{ref} (tons C/hectare) refers to the initial reference SOC value, *Flu* is land use, *Fmg* is the stock change factor for management regime, and *FI* is the stock change factor for input of organic matter. *Flu*, *Fmg* and *FI* adjust the reference SOC value based on the chosen agricultural practices. Values were obtained from the IPCC (2003) and selected to represent the most common cropping production systems in western Kansas. Wheat and sorghum were classified as being grown on "long-term cultivated land," referring to fields that have been continuously farmed with annual crops for over 20 years (Gautam et al., 2020; Life Cycle Assessment Commons, 2024), leading to a selection of 0.82 for the *Flu* variable (dry, temperate, long-term cultivated land use). As the crop production guides

selected for this study were based on no-till practices, Fmg was set at 1.10. Input factor (FI) was set at 1.0, a “medium input (no manure)” system. $NetSOC$ (on a 25 year basis) as then calculated by subtracting SOC_{ref} from the calculated SOC. To account for carbon sequestration, soil carbon data were scaled to align with the grain yields and system boundaries of this study. $NetSOC$ (tons C/hectare) to obtain sequestration in kg CO₂ eq/kg grain. The scaled values were then converted from elemental carbon to CO₂ equivalents using the IPCC-recommended conversion factor (44/12). This approach allowed for direct comparison between net changes in soil carbon and production-related GWP emissions.

As the functional unit adopted in the this is one metric ton of animal weight gain, the amount of grain used in the amount of feed needed was found by multiplying 1000 kg times the % grain composition. $NetSOC$ (kg CO₂ eq/kg of grain) was then scaled to the functional unit by multiplying by the mass.

The soil carbon sequestration data represent accumulation over approximately 25 years, following IPCC methodology. To integrate sequestration into the GWP assessment, a "net GWP" was calculated using the equation:

$$Net\ GWP = LCA\ GWP\ (kg\ CO_2\ eq) - \left(\frac{SOC\ kg\ CO_2\ eq}{25} \right) \quad (7.9)$$

By subtracting the sequestration value, the net GWP provides a more balanced view of the environmental impacts of comparing cereal grains. Including this calculation highlights the importance of considering soil carbon dynamics in LCA studies, which is

especially relevant when evaluating the long-term environmental impacts of transitioning to alternative grains such as sorghum.

Sensitivity Analysis

Sensitivity analysis was performed to evaluate the impact of various input parameters on emission levels. The chosen feed used for sensitivity analysis was the wheat-based trout feed, with three variables selected: grain yield, boiler fuel usage, and electricity use during extrusion processing. These variables were adjusted by $\pm 15\%$, and impact categories were recalculated for each scenario. These variables were selected for sensitivity analysis due to the uncertainty involved; while crop production datasets have defined inputs, actual grain yields can vary based on a variety of factors including soil health, variations in rainfall, and daily environmental conditions. Similarly, extrusion processing of aquatic feed has two primary inputs: mechanical and thermal energy. Mechanical energy is primarily driven by energy use (electricity) needed to convey the extruder screw during processing, while thermal energy is primarily delivered by steam injection, which is created at the boiler.

7.3 Results and Discussion

7.3.1 Specific Thermal Energy - Efficiency

Thermal energy transfer during single screw extrusion takes place primarily through preconditioner steam injection. It is important to note, however, that not all steam is absorbed by the raw diet during this process; some amount of steam and heat is lost to the ambient environment. As such, specific thermal energy (STE, kJ/kg) can be used as a measure of process efficiency.

Processing conditions were held constant across all treatments, with only minor natural variations. Specific thermal energy efficiency (STE) in the preconditioner was assessed for trout, tilapia, and shrimp feeds using wheat- and sorghum-based diets (Tables 7.10–7.12), revealing key differences in process-level efficiency and highlighting opportunities for improvement.

7.3.1.1 Trout

For trout feeds (Table 7.10), which compared wheat and sorghum as base ingredients, sorghum-based diets exhibited higher steam loss (30% of injected steam) compared to wheat (19%). This discrepancy in steam retention directly affected STE values, with sorghum achieving 185 kJ/kg and wheat reaching 212 kJ/kg. These results suggest that sorghum diets are less efficient at utilizing injected steam, likely due to differences in physicochemical properties such as starch structure or fiber content that affect steam absorption during preconditioning.

7.3.1.2 Tilapia

Tilapia feeds (Table 7.11) involved three grind sizes (coarse, medium, fine) for both wheat- and sorghum-based formulations. Across all treatments, wheat-based diets showed greater steam loss, ranging from 42% to 61%, compared to 35% to 53% for sorghum-based diets. This likely has to do with particle size reduction; grains used in this study went through more severe processing conditions during particle size reduction. Despite finer grinding leading to increased surface area which should lead to more steam absorption, fine grinding impaired steam absorption. It is possible that the heat from fine grinding damaged the starch, impairing or limiting the amount of steam that could be

absorbed during preconditioning. Accordingly, STE values declined with decreasing particle size. For wheat, STE dropped from 275 kJ/kg under coarse grind to 219 kJ/kg under fine grind. Sorghum followed a similar trend, declining from 293 to 253 kJ/kg. These results indicate that finer grinding reduces the material's capacity to absorb steam effectively, possibly due to altered surface area or reduced porosity, which can impair heat transfer and moisture penetration during conditioning.

7.3.3 Shrimp

In shrimp feeds (Table 7.12), three levels of steam injection were applied to both wheat- and sorghum-based diets. As steam input decreased, STE improved in both diets, with wheat improving from 237 kJ/kg at high injection to 119 kJ/kg at low injection, and sorghum from 280 to 114 kJ/kg. However, the improvement was more pronounced in wheat-based diets. Despite reduced input, neither grain achieved 100% efficiency, suggesting persistent system-level losses or limitations in heat transfer within the preconditioner. This trend reinforces the idea that excess steam input does not necessarily translate to improved thermal efficiency and may instead increase loss or elevate system humidity beyond optimal levels.

Collectively, these findings underscore differences between wheat and sorghum in steam efficiency and energy use. Sorghum-based feeds tend to lose more steam during conditioning (with the exception of tilapia feeds), resulting in lower STE values across all species. Additionally, particle size reduction diminished steam absorption efficiency, particularly in finer ground diets. In shrimp feeds, lower steam input improved STE, but

sorghum diets remained less efficient overall, highlighting formulation and process interactions that influence thermal energy dynamics.

Pacheco et al. (2018) reported STE values of 35.2 and 64.7 kWh/ton (equivalent to 126.7 and 232.9 kJ/kg) at preconditioner temperatures comparable to those in this study. These values are consistent with the STE ranges observed here, validating the generalizability of the current findings. However, it is important to note that STE is also influenced by diet composition, particularly starch content, as well as physical form and moisture interactions. As such, differences in formulation and feedstock properties must be considered alongside process parameters.

Overall, this analysis points to the need to develop actionable strategies for improving thermal efficiency in extrusion. Enhancing insulation, optimizing preconditioner design, and tailoring steam injection protocols could reduce steam loss and improve heat capture, potentially recovering 20–60 kJ/kg of thermal energy. Such improvements could reduce operational energy demand and support more sustainable feed production practices while maintaining product quality.

7.3.2 LCA Assessment Phase

The impact categories assessed are global warming potential (kg CO₂ eq), eutrophication (kg N eq), acidification (kg SO₂ eq), smog formation (kg O₃ eq), ozone depletion (kg CFC-11 eq), and freshwater ecotoxicity (CTUeco).

GWP results are presented as NetGWP based off carbon sequestration (Table 7.22). Observation of the results shows dryland wheat had the highest impact category values, while sorghum-based feeds were the lowest in all categories. GWP values ranged from

90.18 to 303.52 kg CO₂ eq/metric ton of growth; FWE values ranged from 9.91 to 78.38 CTUeco/metric ton of growth; SF values ranged from 5.87 to 20.31 kg O₃ eq/metric ton of feed. This life cycle assessment consistent shows that extruded wheat-based feeds generate higher environmental impacts than those based on grain sorghum.

7.3.2.1 Trout

In the trout scenario (Table 7.13), wheat feed generates 86.94 kg CO₂ eq kg, 21.93 CTUeco, and 9.07 kg O₃ eq, while sorghum feed produces only 66.98 kg CO₂ eq, 9.91 CTUeco, and 5.87 kg O₃ eq, respectively. Those represent roughly 30–55 percent reductions when substituting sorghum for wheat. This is largely driven by the reduced inputs during grain cultivation and higher sorghum yields as indicated by inventory data (Table 7.4).

7.3.2.2 Tilapia

Impact assessment results for dryland wheat, irrigated wheat, and sorghum-based feeds are shown in Tables 7.14, 7.15 and 7.16, respectively. Dryland wheat values were again the highest across all grains, and sorghum-based feeds were the lowest. Within each grain, three different grinding scenarios which impacted the energy used during processing were considered. In tilapia feeds, finer grinding amplified differences in environmental impacts across grain types. For sorghum-based tilapia feeds, coarse grinding yielded 123.66 kg CO₂ eq/metric ton of growth, medium 128.73 kg CO₂ eq/metric ton of growth, and fine 188.01 kg CO₂ eq/metric ton of growth, a 55 kg CO₂ eq/metric ton of growth jump from coarse to fine. Wheat-based feeds rose from 184.96 to 247.69 kg CO₂ eq over the same grind range, an increase of approximately 63 kg CO₂ eq. Grain choice and grinding

parameters were shown to noticeably impact environmental impacts, with emissions rising as grinding intensified, and wheat-based feeds having overall higher values. FWE values were again lower for sorghum-based feeds (29.64-32.46 CTUeco/metric ton of growth) in comparison to wheat and irrigated wheat-feeds (75.83-78.38 and 70.34-72.64, respectively).

7.3.2.3 Shrimp

Impact assessment results for dryland wheat, irrigated wheat, and sorghum-based shrimp feeds are shown in Tables 7.17, 7.18 and 7.19, respectively. Dryland wheat values were again the highest, with sorghum values the lowest. The three highest categories were again FWE, GWP, and SF. Within each grain, three different preconditioning scenarios were considered. This was done by altering the amount of steam and water injected during processing. Steam injection at high (High PC), medium (Medium PC), and low (Low PC) levels was recorded during processing. Across all grains, reducing steam injection during processing had minimal effect on the chosen impact categories. Reducing steam injection from the high levels to the medium levels did reduce GWP by approximately 6 kg CO₂ eq, but there was minimal to no impact on GWP values by reducing steam injection from medium to low.

This study has several limitations. Transportation of ingredients is not included, and the use of crop production guides specific to Kansas as opposed to existing databases may not accurately reflect the environmental impacts of wheat vs sorghum-based feeds on a national or global basis.

7.3.3 NetSOC Carbon Emissions

Net SOC (kg CO₂ eq/kg grain/annually) are shown in Table 7.20. Due to differences in grain yield, dryland wheat grown in southwest Kansas is estimated to lose 3.76 kg of CO₂ eq/kg grain/year, while sorghum is estimated to lose 2.56 kg CO₂ eq/kg grain/year. Table 7.21 shows that value scaled up to the amount of grain used in each diet based on the inclusion percentage of grain in one metric ton of the respective feed. Table 7.22 shows the Net GWP as calculated from the GWP from LCA analysis. Across all diets and species, as the NetSOC value was negative (representing a loss of carbon from soil to the atmosphere, as opposed to sequestration), the GWP increased. Due to sorghum having a lower estimated soil carbon loss per kg of grain, the differences between wheat and sorghum diets grew larger, as opposed to shrinking, further representing the potential of grain sorghum as a sustainable fish feed ingredients.

Limited research exists on the specific environmental impacts of extruded aquatic feed during feed processing. Wang et al (2022) reported a GWP value of 1333.9 kg CO₂ eq, but comparisons between that study and this one is not completely compatible. Wang et al (2022) looked at the processing of extruded grass carp feed (1 ton); they included numerous transportation steps, many more ingredients, and packaging, all specific to Shaoxing, Zhejiang province, China. The authors found that raw material production accounted for 72.18% of the total GWP, while processing accounted for 18.94%. This aligns with the conclusion found in this study that, while process improvements can reduce environmental impacts, the inherent sustainability of extrusion processing lessens the overall impacts of these changes, and the greatest opportunity for a reduction in

emissions is through optimizing the production and processing of ingredients and by-products. Extrusion processing improves the digestibility of nutrients in aquafeeds, reducing feed conversion ratio and requiring less feed for growth in comparison to lower energy processing such as pellet milling. This is particularly evident in the processing of alternative proteins to replace fishmeal, reducing reliance on unsustainable marine ingredients (Fallahi et al, 2016; Sarker et al, 2023).

7.3.4 Sensitivity Analysis

Sensitivity analysis was performed in order to evaluate the impact of various input parameters on emission levels. Three variables were selected (grain yield, boiler fuel usage, and electricity use during extrusion processing) and were adjusted by $\pm 15\%$, and impact categories were recalculated for each level using the wheat-based trout feed scenario. The results from this analysis are shown in Tables 7.23, 7.24 and 7.25, respectively. Yield adjustments of wheat impacted results to a large degree – increasing the yield of wheat by 15% while keeping inputs the same resulted in all calculated impact categories being reduced by 2.3-12.8%; this also occurred with the 15% reduction in yield resulting in increased emissions, ranging from 3.2-17.3%. Eutrophication and Freshwater Ecotoxicity were the most significantly affected in terms of absolute percentages. An increase/reduction in yield by 15% resulted in Eutrophication values of 0.42 and 0.57 kg N eq, respectively, so while the percentage it changed was large, change in absolute value were small. For GWP and FWE, a 15% increase in yield resulted in a 4.9% and 12.3% decrease, respectively; a 15% reduction in yield resulted in FWE increasing by 16.6% and GWP increasing by 6.6%. Boiler fuel had limited and overall minor effects on emission

levels, indicating a low level of sensitivity. Of all emission categories, the largest change took place in GWP. Increasing boiler fuel usage by 15% increased GWP by 2.5%, while reducing it boiler fuel use by 15% lowered GWP by another 2.5%. Adjusting the electricity by +/- 15% resulted in changes to all categories. The most significant changes (in terms of absolute percentages) were in the Acidification and Human health-particulate matter categories (9.6 and 11.8%, respectively. GWP values increased by 6.8% with an additional 15% electricity usage, while reducing electricity by 15% decreased GWP by 6.8%, again. Based on these results, it appears that grain yield and electricity use have larger degrees of sensitivity than boiler fuel use does in terms of impacting emission values.

7.4 Conclusion

The life cycle assessments conducted in this study highlight key environmental impacts associated with the production of extruded aquatic feeds, specifically wheat and sorghum-based diets for trout, tilapia and shrimp. The results show that sorghum-based feeds tend to have lower overall environmental impacts when compared to wheat-based feeds across the chosen impact categories, with global warming potential, freshwater ecotoxicity and smog formation the three largest impacts. The analysis of thermal energy efficiency during extrusion suggests that while sorghum tends to have higher steam loss and reduced thermal efficiency, its overall lower environmental footprint makes it a strong alternative to wheat in feed production for aquaculture. These findings can be used to inform feed producers about more sustainable ingredient choices, which will help contribute to reduced environmental impacts in the aquaculture industry.

7.5 References

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 - Electricity, at grid, US, 2008 – RNA (2008)
 - Natural gas, combusted in industrial equipment, 1.357 m³/kg, 52.13 Mj/kg – RNA (2023)
 - Cutoff Agrochemicals, at plant – RNA (2019)
 - Diesel, combusted in industrial equipment – RNA (2019)
 - Nitrogen fertilizer, production mix, at plant – RNA (2019)
 - Phosphorous fertilizer, production mix, at plant – RNA (2019)

7.6 Figures and Tables

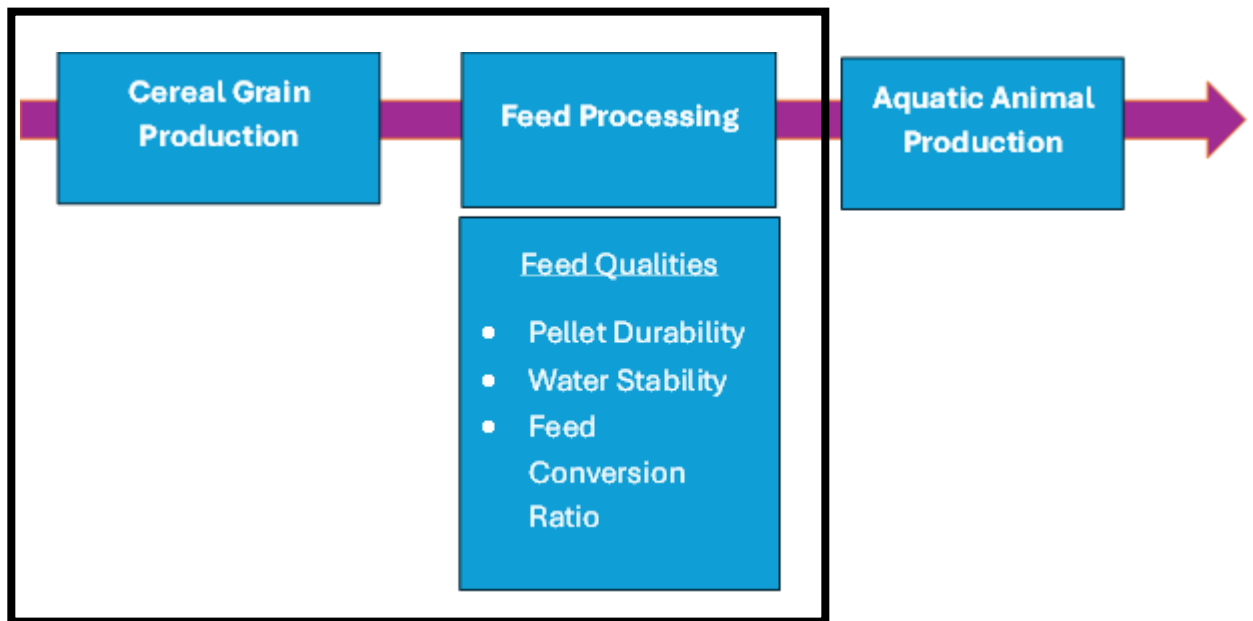


Figure 7.1: System Boundary

Table 7.1: Dietary formulation for wheat and sorghum-based feeds for rainbow trout

Ingredient	Composition %
Menhaden Fishmeal	13.55
Poultry by-product	13.6
Corn Protein Concentrate	5
Blood Meal	5
Feather Meal	5
Soybean Meal	12
Ground Wheat or Sorghum	22.52
Monocalcium Phosphate	2.3
Lysine HCl	2.18
DL-Methionine	0.59
Threonine	0.63
Soy Lecithin	1.5
Stay-C 35	0.2
Vitamin Premix	1
Choline Chloride 50%	1
Yttrium Oxide	0.1
TM ARS 1440	0.1
Astaxanthin	0.08
Taurine	0.5
Calcium Propionate	0.15
Menhaden Fish Oil	6.8
Poultry Fat	6.2

Bolded text indicates inclusion of ingredient into the LCA. Non-bolded ingredients have the same inclusion level across all diets and were not included in LCA.

Table 7.2: Dietary formulation for wheat and sorghum-based feeds for Nile tilapia

Ingredients	Composition % of wheat-based diet	Composition % of sorghum-based diet
Menhaden Fishmeal	6	6
Soybean Meal	44	44.60
Corn Protein Concentrate	9	9
Menhaden Fish Oil	2	2
Soy Oil	3.52	3.00
Soy Lecithin	1	1
Ground Wheat or Sorghum	32.32	32.24
Mineral Premix	0.07	0.07
Vitamin Premix	0.04	0.04
Choline Chloride	0.2	0.2
Rovimix Stay-C 35%	0.1	0.1
Dicalcium Phosphate	1.75	1.75

Bolded text indicates inclusion of ingredient into the LCA. Non-bolded ingredients have the same inclusion level across all diets.

Table 7.3: Dietary formulation for wheat and sorghum-based feeds for Pacific white shrimp

Ingredient	Composition % of wheat-based diet	Composition % of sorghum-based diet
Fishmeal	10.00	10.00
Soybean meal	39.00	39.45
Corn Protein Concentrate	8.00	8.00
Fish oil	2.00	2.00
Soy oil	3.02	2.65
Lecithin	1.00	1.00
Cholesterol	0.12	0.12
Ground Wheat or Sorghum	23.20	23.12
Rice Bran	10.00	10.00
Mineral premix	0.07	0.07
Vitamin premix	0.04	0.04
Choline chloride	0.20	0.20
Stay-C 35% Active	0.10	0.10
CaP-dibasic	1.75	1.75
Bentonite	1.50	1.50

Bolded text indicates inclusion of ingredient into the LCA. Non-bolded ingredients have the same inclusion level across all diets.

Table 7.4: Crop Production Inventory Data

	Sorghum, at field	Wheat, at field	Irrigated Wheat, at field
Inputs			
CUTOFF Agrochemicals, at plant (kg)	0.00303	0.0019	0.0015
Diesel, combusted in industrial equipment (imp gal)	0.00154	0.0032	0.0024
Land use m ² *a	1.87433	2.7503	2.1242
Nitrogen Fertilizer, production mix, at plant (kg)	0.01366	0.0245	0.0231
Phosphorous fertilizer, production mix, at plant (kg)	0.00693	0.0086	0.0081
Outputs			
2,4-D (kg) water (5%)	0.00001	0.00002	0.00001
2,4-D (kg) air (10%)	0.00002	0.00003	0.00002
Dicamba (kg) water (5%)	0.00002	0.00001	0.00001
Dicamba (kg) air (10%)	0.00003	0.00002	0.00002
Glyphosate (kg) water (5%)	0.00004	0.00001	0.00004
Glyphosate (kg) air (10%)	0.00008	0.00012	0.00095
Dinitrogen monoxide (air/low population) (1.5%)	0.00015	0.00028	0.00026
Orthophosphate kg water (1.5%)	0.00010	0.00013	0.00012
Harvested Grain (kg)	1	1	1

All quantities are from Ibendahl, G., & O'Brien, D. (2023). Inputs were sourced from U.S.

LCA Commons dataset and can also be referenced here.

Table 7.5: Extrusion processing inventory data for trout

	Sorghum	Wheat	Irrigated Wheat
Inputs			
CUTOFF Water, at plant (kg)	0.2669	0.2665	0.2665
Electricity, at grid, US, 2008 (kJ)	256.87	320.36	320.36
Natural gas, combusted in industrial equipment (m3)	0.0071	0.0073	0.0073
Irrigated Wheat, at field (kg)	0.0000	0.0000	0.2252
Wheat/Sorghum grains, at field (kg)	0.2252	0.2252	0.0000
Wheat/Sorghum Feed (kg)	1	1	1

All quantities are from Ibendahl, G., & O'Brien, D. (2023). Inputs were sourced from U.S.

LCA Commons dataset and can also be referenced here.

Table 7.6: Extrusion processing inventory data (wheat and irrigated wheat) for tilapia

	Wheat Fine	Wheat Medium	Wheat Coarse
Inputs			
CUTOFF Water, at plant (kg)	0.4463	0.3613	0.3936
Electricity, at grid, US, 2008 (kJ)	778.3	471.48	290.5
Natural gas, combusted in industrial equipment (m3)	0.0121	0.0108	0.01186
Soy oil, refined, at plant (kg)	0.0352	0.0352	0.0352
Dryland/Irrigated Wheat, at field (kg)	0.3232	0.3232	0.3232
Wheat Feed (kg)	1	1	1

All quantities are from Ibendahl, G., & O'Brien, D. (2023). Inputs were sourced from U.S.

LCA Commons dataset and can also be referenced here.

Table 7.7: Extrusion processing inventory data (sorghum) for tilapia

	Sorghum Fine	Sorghum Medium	Sorghum Coarse
Inputs			
CUTOFF Water, at plant (kg)	0.4578	0.3785	0.4018
Electricity, at grid, US, 2008 (kJ)	733.12	435.98	348.49
Natural gas, combusted in industrial equipment (m3)	0.0125	0.0114	0.0117
Soybean meal, solvent-extracted (kg)	0.006	0.006	0.006
Sorghum grains, at field (kg)	0.3224	0.3224	0.3224
Sorghum Feed (kg)	1	1	1

All inventory data was collected during processing of diets at Kansas State University and can be referenced here.

Table 7.8: Extrusion processing inventory data (wheat and irrigated wheat) for shrimp

	Wheat Low PC	Wheat Medium PC	Wheat High PC
Inputs			
CUTOFF Water, at plant (kg)	0.2758	0.2891	0.274
Electricity, at grid, US, 2008 (kJ)	480.44	446.54	448.04
Natural gas, combusted in industrial equipment (m3)	0.0035	0.0056	0.0079
Soy oil, refined, at plant (kg)	0.0037	0.0037	0.0037
Dryland/Irrigated Wheat, at field (kg)	0.232	0.2320	0.232
Wheat Feed (kg)	1	1	1

All inventory data was collected during processing of diets at Kansas State University and can be referenced here.

Table 7.9: Extrusion processing inventory data (sorghum) for shrimp

	Sorghum Low PC	Sorghum Medium PC	Sorghum High PC
Inputs			
CUTOFF Water, at plant (kg)	0.2782	0.2742	0.2891
Electricity, at grid, US, 2008 (kJ)	453.15	423.05	402.75
Natural gas, combusted in industrial equipment (m3)	0.0041	0.0061	0.0098
Soybean meal, solvent-extracted (kg)	0.0045	0.0045	0.0045
Sorghum grains, at field (kg)	0.2312	0.2312	0.2312
Sorghum Feed (kg)	1	1	1

All inventory data was collected during processing of diets at Kansas State University and can be referenced here.

Table 7.10: Preconditioner efficiency for extruded trout feed

	Sorghum	Wheat
Raw Material (kg/hr)	152.07	148.54
PC Steam Input (kg/hr)	13.48	13.54
<i>Q_{pcs}</i> (kJ/kg)	241.77	248.63
Steam Loss (kg/hr)	4.09	2.57
% of input	30%	19%
<i>Q_{sloss}</i> (kJ/kg)	56.78	36.58
<i>STE</i> (kJ/kg)	184.99	212.05

Table 7.11: Preconditioner efficiency for extruded tilapia feed with different grind sizes

	Wheat Fine	Wheat Medium	Wheat Coarse	Sorghum Fine	Sorghum Medium	Sorghum Coarse
Raw Material (kg/hr)	88.7	94.53	85.5	78.26	85.87	87.43
PC Steam Input (kg/hr)	13.54	12.79	12.75	12.32	12.34	12.85
<i>Q_{pcs} (kJ/kg)</i>	<i>416.34</i>	<i>369.00</i>	<i>406.71</i>	<i>429.35</i>	<i>391.93</i>	<i>400.84</i>
Steam Loss (kg/hr)	8.31	5.12	5.31	6.54	4.61	4.48
% of input	61%	40%	42%	53%	37%	35%
<i>Q_{sloss} (kJ/kg)</i>	<i>197.76</i>	<i>114.38</i>	<i>131.24</i>	<i>176.53</i>	<i>113.38</i>	<i>108.26</i>
<i>STE (kJ/kg)</i>	<i>218.58</i>	<i>254.62</i>	<i>275.47</i>	<i>252.81</i>	<i>278.54</i>	<i>292.58</i>

Table 7.12: Preconditioner efficiency for extruded shrimp feed with different steam injection levels

	Wheat Low PC	Wheat Medium PC	Wheat High PC	Sorghum Low PC	Sorghum Medium PC	Sorghum High PC
Raw Material (kg/hr)	134.86	132.78	135.58	135.94	133.79	132.65
PC Steam Input (kg/hr)	5.85	9.27	13.51	7.01	10.18	16.35
<i>Q_{pcs}</i> (kJ/kg)	118.3	190.40	271.76	140.63	207.52	336.15
Steam Loss (kg/hr)	0.35	1.33	2.51	1.46	2.16	3.96
% of input	6%	14%	19%	21%	21%	24%
<i>Q_{sloss}</i> (kJ/kg)	4.9	18.85	34.97	20.19	30.45	56.3
<i>STE</i> (kJ/kg)	113.4	171.55	236.79	120.45	177.08	279.85

Table 7.13: Impact categories for one metric ton of animal weight gain in rainbow Trout

Category	Unit	Sorghum Feed	Wheat Feed	Irrigated Wheat Feed
Acidification	kg SO2 eq	0.43	0.59	0.56
Eutrophication	kg N eq	0.36	0.48	0.45
Freshwater ecotoxicity	CTUeco	9.91	21.93	17.84
Global warming	kg CO2 eq	66.98	86.94	83.94
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.02	0.02	0.02
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	5.87	9.07	8.00

Table 7.14: Impact categories for one metric ton of animal weight gain in Tilapia

Category	Unit	Wheat - Fine	Wheat - Medium	Wheat - Coarse
Acidification	kg SO2 eq	1.48	1.05	0.99
Eutrophication	kg N eq	0.69	0.68	0.69
Freshwater ecotoxicity	CTUeco	78.38	75.83	77.04
Global warming	kg CO2 eq	247.69	187.93	184.96
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.06	0.03	0.03
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	20.31	16.31	16.11

Table 7.15: Impact categories for one metric ton of animal weight gain in Tilapia

Category	Unit	Irrigated Wheat - Fine	Irrigated Wheat - Medium	Irrigated Wheat - Coarse
Acidification	kg SO2 eq	1.42	0.99	0.94
Eutrophication	kg N eq	0.65	0.63	0.64
Freshwater ecotoxicity	CTUeco	71.69	72.64	70.34
Global warming	kg CO2 eq	241.08	181.43	178.35
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.06	0.03	0.03
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	18.55	14.59	14.36

Table 7.16: Impact categories one metric ton of animal weight gain in Tilapia

Category	Unit	Sorghum - Fine	Sorghum - Medium	Sorghum - Coarse
Acidification	kg SO2 eq	1.16	0.73	0.66
Eutrophication	kg N eq	0.51	0.47	0.50
Freshwater ecotoxicity	CTUeco	32.46	29.64	31.35
Global warming	kg CO2 eq	188.01	128.73	123.66
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.04	0.03	0.02
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	13.53	9.56	9.26

Table 7.17: Impact categories for one metric ton of animal weight gain in Shrimp

Category	Unit	Wheat – Low PC	Wheat - Medium PC	Wheat - High PC
Acidification	kg SO2 eq	1.22	1.15	1.21
Eutrophication	kg N eq	0.64	0.63	0.66
Freshwater ecotoxicity	CTUeco	71.74	71.23	74.36
Global warming	kg CO2 eq	193.35	192.20	209.50
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.04	0.04	0.05
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	16.83	16.55	17.66

Table 7.18: Impact categories one metric ton of animal weight gain in Shrimp

Category	Unit	Irrigated Wheat - Low PC	Irrigated Wheat - Medium PC	Irrigated Wheat - High PC
Acidification	kg SO2 eq	1.16	1.11	1.17
Eutrophication	kg N eq	0.61	0.60	0.63
Freshwater ecotoxicity	CTUeco	65.54	65.07	67.93
Global warming	kg CO2 eq	187.20	186.11	203.13
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUnoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.04	0.04	0.05
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	15.20	14.94	15.97

Table 7.19: Impact categories one metric ton of animal weight gain in Shrimp

Category	Unit	Sorghum - Low PC	Sorghum - Medium PC	Sorghum - High PC
Acidification	kg SO2 eq	0.98	0.94	0.89
Eutrophication	kg N eq	0.50	0.50	0.48
Freshwater ecotoxicity	CTUeco	31.16	31.43	30.20
Global warming	kg CO2 eq	149.78	151.76	154.27
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.05	0.05	0.04
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	11.34	11.33	11.15

Table 7.20: Carbon Stock Calculations

Crop	SOCref (tons C/hectare)	Flu	Fmg	F1	SOC (tons C/hectare)	Net SOC (tons C/hectare)	Yield (kg/hectare)	Carbon Sequestered (kg CO2 eq/kg/year)
Wheat	38.00	0.82	1.10	1.00	34.28	-3.72	3630.01	-3.76
Sorghum	38.00	0.82	1.10	1.00	34.28	-3.72	5332.98	-2.56

Table 7.21: Carbon Stock Losses Scaling to Grain Yields

Species	Grain	Processing Input Variable	kg of grain in feed needed for 1 MT of animal growth	kg CO2 eq losses/ton growth over 25 years	kg CO2 eq losses/ton growth annually
Trout	Wheat	n/a	225.11	-846.41	-33.86
	Sorghum	n/a	226.60	-580.11	-23.20
Tilapia	Wheat	Fine Grind	371.19	-1395.68	-55.83
		Medium Grind	364.62	-1370.97	-54.84
		Coarse Grind	371.19	-1395.68	-55.83
	Sorghum	Fine Grind	355.68	-910.55	-36.42
		Medium Grind	336.10	-860.43	-34.42
		Coarse Grind	358.95	-918.90	-36.76
Shrimp	Wheat	Low PC	344.31	-1294.61	-51.78
		Medium PC	342.14	-1286.45	-51.46
		High PC	356.56	-1340.67	-53.63
	Sorghum	Low PC	347.86	-890.53	-35.62
		Medium PC	351.57	-900.03	-36.00
		High PC	336.87	-862.38	-34.50

Table 7.22: Net Global Warming Potential

Species	Grain	Processing Input Variable	GWP - LCA (kg CO2 eq)	Carbon	Net GWP
				Sequestration (kg CO2 eq/ton growth annually)	(kg CO2 eq/ton growth)
Trout	Wheat	n/a	86.94	-33.86	120.80
	Sorghum	n/a	66.98	-23.20	90.18
Tilapia	Wheat	Fine Grind	247.69	-55.83	303.52
		Medium Grind	187.93	-54.84	242.77
		Coarse Grind	184.96	-55.83	240.79
	Sorghum	Fine Grind	188.01	-36.42	224.44
		Medium Grind	128.73	-34.42	163.15
		Coarse Grind	123.66	-36.76	160.42
Shrimp	Wheat	Low PC	193.35	-51.78	245.13
		Medium PC	192.20	-51.46	243.66
		High PC	209.50	-53.63	263.12
	Sorghum	Low PC	149.78	-35.62	185.40
		Medium PC	151.76	-36.00	187.76
		High PC	154.27	-34.50	188.77

Table 7.23: Impact of grain yield sensitivity on the emissions of one metric ton weight gain for trout fed wheat-based trout

Category	Unit	Plus 15	Baseline	Minus 15
Acidification	kg SO2 eq	0.56	0.59	0.62
Eutrophication	kg N eq	0.42	0.48	0.57
Freshwater ecotoxicity	CTUeco	19.23	21.93	25.57
Net Global warming potential	kg CO2 eq	82.66	86.94	92.72
Human health – cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUnoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.02	0.02	0.02
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	8.40	9.07	9.95

Table 7.24: Impact of boiler fuel sensitivity on the emissions of one metric ton weight gain for trout fed wheat-based trout

Category	Unit	Plus 15	Baseline	Minus 15
Acidification	kg SO2 eq	0.59	0.59	0.58
Eutrophication	kg N eq	0.48	0.48	0.48
Freshwater ecotoxicity	CTUeco	21.96	21.93	21.89
Net Global warming potential	kg CO2 eq	89.12	86.94	84.74
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUnoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.02	0.02	0.02
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	9.18	9.07	8.95

Table 7.25: Impact of electricity use sensitivity on the emissions of one metric ton weight gain for trout fed wheat-based trout

Category	Unit	Plus 15	Baseline	Minus 15
Acidification	kg SO2 eq	0.64	0.59	0.53
Eutrophication	kg N eq	0.50	0.48	0.48
Freshwater ecotoxicity	CTUeco	22.08	21.93	21.76
Net Global warming potential	kg CO2 eq	92.87	86.94	81.00
Human health - cancer	CTUcancer	0.00	0.00	0.00
Human health - non-cancer	CTUoncancer	0.00	0.00	0.00
Human health - particulate matter	PM 2.5 eq	0.02	0.02	0.02
Ozone depletion	kg CFC-11 eq	0.00	0.00	0.00
Smog formation	kg O3 eq	9.55	9.07	8.57

Chapter 8 - Processing, Pellet Qualities, and Environmental Impacts of Extruded Aquatic Feed with Distillers Dried Grains

Abstract

This study evaluated the impact of sorghum distillers dried grains (sDDGs) inclusion on extrusion energy, pellet quality, and environmental outcomes in aquafeed production. Diets containing 0%, 20%, and 40% sDDGs were formulated and compared to a fishmeal Fishmeal diet. Increasing sDDG inclusion elevated total grinding energy from 126.7 kJ/kg in the Fishmeal diet to 162.1 kJ/kg in the PP-40ddgPP-40ddg diet. During extrusion, specific mechanical energy (SME) decreased from 279 kJ/kg in PP-0ddg to 213.8 kJ/kg in PP-40ddgPP-40ddg, while specific thermal energy (STE) increased from 182.8 kJ/kg to 239 kJ/kg. Pellet expansion (SEI) declined sharply, from 2.64 in the Fishmeal diet to 0.95 in PP-40ddgPP-40ddg, with dry bulk density rising from 391.5 g/L to 487.0 g/L. Floatability dropped correspondingly, 100% float at 30 min for the Fishmeal diet versus 0% for PP-40ddgPP-40ddg. Pellet durability improved with higher sDDG, from 83.86% (Fishmeal diet) to 91.10% (PP-40ddg). Water stability declined slightly (from 89.16% to 79.18%). Adjusting preconditioning inputs in PP-20ddg diets showed high water-to-steam ratios increased floatability (56%) and durability (91.46%). Dryer efficiency peaked at 15.35% in the PP-20ddg treatment but declined to 12.65% in PP-40ddg, despite higher moisture removal (32.09 kg/hr). Life cycle assessment revealed rising environmental burdens with sDDG: global warming potential increased from 1204.9 kg CO₂ eq (Fishmeal diet) to 2097.1 kg CO₂ eq (PP-40ddg), eutrophication from 0.089 to 0.567 kg N eq, and freshwater ecotoxicity from

15.63 to 3045.6 CTUeco. These results suggest that while sDDGs enhance pellet durability and reduce extrusion SME, their inclusion demands more processing energy and poses environmental trade-offs that must be weighed against their co-product sustainability value.

8.1 Introduction

As the global population grows, the demand for high-quality protein has increased, leading to a 3% annual rise in fish consumption between 1961 and 2019, nearly twice the rate of population growth (FAO, 2022). Aquatic species, both wild-caught and farmed, now provide about 17% of the world's animal protein intake, with approximately 3 billion people relying on fish for at least 20% of their daily protein needs (FAO, 2022). Aquaculture alone produced 88 million tons of seafood in 2020, representing roughly half of global seafood consumption (FAO, 2020). This share continues to grow as concerns over declining wild fish stocks increase. Consequently, aquaculture production has accelerated, growing at an average annual rate of 5.3% from 2001 to 2018 (FAO, 2020).

Currently, 70% of aquaculture farms depend on commercially manufactured compound feeds. From 2021 to 2022, the aquafeed sector grew by 2.72%, while feed production for most other livestock species declined, with poultry (1.27%) and laying hens (0.31%) being the only exceptions (Global Feed Survey Archives, 2023). In 2010, feed costs accounted for 50–70% of total production expenses on fish farms (FAO, 2010).

Increasing costs are primarily driven by ingredients, most often attributed to the cost and inclusion of ingredients such as fish meal (El-Sayed 1999; Hardy 2006; Garza de Yta et al, 2012), creating an ongoing search for cheaper plant protein sources (Amaya et al., 2007).

DDGS in fish feed have been researched in the past; a 2012 study by Garza de Yta et al. found that DDGS were similar in growth rates for red-claw crayfish as poultry by-product and fishmeal. Pacific white shrimp had similar growth rates when fed a combination of DDGS and soybean meal (Sookying & Davis, 2012). These studies were focused on DDGS created from corn. As the number of ethanol plants that utilize sorghum has increased, there is an increasing need for more research to look at sorghum DDGS (sDDGS). A previous study by Adedeji et al (2015) found that sDDGS in diets for Pacific white shrimp could be included in levels up to 40% with no negative impacts on growth, survival and feed conversion ratio. The authors reported some pellet quality differences; bulk density, expansion ratio, pellet durability and water stability were impacted by the inclusion level of sDDGS.

The handling and transport of aquatic feeds, most often by pneumatic systems, subject pellets to significant physical stress, necessitating high pellet durability to minimize breakage and fines. Poor mechanical durability not only increases feed waste and farm costs but also exacerbates environmental impacts by introducing excess organic material into aquatic systems. In salmonid aquaculture, even a 1% loss in feed can result in financial losses of up to USD \$30 million annually and contribute as much as 30,000 tons of organic waste to surrounding waters (Haubjerg, 2016).

Extrusion has become the dominant method for producing aquatic feeds, offering several advantages over traditional techniques like pellet milling. Unlike older methods, extrusion applies significantly higher levels of thermal energy, pressure, and moisture, resulting in greater starch gelatinization (Adedeji et al., 2021; Robinette, 1977). The process

includes two main components: preconditioning and mechanical transformation via screw-driven extrusion (Riaz, 2000). In single-screw extrusion systems, most of the thermal energy input occurs during preconditioning (Monti et al., 2016; Pachecho et al., 2018), where raw ingredients are combined with steam and water. This step initiates cooking and starch gelatinization by hydrating and softening the feed material, thereby improving product quality and enhancing extruder stability (Guy, 2001; Gibson & Alavi, 2013).

The final cooking and starch gelatinization are completed in the extruder barrel through mechanical shear and pressure generated by rotating screws (Levine, 2014). Both steam conditioning and diet composition significantly influence pellet quality. For example, steam conditioning of wheat-based diets for nursery pigs increased dry matter digestibility compared to unprocessed mash. Although starch and protein digestibility remained mostly unchanged, increasing the intensity of steam conditioning led to reduced bulk density, while pellet durability index and starch gelatinization improved (Lundblad et al., 2011). Drying represents one of the most energy-intensive steps in both food and feed processing, which creates a strong need for dryer optimization (Mouhoubi et al, 2019; Akgun & Kontas, 2024). This optimization, however, must maintain balance between reducing energy consumption and preserving the desired final product quality (Haubjerg, 2016). A dryer's energy use is directly determined by its operating parameters, such as air temperature, air velocity, and the total drying time (Nwakuba et al., 2016). Research published in 2018 demonstrated that optimizing drying conditions could improve the mechanical durability of pellets, thus minimizing the creation of fines post-testing.

Specifically, pellets subjected to a more rigorous drying process, resulting in a lower final moisture content, showed a higher percentage of fines (3-5%) after durability testing. Conversely, less severe drying led to a lower percentage of fines (2-3%). The study also concluded that drying with humid air and higher temperatures yielded fewer fines compared to drying with dry air and lower temperatures (Haubjerg & Veje, 2018). Some research focuses on the drying phase following extrusion processing, notably in pasta. Drying extruded goods is complex, and the glass transition hypothesis has been applied in an attempt to connect the drying process with the resultant product quality (Li et al., 2018; Kasapis, 2005). The connection between drying and product quality has been investigated across various products, including rice, noodles, pasta, and apricot (Cnossen et al., 2001; Dai et al., 2015; Mercier 2019). It is generally proposed that employing higher temperatures and high humidity during the initial drying stage can help reduce the formation of crust on the product, thereby enhancing product quality (Dai et al., 2015).

It was hypothesized that inclusion of sorghum DDGs would significantly alter energy use during processing due to compositional differences when compared to traditional protein sources. It was hypothesized that increasing inclusion would increase bulk density, pellet durability due to reduced expansion after extrusion, and reduce floatability of the pellet. It was also hypothesized that sDDGs would reduce the overall environmental impact of aquafeed production as assessed through life cycle assessment (LCA) due to it being a by-product from ethanol production. The objective of this study was two-fold; to determine how sDDGS inclusion levels impacted the energy use during processing of these diets, specifically particle size reduction, preconditioning, and extrusion processing,

and how sDDGS impacts pellet qualities after drying. The second objective was to conduct a comprehensive LCA based off protein source and ground wheat and determine how the inclusion of sDDGS impacted the environmental impacts.

8.2 Materials and Methods

8.2.1 Experimental Design

Four treatment diets were formulated to assess the impact of different protein sources and on energy use during extrusion processing, extrudate properties, and efficiency during drying and cooling. Diets were formulated to have approximately 35-37% crude protein and approximately 8-9% fat. Diets were formulated by creating a basal mix that was kept identical across all diets, and then protein and fat levels were reached by adjusting the amount of protein source, ground whole wheat, and fish oil. Three proteins were included: fishmeal, soybean meal, and sorghum DDGs. The fishmeal-based diet was used as the control; the second diet was a blend of soybean meal and fishmeal (PP-0ddg); the third diet (PP-20ddg) was formulated with 20% sorghum DDG; and a third diet (PP-40ddg) was formulated with 40% sorghum DDG. Dietary formulations are shown in Table 8.1. A second experiment was created using the PP-20ddg diet based on preconditioner inputs. PP-20ddg-60/40 was processed similarly to the other diets with a ratio of 60:40% for water and steam, respectively, injected in the preconditioner PP-20ddg-66/33 had a higher ratio of water to steam input during preconditioning, while PP-20ddg-23/77 had a higher ratio of steam to water input during preconditioning. In-barrel moisture content was kept constant at approximately 30%.

8.2.2 Raw Materials

Ingredients were procured through a local feed mill (Fairview Mills, Seneca, KS, USA). The sorghum DDGs were donated by the Sorghum Checkoff program. The Emphyreal 75 – Corn Protein Concentrate was procured through Cargill, Inc (Wayzata, MN, USA). Protein (AOAC 990.03, nitrogen combustion method), fat (AOAC 920.39, ether extract method), ash (AOAC 942.05), and total dietary fiber (AACC method 32-07) were analyzed through proximate analysis conducted by Midwest Laboratories (Omaha, NE, USA).

8.2.3 Grinding, Energy Collection, and Mixing

The whole wheat grains ground using a pilot-scale hammer mill (Model D Comminuter, Fitzpatrick, Westwood, MA, USA). Grain grinding was done using a screen with a 1.65 mm hole size. The dry ingredients were mixed in a double-ribbon blender (Wenger Manufacturing, Sabetha, KS, USA). Diets were mixed for 3 minutes for uniformity. Post mixing, all diets were then ground through the same hammermill with a 1.02 mm screen to provide a uniform particle size within each diet. Energy use during grinding and mixing was measured using a three-phase power quality logger (Fluke Corporation, Everett, WA, USA). Based on the energy usage rate and throughput, energy use in kJ/kg was calculated for both grain- and post-batch grinding.

8.2.4 Particle Size Analysis

Particle size analysis was performed using a rotating-tapping sieve stack (Ro-Tap, Tyler Co., Mentor, OH, USA) according to the ASAE S319.2 standard method (ASAE, 1995) to determine the fineness of feed materials through sieving. Flow agent and sieve agitators (brushes and rubber balls) were used. The Ro-Tap test utilizes a sieve stack with

decreasing screen sizes; it begins with a #6 screen and ends with a #270 screen. One hundred grams of material is placed in the top sieve. The shaker is then turned on for ten minutes. The remaining material on each sieve is measured and the geometric mean diameter (dgw) and the geometric standard deviation (sgw), as well as the percentage of particles that pass through each sieve, are calculated. The dgw is calculated in microns, while the sgw is unitless; multiplying the dgw by the sgw will give you the dgw for the 84th percentile, while dividing by sgw will give you the 16th percentile.

8.2.5 Rapid visco analysis

The pasting properties of formulations were studied using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Waltham, MA, USA) by measuring viscosity changes in slurry form during a controlled heating and cooling process with the application of moderate shear. AACC standard method 76-21.02 was followed for the test (AACC Method 76-21.02 STD1). Briefly, ~3g of each sample was combined with ~25 ml deionized water to create a 14% (w/v basis) suspension and placed in the RVA. Stirring was carried out throughout the test as specified in the standard. The temperature cycle involved holding at 50°C for 1 min, heating 95°C at 12.2°C per min, followed by holding again at 95°C for 2.5 min, and cooling to 50°C at 11.8°C per min before a final holding period for 2 min. Parameters such as pasting temperature and peak viscosity were recorded using the pasting profiles that were generated from each test. RVA analyses were conducted in duplicate for each formulation.

8.2.6 Feed Rate Calibration

Prior to extrusion, flow rate of the Fishmeal diet, PP-0ddg and PP-40ddg formulations delivered by the volumetric feeding system of the extruder were calibrated at three feeder screw speeds, 8, 11, and 14 rpm. Measurements were done in duplicate and averaged over both high and low fill levels of the live-bottom feed hopper and are shown in Table 8.2. Formulations were processed using a 37.3 kW (50 hp) pilot-scale single screw extruder (X-20, Wenger Manufacturing, Sabetha, KS, USA) with a screw diameter of 82.6 mm (3.25 in) and L/D ratio of 8:1. The extruder barrel consisted of 6 heads divided into three heating zones, with the inlet head having a smooth liner and rest of the heads having spiral liners. The screw profile (Figure 8.1) had single-flighted full pitch screws in the feed intake section, transitioned to double-flighted half pitch screws for creating compression, and ended with a cone screw at the discharge for directing flow into the die opening. Two spacers and two steam locks were interspersed between the screw elements to provide resistance to flow, increase material fill and lead to desired mechanical energy input.

8.2.7 Extrusion Processing

During the experiment, the hopper was filled completely and allowed to run down to the 'low' level before being refilled with material. The feeder screw speed was set at 12-13 rpm, which corresponded to dry mash feed rate of 101.70-108.05 kg/h as obtained from the calibration for each formulation. The mash was conditioned to approximately 86-89°C in a Wenger model 2 DDC pre-conditioner, with water and steam injection at 16.15-17.00 kg/h and 10.25-10.40 kg/h, respectively. Water was injected into the extruder barrel at an average rate of 4.3 kg/h, to achieve an in-barrel moisture of approximately 30% wb. The

extruder barrel temperatures were set at 40, 70 and 90°C from inlet to discharge, and the screw speed at 482 rpm. Water and steam usage for barrel heating was collected by using two flow meters at the inlet and the discharge (GPI 01N31GM electronic water meter). Die temperature was collected during extrusion with a probe inserted into the material flow area 5 cm (approximately 1.97 inches) behind the die exit. A die with five-2.50 mm openings was used. Upon exiting the die, the product was cut using 6 hard knives. A thermocouple probe was inserted into the material stream immediately downstream of the discharge screw to measure the die temperature. Process data were recorded manually at the beginning and end of each run period of 15 minutes. The extruded feeds were conveyed using a negative pressure pneumatic system to a pilot-scale Wenger series 4800 two-pass gas-fired continuous dryer and single-pass cooling system. Drying temperature and time were 104.4°C (220°F) and 16 min, while cooling was done with room temperature air at for 5 min.

The second experiment involved different preconditioner settings using the PP-20ddg diet. For the baseline (PP-20ddg) diet, processing conditions are described in the paragraph above. Input settings were then modified to create a high water to steam ratio (PP-20ddg-66/33) and a high steam to water ratio (PP-20ddg-23/77). Processing conditions were identical to described above except for preconditioner inputs, which were 17.85 kg/hr of water, 9.00 kg/hr of steam, and a downspout temperature of 78°C for the PP-20ddg-66/33 treatment. The PP-20ddg-23/77 treatment had 6.15 kg/hr of water, 20.65 kg/hr of steam, and a downspout temperature of 99°C.

Samples were collected at several locations: preconditioned mash at the extruder inlet; extruded pellets off the die; extruded pellets exiting the pneumatic conveying system; hot pellets exiting the dryer; and cooled pellets exiting the cooler.

8.2.8 Dryer Collection

Product was dried using a Wenger 4800 two-pass gas-fired continuous dryer with one heating zone and single-pass cooling system. Product is conveyed using a negative pressure pneumatic conveying system and discharged through a sloped oscillating spout spreader. Product is carried through the dryer and cooler on a stainless-steel woven screen belt conveyor. Ambient air, dryer exhaust air, and cooler exhaust air were collected using an Amprobe THWD-5 Relative Humidity Temperature Probe. Ambient air was collected at the dryer intake below the feed spreader, and exhaust air was collected by inserting a probe through a hole in the dryer and cooler exhausts. Readings were taken at the beginning and end of each collection period. Energy use during drying was measured using a three-phase power quality logger (Fluke Corporation, Everett, WA, USA). Product was collected off the extruder, off the spreader, off the dryer, and off the cooler for moisture content analysis as well as product temperature readings. Gas usage was determined by visual observation of the gas meter at the beginning and end of each collection. Sampling was staggered in time in order to allow for processing time to elapse; as the dryer belts take 16 minutes to convey product through the system at the chosen settings, hot pellet collection was done 16 minutes after sampling at the pneumatic conveyor. Similarly, cooler retention time was set at 5 minutes, so cooled pellet sampling was done 5 minutes after hot pellet collection. As the extruder run time was 15 minutes for

each treatment, these samples were done twice, 15 minutes apart to capture samples from the beginning and end of processing.

8.2.9 Extrudate Drying and Cooling

Product was dried using a Wenger 4800 two-pass gas-fired continuous dryer with one heating zone and single-pass cooling system. Product is conveyed using a negative pressure pneumatic conveying system and discharged through a sloped oscillating spout spreader. Product is carried through the dryer and cooler on a stainless-steel woven screen belt conveyor. Ambient air and dryer exhaust air temperature and humidity were collected using an Amprobe THWD-5 Relative Humidity Temperature Probe (Amprobe, Everett, WA, USA). Ambient air data was collected at the dryer intake below the feed spreader, and exhaust air data was collected by inserting a probe through a hole in the dryer exhaust. Volumetric flow of the exhaust air was collected at the same location. Readings were taken at the beginning and end of each collection period. Product was collected off the extruder, off the spreader, and off the cooler for moisture content analysis. Gas usage (cubic feet) was determined by visual observation of the gas meter at the beginning and end of each collection.

The total mass of material entering a system must equal the total mass exiting the system. Across all treatments, the sole input streams are the raw material, steam, and water; the sole exit streams are raw material, steam, and in the case of drying, moisture loss. Mass balancing was performed as described by Riaz (2000) and Monti, et al. (2016). The mass flow rate of the dryer is calculated through the following equation:

Dryer energy analysis was performed according to the following equation:

$$md = m_{ex}((1 - xw_{ex})/(1 - xw_d)) \quad (8.1)$$

Where md is the mass of raw material exiting the drying system, m_{ex} is the extruded material flow rate entering the dryer in kg/hr, xw_{ex} is the moisture content of the extruded product, and xw_d is the dried product moisture content. This equation was used to find the moisture loss during every step in the process; from the extruder to off the spreader conveyor and from product entering the dryer off the spreader to product exiting the dryer.

As total energy into a system must equal the total energy out, energy analysis was performed according to the following equation:

$$Q_g(\frac{kJ}{h}) + Q_{dia}(\frac{kJ}{h}) + Q_{cia}(\frac{kJ}{h}) + Q_{wprod}(\frac{kJ}{h}) = Q_{dprod}(\frac{kJ}{h}) + Q_{dea}(\frac{kJ}{h}) + Q_{cea}(\frac{kJ}{h}) + Q_{loss}(\frac{kJ}{h}) \quad (8.2)$$

Where Q_g is the energy supplied by natural gas in the dryer; Q_{dia} is the inlet air of the dryer before being heated; Q_{cia} is the inlet air of the cooler; Q_{wprod} is the extruded product entering the dryer; Q_{dprod} is the dried product exiting the cooler; Q_{dea} is the air exiting the dryer exhaust; Q_{cea} is the cooler air exiting the exhaust; Q_{loss} is the overall energy lost during the process due to air leakage and heat loss through the dryer walls.. After finding the kJ/hr values for each of the above variables, variables were then converted to a kJ/kg basis by dividing by the dry material feed rate.

Q_g was solved by the following equation, using a standard rate of 1055 kJ/cubic foot of natural gas and multiplying that by the amount of gas used in one hour.

$$Q_g(\frac{kJ}{h}) = Cubic \frac{ft}{hr} \times \frac{1055 kJ}{1 cubic ft} \quad (8.3)$$

Q_{wprod} and Q_{dprod} was solved using the following equation:

$$Q_{wprod}(\frac{kJ}{h}) = msf * Cp * ^\circ C \quad (8.4)$$

Where msf is the flow rate (kg/hr) of product entering/exiting the dryer; Cp is the specific heat of the product (found by multiplying 4.18 times the moisture content and adding 1.5 times the dry solids of the product); and °C is the temperature of product entering/exiting the dryer (assumed to be 40°C/26.7°C for all treatments).

Q_{dia} , Q_{cia} , Q_{dea} and Q_{cea} were found through the following equation:

$$Q_{dia}(\frac{kJ}{h}) = mf * \Delta H \quad (8.5)$$

Where mf is the mass feed rate of air into the system (kg/hr) and ΔH is the enthalpy of air in kJ/kg. The enthalpy of air was determined by using the measured temperature and humidity of the ambient air and the utilization of a psychometric calculator (Psychometric Calculator).

The mass feed rate of air (mf) was determined by taking the measured volumetric air flow in CFM and using the following equations:

$$Volume\ flow\ (\frac{m^3}{hr}) = CFM * .0283(\frac{m^3}{min}) \quad (8.6)$$

$$Mass\ flow\ (\frac{kg}{hr}) = Volume\ Flow * Density(\frac{kg}{m^3}) \quad (8.7)$$

The ambient air density was found by using the following equation:

$$Density\ (\frac{kg}{m^3}) = \frac{Pd*1000}{287.05*Tk} + \frac{Pv*1000}{461.50*Tk} \quad (8.8)$$

Where Pd is the partial pressure of dry air; Pv is the actual vapor pressure and Tk is the temperature of the ambient air in Kelvin.

Pv is found through the following equation:

$$P_v = RH * \left[0.61078 * \exp \left(\frac{17.27 * T_c}{T_c + 237.3} \right) \right] \quad (8.9)$$

Where RH is the relative humidity of the ambient air which is multiplied by the Tetens equation for saturation vapor pressure in brackets (Tc is the temperature in Celsius). Pd is then found by the following equation:

$$P_d = P_{atm} - P_v \quad (8.10)$$

Where Patm is the atmospheric pressure (assumed to be 101.325 kPa).

After solving for each variable, Qloss can be determined through the following equation:

$$Q_{loss} = (Q_g + Q_{dia} + Q_{cia} + Q_{wprod}) - (Q_{dprod} + Q_{dea} + Q_{cea}) \quad (8.11)$$

While the dryer efficiency can be determined through the following equation:

$$Efficiency(\%) = \frac{Q_{dprod} + Q_{dea} + Q_{cea}}{Q_g + Q_{dia} + Q_{cia} + Q_{wprod}} * 100 \quad (8.12)$$

Specific Energy Consumption (SEC) was calculated through the following equation:

$$SEC \left(\frac{MJ}{kg H_2O} \right) = \left(\frac{Q_g \left(\frac{kJ}{kg} \right)}{Xr_{kg} \left(\frac{kg}{kg} \right)} \right) \div 1000 \quad (8.13)$$

Where Qg is the energy used by natural gas per kg of material, and Xr_{kg} is kilograms of moisture removed per kilogram of material. Efficiency was also calculated using the latent heat of evaporation method through the following equation:

$$Efficiency (Latent Heat)(\%) = \left(\frac{Xr_{kg} \left(\frac{kg}{kg} \right) * 2600 \left(\frac{kJ}{kg} \right)}{Q_g \left(\frac{kJ}{kg} \right)} \right) \quad (8.14)$$

Finally, efficiency was calculated again through an energy balance using the following equations:

$$M_1 C_{p1} T_1 - M_2 C_{p2} T_2 + \Delta E_g = +M_{ad2} h_{ad2} - M_{ad1} h_{ad1} \quad (8.15)$$

Where M_1 is the mass of material entering the dryer, Cp_1 is the specific heat, and T_1 is the temperature of the product entering the dryer. $M_2Cp_2T_2$ is the mass, specific heat, and temperature of product exiting the dryer. ΔE_g is the energy contribution from gas. $M_{ad1}h_{ad1}$ is the mass and energy from air entering the dryer. Variables with a subscript of 2 are product/air/energy exiting the dryer. Using enthalpy values from a psychometric chart, the feed rate where the equation balanced itself to have zero losses or inefficiencies was found. The actual feed rate was then divided by the calculated feed rate to determine the overall efficiency of the drying system.

Finally, an overall drying and cooling system efficiency that takes into account both thermal and mechanical energy was calculated using the following equation:

$$Overall\ Efficiency(\%) = \frac{h_{fg}}{\left(\frac{E_g + E_{mech}}{M_w}\right)} \quad (8.16)$$

Where h_{fg} is the latent heat of evaporation of water at atmospheric pressure, E_g is the total gas consumed (kJ/hr), E_{mech} is the total energy input for the dryer and cooler fans, as well as the energy needed to drive the belts, and M_w is the rate of moisture removal in kg/hr.

8.2.10 Mass Balance

The total mass of material entering a system must equal the total mass exiting the system. Across all treatments, the sole input streams are the raw material, steam, and water; the sole exit streams are raw material, steam, and in the case of drying, moisture loss. Accordingly, it is possible to calculate parameters such as steam lost during preconditioning. Mass balancing was performed as described by Riaz (2000) and Monti, et

al. (2016). The mass flow rate of the preconditioner is found through the following equation:

$$m_{pc} = m_r ((1 - x_{wr})/(1 - x_{wpc})) \quad (8.17)$$

Where m_{pc} is the mass of raw material exiting the preconditioner, m_r is the raw material flow rate entering the preconditioner in kg/hr, x_{wr} is the moisture content of the raw material, and x_{wpc} is the conditioned mash moisture content. Preconditioner steam loss can be calculated through the following equation:

$$m_{slpc} = (m_r + m_{spc} + m_{wpc}) - m_{pc} \quad (8.18)$$

Where m_{slpc} is the mass flow rate of steam lost to the ambient environment, m_{spc} is the mass flow rate of steam addition, m_{wpc} is the mass flow rate of water, all in kg/hr. Steam loss is also present in the extrusion process, and can be calculated using equations 7 and 8, and the appropriate input/output streams. Total mass balance can be used to determine the actual extrusion production rate regarding material exiting the die using the following equation:

$$m_{ex} = m_r ((1 - x_{wr})/(1 - x_{wex})) \quad (8.19)$$

Where m_{ex} is the mass of the material exiting the die, and x_{wex} is the moisture content of the extrudate. From there, the final rate of product exiting the dryer can be calculated using the following equation:

$$mdry = mex ((1 - xwex)/(1 - xwdry)) \quad (8.20)$$

8.2.11 Specific Mechanical Energy and Thermal Energy

Specific mechanical energy (SME) transferred from the screw to the material during the extrusion process was calculated as described below from the power consumed by the extruder motor measured using a wattmeter (Flory et al., 2023).

$$SME \left(\frac{kJ}{kg} \right) = \frac{kW - kW_0}{mr} \quad (8.21)$$

where W is the power consumed during processing (kW), W₀ is power consumed at no load with the screw running and no material flowing through (kW) and m_f is the dry feed rate or rate of material delivered by the feeder screw in kg/sec as determined using volumetric feed delivery calibration.

Specific thermal energy (STE) input during the extrusion process was calculated for selected treatments from net heat transferred to the material due to steam absorbed during preconditioning, as follows.

$$STE \left(\frac{kJ}{kg} \right) = \frac{Q_{thermal}}{m_f} \quad (8.22)$$

where Q_{thermal} is the net rate of thermal energy or heat absorbed from steam (kJ/s), which was calculated using the below equation from the enthalpies of steam injected and unabsorbed steam lost to atmosphere at the preconditioner discharge.

$$Q_{thermal} \left(\frac{kJ}{s} \right) = (m_s \cdot h_s - m_s \cdot 4.18 \cdot T_r) - (m_{sl} \cdot h_{sl} - m_{sl} \cdot 4.18 \cdot T_r) \quad (8.23)$$

where h_s and h_{sl} are enthalpies of steam injected and steam lost to atmosphere (kJ/kg), respectively, as obtained from the steam tables, m_s and m_{sl} are rates of steam injection and loss (kg/s), respectively, the constant 4.18 is the specific heat of water at room

temperature in kJ/kg°C and T_r is room temperature (°C). The rate of steam loss from the preconditioner was calculated using mass balance as described below.

$$m_{sl} \left(\frac{kg}{s} \right) = (m_f + m_s + m_w) - m_f \left(\frac{1 - X_{wf}}{1 - X_{wpc}} \right) \quad (8.24)$$

where m_w is the water injection rate into the preconditioner (kg/s) and the second term on the right of the equation is the rate of conditioned mass exiting the preconditioner with X_{wf} and X_{wpc} representing the fractional wet basis (or as is) moisture contents of the feed material and the conditioned mass, respectively, measured using oven drying method (135°C, 2 h).

8.2.12 Bulk Density

Extruded product bulk density (g/L) was measured by recording the mass of pellets filling a 1-liter cup to the brim. Two replicate measurements were made for both wet product directly off the extruder discharge and dry product after the dryer.

8.2.13 Extrudate Characterization

Twenty replicates were measured from each treatment. Individual mass, diameter and length of each pellet were recorded. These data were used for calculating sectional expansion ratio (ER), specific length (L_{sp}) and piece density (P_d) as described below (Karkle et al., 2012).

Expansion ratio (ER) is measured by taking the square of the extrudate diameter and dividing that by the square of the die diameter. Where D_e is the extrudate diameter, and

D_d is the diameter of the die.

$$ER = \frac{D_e^2}{D_d^2} \quad (8.25)$$

Specific length is the length of one pellet divided by the weight of the same pellet.

This is recorded in millimeters per gram (mm/g).

$$L_{sp}(\frac{mm}{g}) = \frac{L_e}{m_e} \quad (8.26)$$

Where L_e is the measured length of the extrudate, and m_e is the mass of the extrudate.

Piece density of the dried pellets was calculated from the measurements collected on the individual pellets. Each pellet had its diameter (cm) and length (cm) measured (at the widest point) using digital calipers. The weight (g) was also measured. Piece density was calculated through the following equation.

$$Piece\ Density\ (\frac{g}{cm^3}) = \frac{m_e}{V_e} \quad (8.27)$$

Where m_e is the mass of the pellet and V_e is the volume of that same pellet. The volume is calculated by dividing the width in half to get the radius (r), then multiplying r^2 by π and the pellet length.

8.2.14 Floatability

Floating/sinking of the extruded pellets was determined by randomly selecting fifty pellets from the first and the last bag of each treatment and immersing them in water. The number of pellets that sank was counted every five minutes for thirty minutes. Three replicates were done per treatment.

8.2.15 Pellet Durability Index

Pellet durability index (PDI) is the standard for measuring the number of broken pellets, dust, and fines that are generated through repeated handling. PDI was tested using a Holmen NPH100 pneumatic tester. Pellets were first sieved using a #10 screen to remove broken pellets and fines. One hundred grams of sieved pellets were tested. Samples were placed in the chamber and then air-agitated for 60 seconds using 70 mbar of pressure. Upon test completion, the fines were automatically sieved from the tested sample. PDI is defined as the percentage of pellets remaining after the conclusion of the test. Three replicates were done per treatment.

8.2.16 Water Stability

Water stability of the extruded pre-test pellets was measured using a modified method of the version described by Adediji, et al. (2017). Five grams of feed were weighed and then placed in a mesh tea ball before being placed in a beaker of distilled water at room temperature. The samples were soaked for 30 minutes, removed from the container, and then dried in a convection oven for 24 hours at 90°C. Sample weight was collected post-drying, with the difference in mass between pre and post-soak weights after drying being defined as ‘water stability’, as shown in the following equation:

$$\text{Water Stability} = \frac{\text{Post soak weight} \times (1 - X_{w\text{post}})}{\text{Pre soak weight} \times (1 - X_{w\text{pre}})} \times 100 \quad (8.28)$$

Where the initial MC% is the moisture content of the dried pellet before the water stability test, and the final MC% is the moisture content of the dried pellets that underwent testing. This moisture content was determined using the standard AACC 44-19.01 method of two hours drying at 135° C (AOAC, 1999). The corrected water stability was calculated by taking

the original water stability value and multiplying it by the moisture correction factor for each test. Three replicates were done per treatment.

8.2.17 Life Cycle Assessment

In this study, a life cycle assessment from field to animal feed gate was performed to assess the environmental impacts and footprints of feed production for aquatic feed using different protein sources. Additionally, several key metrics were evaluated to determine their environmental impacts, including the amount of energy used during particle size reduction of the raw diet and the amount of thermal energy (through preconditioner steam injection) during processing, as well as energy use during drying and cooling of the product. Extrusion processing of the feed was conducted at Kansas State University; energy and water use during processing were collected. Analyzed impact categories include the global warming potential (GWP), eutrophication potential, acidification, smog formation, ozone depletion, and freshwater ecotoxicity. The study aims to analyze the environmental impacts of extruded feeds (wheat-based and sorghum-based) for aquaculture to help producers make informed decisions when considering how to reduce their environmental footprint. This study will be released to the public. Environmental impacts of sorghum and wheat were compared for the following three aquaculture systems. All life cycle inventory data for ingredients were obtained from datasets compiled in the U.S. LCA data commons.

Allocation was performed based on the physical mass ratio of dried distillers grains and ethanol produced during ethanol production. The first scenario attributed all emissions to the dried distillers grains, treating the feedstock as the primary product of

interest. The second method employed the physical allocation approach, distributing the environmental burden between the grain and the straw. This was based on their mass ratio, taking into account that dried distillers grains are a by-product of ethanol production, not the main or primary product of interest. The LCA Commons Dataset has a physical ratio of 0.5017:0.4983 for distillers grains and ethanol, respectively, built into the dataset for physical allocation. By comparing the two allocation methods, this study provides a range of environmental impact potentials and demonstrates how physical allocation, or the lack of, can distort the overall estimated impacts.

8.2.18 Statistical Analysis

Statistical analyses were performed on SAS software (SAS Institute, Inc, Cary, NC, USA). One-way ANOVA ($P < 0.05$) using the general linear model was employed to determine statistical difference between treatment means. Tukey's post-hoc test ($P < 0.05$) was performed for pairwise comparison between means after a significant ANOVA result.

8.3 Results and Discussion

8.3.1 Grinding, Energy Collection, and Mixing

Energy use was collected during grinding of the wheat grain used in the diets prior to mixing, during mixing, and then during the post-batch grinding of the mixed diets. Dietary formulations are shown in Table 8.1. Table 8.3 shows the energy used during grinding and mixing. PP-40ddg required significantly more energy for mixing than all other diets. This is most likely due to the increased mixing chamber fill due to ingredient composition which resulted in a lower bulk density of the raw material. There was a significant difference of energy use during post-batch grinding as well, with the Fishmeal diet requiring significantly

less energy during particle size reduction than the sDDG diets, and overall less total energy on kJ/kg basis (Table 8.3), most likely due to the increased amount of wheat in the diet. Whole wheat was ground prior to mixing to increase mixer uniformity; as more of the fishmeal diet ingredients had gone through the hammermill once, this in turn reduced the total amount of energy needed during the secondary post-batch grinding step

8.3.2 Particle Size

Cumulative particle size distribution is shown in Figure 8.2. The Fishmeal diet had the overall lowest particle size (233 microns). The largest was the PP-0ddg (297.5 microns), and as sDDGs inclusion increased, particle sized decreased (297.5-275.5 microns).

8.3.3 Extrusion Processing

Specific Thermal Energy (STE) facilitates the initial cooking and softening of ingredients before they enter the extrusion chamber (Karkle et al., 2012; Monti et al., 2016; Maichel, 2021; Graff et al., 2024; Chen et al., 2025). The data for these measurements are presented in Table 8.4. In the first experiment, the Fishmeal diet registered the highest steam absorption, while the PP-0ddg diet showed the lowest. As the inclusion level of soluble Distillers Dried Grains (sDDG) increased in the PP-20ddg and PP-40ddg diets, STE increased; this was associated with a corresponding decrease in Specific Mechanical Energy (SME), which subsequently led to a drop in die temperature (from 118°C to 108°C). Limited studies exist concerning specific thermal energy in aquatic feed production, though preconditioning is an integral step. Several studies on the extrusion of cat and dog food (Monti et al., 2016; Pacheco et al., 2018; Maichel, 2021; Chen et al., 2025) report STE values ranging from 161 to 430 kJ/kg; the differences observed may be due to variations in

raw material characteristics and formulation. In the second experiment, increasing the water input led to a rise in both SME and STE, most likely because the improved steam condensation allowed for better absorption. A high steam input resulted in a much greater STE, indicating a more energy-intensive process overall. The increase in sDDG inclusion suggested improved preconditioning performance, evidenced by greater steam absorption (Table 8.4).

Specific Mechanical Energy (SME) was highest in the PP-0ddgs diet and decreased as the inclusion of sDDGS increased, with die temperature correspondingly falling (118°-108°C). This finding contrasts with results from Adedeji et al. (2015), who reported that SME increased with sDDGS inclusion due to the increase in fiber content as sDDGS inclusion increased. Fiber content typically found in distillers grain can provide more resistance to flow and potentially contribute to higher SME (Karkle et al., 2012). A possible explanation for the results in this study is the reduced amount of ground wheat in the diets as sDDGS inclusion rose; increasing wheat flour and gluten can raise the SME due to higher dough viscosity and protein network formation, which increases resistance to extrusion and requires more mechanical energy to convey forward (Zhang et al., 2023). Very few studies report SME values during the extrusion of aquatic feeds (Pennells et al., 2025). Rapid Visco Analysis (RVA) showed that wheat had a lower pasting temperature (71.75°C) compared to sorghum DDGs (94.4°C). The experimental diets' pasting temperatures rose as sDDG inclusion increased (72.85° to 76.75°C). These RVA differences are consistent with the decreased starch content in the sDDG formulations, which requires a higher thermal energy input to initiate the necessary viscosity increase.

Total Specific Energy (TSE) remained similar across all treatments in Experiment 1 despite the differences observed in STE and SME, demonstrating an inverse relationship between the two metrics: as STE increased, SME generally decreased, resulting in overall similar total energy levels. In Experiment 2, increasing the water usage led to lower overall steam loss and a resultant higher TSE due to increased mechanical energy due to the raw material not being softened enough. However, while the TSE for the high steam treatment increased significantly to 570.6 kJ/kg, the associated steam loss also rose to 41% , indicating a high degree of inefficiency during that specific processing condition.

8.3.4 Extrudate Characterization

Pellet expansion characteristics are shown in Table 8.5. There were significant differences between both wet and bulk density of the diets. Wet bulk density ranged from 424.75-499.50 g/L. This is most likely attributable to the decreased inclusion amount of wheat as sDDG inclusion increased. Wheat flour's starch and gluten create more viscosity during extrusion, resulting in a higher mechanical energy and die temperature, which in turn created a more porous, expanded product. The Fishmeal diet, with the largest wheat inclusion, had the lowest bulk density and the highest radial expansion (SEI) at 2.64. PP-40ddgs had the lowest inclusion of ground wheat, the highest bulk density (499.5 g/L) and the smallest SEI 0.95. This trend was reversed for Specific Length (SL), which is an indicator of longitudinal expansion. As radial expansion (SEI), SL subsequently decreases in all treatments, mirroring each other. Piece density mirrored these trends, reflecting the bulk density trends with the Fishmeal diet having the lowest density, and the PP-40ddg diet having the highest.

Bulk density (both wet and dry), SEI, and piece density were not significantly impacted by processing conditions in the case of the PP-20ddg experiment. SL was significantly impacted with the PP-20ddg-66/33 having a significantly higher SL (291.73 mm/g) than the PP-20ddg-23/77 treatment (249.72 mm/g). This is due to the plasticizing effect of water addition; additional moisture during processing can lead to thinner viscosity, reducing radial expansion, and leading to higher longitudinal expansion. This is reflected in the SEI's (1.37 for PP-20ddg-66/33, 1.48 for PP-20ddg-23/77), despite not being significantly different. The higher pasting temperatures in sDDG-containing diets likely contributed to the reduced expansion and increased pellet density seen in those treatments. RVA analysis conducted on ground, post-dried extruded pellets revealed progressive changes in pasting behavior across treatments (Figure 8.5), reflecting the degree of starch transformation during processing. The Fishmeal diet exhibited the highest peak viscosity among all treatments. This result corresponds with its higher wheat content and lower pasting temperature, suggesting that some starch granules remained intact and retained their swelling capacity during RVA testing.

With increasing inclusion of sorghum DDGs, peak viscosity declined markedly. The PP-0ddg diet showed moderate viscosity development, while the PP-20ddg treatment had further reduced viscosity, consistent with reduced starch input and higher preconditioning energy. The PP-40ddg diet displayed almost no viscosity development, due to the very low level of starch present in the diet. This observation aligns with its high specific thermal energy input (239 kJ/kg), lowest wheat content (13.82%), and poorest floatability (0%).

8.3.5 Floating/Sinking Percentage

Floatability of the extruded diets was significantly influenced by both the inclusion level of sorghum distillers dried grains (sDDGs) and the ratio of steam/water inputs used during preconditioning. As expected with its high expansion and wheat content, the Fishmeal diet exhibited excellent floatability, maintaining 100% floating pellets over the 30-minute observation period. Inclusion of soybean meal and reduced fishmeal in the PP-0ddg resulted in a slight but statistically insignificant decline, with 90% of pellets remaining afloat. However, increasing sDDG to 20% and 40% caused a marked reduction in floatability, particularly in the PP-40ddg diet, which lost buoyancy almost immediately and showed complete sinking by 15 minutes. The PP-20ddg diets exhibited intermediate floatability, but differences emerged based on preconditioning conditions: pellets preconditioned with more water (PP-20ddgs-66/33) retained the highest float percentage at 30 minutes (56%), followed closely by the standard steam-water condition (PP-20ddgs; 52%), while the steam-dominant treatment (PP-20ddgs-23/77) floated poorly (11%).

These floatability outcomes align with physical pellet characteristics observed during processing. Correlation analysis showed that dry bulk density had an inverse relationship with floatability ($r=-0.94$), indicating that as the dry bulk density of a product increases, the floatability of the product decreases. The PP-40ddg diet exhibited the highest bulk density (499.5 g/L) and the lowest sectional expansion index (SEI = 0.95), consistent with its rapid sinking. In contrast, the fishmeal diet had the lowest density and highest SEI (2.64), corresponding with excellent buoyancy. Among the PP-20ddg diets, SEI and bulk density were similar across processing conditions, suggesting that visual or

dimensional pellet expansion alone was insufficient to predict floating behavior. However, the floatability trend suggests that higher water input in the preconditioner (PP-20ddg-66/33) may improve internal pellet porosity or starch gelatinization to a degree that enhances air entrapment and buoyancy, even when SEI remains constant. These results emphasize that both ingredient composition and preconditioning conditions jointly impact functional pellet properties such as floatability, and that subtle processing adjustments may optimize performance even at moderate inclusion levels of high-fiber co-products like sDDGs.

8.3.6 Pellet Durability Index

Pellet durability (PDI) increased significantly with higher inclusion levels of sorghum DDGs, rising from 83.86% in the Fishmeal to 91.10% in the PP-40ddgs diet ($P = 0.0178$). This trend likely reflects the combined effects of formulation and physical structure. As sDDGs replaced wheat and soybean meal in the diet, the overall fiber content increased, and starch content decreased. The reduction in wheat (from 45.67% in the Fishmeal to 13.82% in PP-40ddgs) likely lowered the overall starch availability, resulting in reduced expansion during extrusion (SEI: Fishmeal = 2.64 vs. PP-40ddgs = 0.95) and increased pellet density. These denser, less-expanded pellets inherently resist mechanical degradation more effectively, leading to higher PDI values. Additionally, the high-protein, fibrous nature of DDGs likely disrupted the internal pellet matrix, further enhancing durability by creating a more compact, less expanded pellet.

Among the PP-20ddg diets, which shared the same formulation, preconditioning conditions had a substantial impact on pellet durability. The PP-20ddgs-66/33 diet,

processed with more water in the preconditioner, achieved the highest PDI (91.46%), while the high-steam variant (PP-20ddgs-23/77) showed a significant drop to 84.57% ($P = 0.0019$). These differences mirror patterns seen in floatability, where PP-20ddgs-66/33 outperformed PP-20ddgs-23/77 (56% vs. 11% floating at 30 minutes). Despite identical formulations, the increased steam input in PP-20ddgs-23/77 may have led to excessive protein denaturation, weakening pellet cohesion and compromising both floatability and durability. A study using increasing levels of corn ddgs in European catfish diets found that as ddgs increased from 100-300 g/kg, pellet durability increased, with reported values of 93.94-95.57%, along with a corresponding increased in bulk density and decreased expansion ratio (Ilic et al., 2023). These results are in line with the results presented in this study.

It is important to note that the Holmen durability tester, as used in this study, primarily uses air agitation to initiate abrasive rubbing of the pellets, which for some common feed transport modes in aquaculture such as bulk handling or silo loading, will represent only a small part of the overall testing of the pellet's durability (Aas, et al., 2011; Haubjerg, 2016). Taken together, these results emphasize that while inclusion of high-fiber co-products like DDGs can enhance mechanical integrity, fine-tuning preconditioning moisture, particularly favoring water over steam, can preserve both floatability and structural strength, even when expansion remains low.

8.3.7 Water Stability

Water absorption and water stability results provide further context for understanding how diet formulation and processing conditions influence pellet

functionality. Water absorption decreased significantly with higher sDDG inclusion, with the PP-40ddgs diet absorbing the least water (283.20%) and the PP-0ddg diet absorbing the most (321.65%; $P = 0.004$). This trend aligns with reduced starch levels across increasing DDG diets and lower expansion indices (SEI), indicating a denser, less porous structure with fewer sites available for water uptake. Despite lower water absorption, water stability remained statistically similar among the Fishmeal, PP-0ddg, and PP-20ddg diets (all >85%), but dropped significantly in the PP-40ddg diet (79.18%; $P = 0.0019$), suggesting that excessively dense pellets may resist water uptake yet still disintegrate due to weakened internal cohesion, possibly from fiber overloading or protein structure disruption during extrusion. Within the PP-20ddg group, water absorption was again influenced by preconditioning: PP-20ddgs-23/77 absorbed significantly more water (310.89%) than PP-20ddgs-66/33 (262.67%), likely due to greater internal porosity or fissuring caused by rapid steam-driven expansion. However, water stability at 30 minutes did not differ significantly between these two (86.14% vs. 83.71%) unlike the floatability did, indicating that floating characteristics are more sensitive than water stability to fine structural changes induced by preconditioning.

These results demonstrate that floatability, pellet durability, water absorption, and water stability are interconnected but not always correlated. High sDDG levels and steam-heavy preconditioning tend to reduce expansion and increase density, improving durability but harming floatability and, at higher levels, water stability. In contrast, using more water in the preconditioner moderates density, maintains structural integrity, and improves floatability without sacrificing durability or stability. These findings underscore the

importance of both ingredient composition and thermal-moisture balance during extrusion in determining the final physical and functional quality of aquafeeds.

8.3.8 Dryer Efficiency

Input and output streams for the dryer and cooler, as well as moisture contents of the products at various points are shown in Tables 8.10, 8.11, and 8.12. The thermal and mass balance data from drying and cooling processes underscore clear patterns associated with diet composition and preconditioning conditions. Dryer efficiency peaked with the PP-20ddgs formulation (15.35%) but declined notably in the PP-40ddgs diet (12.65%), despite the latter exhibiting the highest moisture loss rate (32.09 kg/hr). This apparent contradiction between greater moisture removal and lower efficiency suggests increasing fiber content and reduced starch may slow heat penetration or trap moisture differently, complicating energy transfer. Cooler output/input ratios also rose with sDDGs inclusion, reaching 129.60% in the PP-20ddgs treatment and even exceeding 130% in PP-20ddgs-66/33. These values, output exceeding input, are physically implausible and indicate measurement discrepancies, likely stemming from air mass flow calculations, temperature-humidity sensor limits, or simplifications in estimating enthalpy. Since energy and mass should be conserved, such imbalances reinforce the limitations of current methods and signal a critical need for refined, standardized procedures in capturing thermal energy flows during the drying of extruded products.

Quantifying energy dynamics across dryer and cooler systems was foundational to the manuscript's broader life cycle and process efficiency objectives. Yet the data suggest that even well-instrumented systems struggle to close the loop between energy in and

energy out. For example, the cooler in the PP-20ddgs-66/33 system recorded a 130.60% output/input ratio, suggesting that more energy left the system than entered, physically impossible under conservation laws. Such anomalies call into question the assumptions used in modeling enthalpy (e.g., steady-state airflow, uniform temperature distribution) and highlight the sensitivity of these calculations to sensor precision and airflow estimates. Total thermal input (Q_{gas} , Q_{dia} , Q_{wprod}) should equal the sum of output streams (Q_{dprod} , Q_{dea}) plus losses (Q_{loss}), this is unprecise; Q_{loss} is either unmeasured heat sinks (e.g., metal walls, belt surfaces) or underestimations of exhaust air properties. These errors are not unique to this study but reflect a larger challenge in feed processing research: few validated models exist for real-time, continuous quantification of drying/cooling thermodynamics in pilot or industrial settings.

Product temperature data taken immediately post-drying reveal why some treatments lost moisture in the cooler while others gained it. Diets with higher off-dryer temperatures, such as PP-20ddgs (61.05°C) and PP-20ddgs-66/33 (60.4°C), exhibited net moisture *loss* in the cooler (+0.19 and +1.17 kg/hr, respectively). These hotter pellets likely entered the cooler with sufficient surface and internal heat to continue driving moisture outward, resulting in further evaporation during ambient air exposure. Their structures, moderately expanded and less dense than PP-40ddgs, likely supported vapor migration and moisture removal.

In contrast, diets like Fishmeal (59.5°C), PP-0ddgs (59°C), and PP-40ddgs (58.4°C) had marginally lower off-dryer temperatures and showed *negative* moisture loss values in

the cooler (i.e., net moisture gain). These slightly cooler pellets were more susceptible to absorbing moisture from the surrounding air.

Dryer energy consumption and post-drying moisture profiles are not just indicators of thermal process efficiency, they are deeply entangled with the physical and functional properties of the resulting pellets. Diets with higher sorghum DDGS inclusion, like PP-40ddgs, required more intense drying ($Q_{\text{gas}} = 2,837,586 \text{ kJ/hr}$) and produced pellets with the highest off-dryer moisture content (4.01%). These denser, fiber-heavy pellets also exhibited the highest dry bulk density (487 g/L) and lowest expansion ratio ($\text{SEI} = 0.95$), suggesting limited internal void space and low vapor permeability. As fiber displaces starch in these formulations, less gelatinization occurs during extrusion, leading to minimal radial expansion and tighter pellet matrices that resist heat penetration, likely necessitating more energy to achieve the same moisture removal as lower-DDGS, more porous diets.

Conversely, the Fishmeal diet, rich in wheat and fishmeal, exhibited the highest SEI (2.64) and lowest bulk density (391.5 g/L), both signs of high expansion and porous internal structure. These attributes promote faster moisture migration during drying, likely reducing the energy needed to drive water from the core of the pellet to the surface. The Fishmeal diet's lower dryer energy input ($Q_{\text{gas}} = 2,619,310 \text{ kJ/hr}$) and low off-dryer moisture (3.05%) point to a more efficient drying phase, where internal vapor channels formed during expansion facilitated rapid moisture escape.

This same structural divergence is echoed in water stability and absorption metrics. Pellets from high-DDGS diets, especially PP-40ddgs, had reduced water absorption

(283.20%) and lower water stability (79.18%), despite their denser form. This paradox of tough, dense pellets that still break down in water, suggests that the drying process may have over-hardened surface layers while leaving internal bonding compromised due to low starch or protein denaturation levels. In contrast, the Fishmeal diet absorbed more water (295.63%) and maintained higher stability (89.16%), a profile aligned stronger gelatinized matrices.

Further supporting this is the impact of preconditioning strategy in the PP-20ddgs treatments. The PP-20ddgs-66/33 pellets, conditioned with more water, retained higher off-dryer moisture (4.23%) but dried with less energy loss ($Q_{loss} = 2,591,469$ kJ/hr vs. $2,296,495$ kJ/hr in HS). These pellets had greater specific length (291.73 mm/g), moderate SEI (1.37), and higher floatability and PDI, indicating that controlled moisture at the drying input point contributed to both process efficiency and better structural integrity. In contrast, PP-20ddgs-23/77, driven by a high steam-to-water ratio, exited the dryer with lower moisture (2.28%) but exhibited poorer floatability (11%) and slightly lower water stability (83.71%), likely from over-dried or internally fractured pellets, a condition where excessive drying energy may have degraded the pellet matrix.

To better understand the drying efficiency of the current system, specific energy consumption (SEC), latent heat of evaporation, heat loss, and dryer efficiency by finding the ideal feed rate was calculated. This data is shown in Table 8.14. Comparing the fishmeal control diet to diets with increasing amounts of sDDGs showed that SEC values peaked in the PP-0ddgs diet at 94.39 MJ/kg water, and were lowest in the PP-40ddgs diet at 88.44 MJ/kg. PP-0ddgs had the lowest efficiency as measured by the latent heat of

evaporation method at 2.39%. Efficiency measured by heat loss ranged from 12.65-15.35%, with PP-20ddgs being the most efficient, and PP-40ddgs being the least. Comparing the PP-20ddgs diet produced with three different ratios of water to steam showed large differences in energy efficiency. SEC was lowest in the 60/40 ratio of water to steam during preconditioning at 89.24 MJ/kg of water; the 66/33 ratio of water to steam led to an increased SEC value of 99.96 MJ/kg, and the 23/77 ratio led to an increased value of 125.51 MJ/kg. Efficiency values as measured by latent heat loss (2.53, 2.26, and 1.80% respectively), and the real/calculated feed rate method (4.20, 3.76, and 3.05%) followed the same trends. These data are similar to results from existing literature. For instance, a 2014 study that compared energy parameters in various dryers reported SEC (MJ/kg water) values ranging from 100 to 200 MJ/kg water (Motevali et al., 2014), which is comparable to the results from this study. Dryer efficiency values from that same study ranged from 2-6.5% (Motevali et al., 2014). It was also reported that as dryer energy temperatures increased from 40°C to 60°C, SEC values decreased while efficiency increased (Motevali et al., 2014). This contrasts with the results of the current study, which experienced higher SEC values and decreased efficiency at 115°C. In a similar vein, another study in 2016 reported the energy requirement for drying 1 kg of product at different temperatures using a convective parallel airflow dryer. That study found that SEC values decreased as temperature increased (50-70°C), with values ranging from 54 to 144 MJ/kg water (Nwakuba et al., 2016).

Drying efficiency, when taking into account both thermal and mechanical energy used in both the drying and cooling systems were low. Overall efficiency ranged from 2.39-

2.56% for experiment one with increasing levels of sorghum ddgs. In experiment two with the PP-20ddg diet with different preconditioning intensities, values ranged from 2.39-1.80%, with efficiency being lowest in the 23/77 ratio diet.

Overall, drying behavior is not independent, it reflects the compounded effects of extrusion expansion, ingredient interactions, and moisture migration dynamics shaped in the preconditioner. Highly expanded pellets dry faster and more uniformly due to increased surface area and porosity, but are also more prone to cracking if overdried. Dense pellets resist moisture loss and require higher thermal input, but may hide internal cohesion weaknesses that manifest during aquatic exposure. This linkage highlights the need for holistic control, formulation, preconditioning, extrusion, and drying must be co-optimized, not isolated.

8.3.9 Life Cycle Assessment

The shift in ingredient composition across diets, from wheat and fishmeal in the Fishmeal diet to soybean meal and increasing levels of sorghum DDGS, had substantial effects on both processing energy use and environmental impact metrics. As wheat inclusion dropped from 45.67% in the Fishmeal to just 13.82% in the PP-40ddgs diet, and sDDGs rose to 40%, grinding energy requirements climbed sharply (from 126.7 to 162.12 kJ/kg), reflecting the additional input needed to size-reduce fibrous and coarse ingredients like DDGS. Extrusion energy followed a more nuanced pattern, peaking at 300.98 kJ/kg in the PP-0ddgs diet, rich in soybean meal, and dropping sharply to 236.43 kJ/kg in the PP-40ddgs treatment, consistent with lower SME observed for high-DDGS formulations. Boiler fuel and natural gas inputs fluctuated modestly, but the net energy input across grinding,

extrusion, and drying generally increased with sDDGs inclusion, reinforcing the idea that high-fiber, low-starch diets are more demanding to process thermally and mechanically.

These processing shifts were mirrored in the life cycle assessment data. Global warming potential (GWP) rose dramatically from 1204.9 kg CO₂ eq in the Fishmeal to 2097.1 kg CO₂ eq in the PP-40ddgs diet, while acidification nearly doubled (1.94 to 3.40 kg SO₂ eq), and eutrophication climbed more than sixfold (0.089 to 0.567 kg N eq). The largest difference, however, was in freshwater ecotoxicity: the Fishmeal diet registered just 15.63 CTUeco, whereas the PP-40ddgs diet skyrocketed to over 3045.6 CTUeco. These massive increases were not due to energy use alone but are driven by upstream agricultural impacts associated with DDGS production, primarily production of ethanol. These results suggest that although sDDGs provide a cost-effective alternative to fishmeal and wheat, their large-scale inclusion introduces considerable environmental trade-offs that must be carefully weighed. These are balanced, of course, by the dual nature of DDG production. DDGs are produced during the production of ethanol. An economic of physical allocation would reduce these impacts significantly; however, it is important to note, that regardless of whether or not a farm chooses to use sorghum or corn based DDGs in feed, ethanol plants will still produce DDGs as a by-product due to demand for ethanol. Sustainability is more than estimated environmental impacts; using by-products such as DDGs is a form of sustainability as it creates use out of a product that would be otherwise discarded, causing more environmental damage.

Within the PP-20ddgs treatments, variations in preconditioner settings also affected impact outcomes. The high-steam (PP-20ddgs-23/77) treatment had the lowest extrusion

energy (226.94 kJ/kg), but also consumed the most boiler fuel (0.0203 m³/kg), and exhibited the highest natural gas input (20.25 cf/kg). Despite its low SME, the increased steam consumption raised the total energy demand, which translated to relatively higher GWP (1681.01 kg CO₂ eq) compared to the high-water (PP-20ddgs-66/33) treatment (1658.94 kg CO₂ eq). Conversely, PP-20ddgs-66/33 used more electrical energy during extrusion (294.59 kJ/kg) but less boiler fuel (0.0089 m³/kg), suggesting more efficient heat transfer and better steam retention. This ultimately lowered its environmental impacts across most categories, reducing acidification (2.762 kg SO₂ eq), eutrophication (0.330 kg N eq), and smog formation (61.10 kg O₃ eq) compared to the HS variant.

Notably, freshwater ecotoxicity remained essentially unchanged across all PP-20ddgs variants (~1531 CTUeco), indicating that ingredient composition, not processing energy, was the primary driver for this category. This highlights a critical trade-off in formulation and processing design: while altering steam-to-water ratios during preconditioning can marginally reduce emissions and energy use, the overwhelming environmental load originates from the raw ingredient inventory itself, especially the heavy reliance on soybean meal and DDGS.

When physical allocation is applied, distributing the environmental burden between ethanol and DDGS based on their respective mass contributions, the narrative around DDGS inclusion shifts dramatically. Under this allocation, global warming potential (GWP) for the PP-40ddgs diet drops from 2097.08 to 1587.86 kg CO₂ eq, a 24% reduction. Likewise, freshwater ecotoxicity is halved, falling from 3045.58 to 1535.02 CTUeco. Similar trends emerge for PP-20ddgs, where GWP drops from 1967.91 to 1513.30 kg CO₂ eq and

ecotoxicity from 1534.94 to 779.67 CTUeco. These reductions highlight that the previously reported surges in environmental burden were not inherent to the feed formulations themselves but stemmed from an attribution method that placed the full weight of ethanol-related emissions on the DDGS component.

Compared to the PP-0ddgs diet, which had a GWP of 1232.13 kg CO₂ eq and freshwater ecotoxicity of just 16.24 CTUeco even without allocation, these rebalanced figures for 20s and PP-40ddgs still represent higher impacts, but the magnitude of difference is less severe and could be minimized by more accurate physical allocation, as opposed to using the default values in the US Commons Database. More importantly, when considering that DDGS are by-products of ethanol production, physical allocation provides a more realistic estimate of their true marginal burden. Notably, while PP-40ddgs still carries higher GWP and ecotoxicity than the Fishmeal or PP-0ddgs diets, the gap narrows enough to justify DDGS use from a circular economy standpoint, transforming a high-burden narrative into one of shared responsibility and resource recovery.

Processing method comparisons under physical allocation (Table 8.17) reinforce this view. The PP-20ddgs-66/33 treatment, with high water and moderate steam, achieved the lowest GWP (1404.33 kg CO₂ eq) and acidification (2.37 kg SO₂ eq), while maintaining ecotoxicity near 775 CTUeco. These values not only outperform the PP-20ddgs diet but also come within striking distance of the PP-0ddgs impacts, especially when weighed against the functional advantages of DDGS in pellet durability and density.

Incorporating these allocation-adjusted results clarifies that the environmental cost of DDGS use is not fixed, but sensitive to modeling assumptions. When burdens are split

with ethanol, a product for which DDGS would be generated regardless, the sustainability case for DDGS-enriched aquaculture diets becomes much stronger. This reframing highlights how methodological nuance in LCA can materially shift the outcome of sustainability assessments, and underscores the importance of treating co-products like DDGS not as environmental liabilities, but as strategic assets in feed design.

8.4 Conclusion

Inclusion of sorghum DDGs in aquaculture diets introduced notable shifts in both processing behavior and end-product quality. Higher sDDG levels improved pellet durability through reduced expansion and increased density, yet simultaneously decreased floatability and water stability, two functional traits critical for performance in aquatic environments. Preconditioning strategies provided a key tool for managing these trade-offs: high water input enhanced structural cohesion and floatability without compromising energy efficiency, while excessive steam addition undermined both.

From an energy standpoint, increased sDDG inclusion raised demands during grinding and drying due to higher fiber content and moisture removal needs. However, extrusion-specific mechanical energy (SME) decreased, balancing total specific energy use across treatments. This highlights the potential to maintain energy efficiency while modifying physical quality through formulation and process controls.

Environmental assessments, however, added complexity. Initially, life cycle impacts, especially global warming potential (GWP) and freshwater ecotoxicity, rose sharply with increasing sDDG levels due to the full attribution of ethanol production burdens to DDGS in the default approach. Under this method, the PP-40ddgs diet reached

2097.08 kg CO₂ eq GWP and 3045.58 CTUeco in freshwater ecotoxicity. However, applying physical allocation, distributing impacts between ethanol and DDGS by mass, dramatically reshaped the narrative. GWP for the PP-40ddgs diet dropped 24% to 1587.86 kg CO₂ eq, and ecotoxicity was halved to 1535.02 CTUeco. Similar trends were seen in the PP-20ddgs diets, with GWP falling from 1967.91 to 1513.30 kg CO₂ eq and ecotoxicity from 1534.94 to 779.67 CTUeco.

Compared to the PP-0ddgs diet (1232.13 kg CO₂ eq GWP and 16.24 CTUeco), DDGS diets still carry higher impacts, but the gap narrows considerably under physical allocation. More notably, the PP-20ddgs-66/33 treatment, with high water and moderate steam, achieved the lowest impacts among sDDG diets (GWP of 1404.33 kg CO₂ eq, acidification of 2.37 kg SO₂ eq, and ecotoxicity of ~775 CTUeco), approaching the performance of PP-0ddgs while offering superior physical pellet characteristics depending on the desired pellet qualities.

These findings illustrate that the environmental cost of DDGS use is not fixed but highly sensitive to life cycle modeling assumptions. When burdens are shared with ethanol, a product whose production inherently generates DDGS, the environmental burdens caused from ethanol production and DDGs decreases dramatically. This underscores the importance of methodological nuance in life cycle assessments, and positions DDGS not as liabilities, but as potential co-products supporting circularity, resource recovery, and feed innovation in aquaculture.

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8.6 Figures and Tables

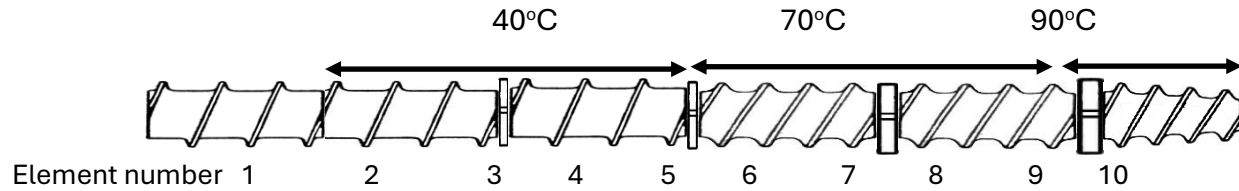


Figure 8.1: Pilot-scale single screw extruder screw profile and set barrel temperatures

1= inlet screw; 2 =single flight full pitch screw; 3=small steam lock; 4=single flight full pitch screw; 5=small steam lock; 6=double flight half pitch screw; 7=medium steam lock; 8=double flight half pitch screw; 9=large steam lock; 10=double flight half pitch cone screw.

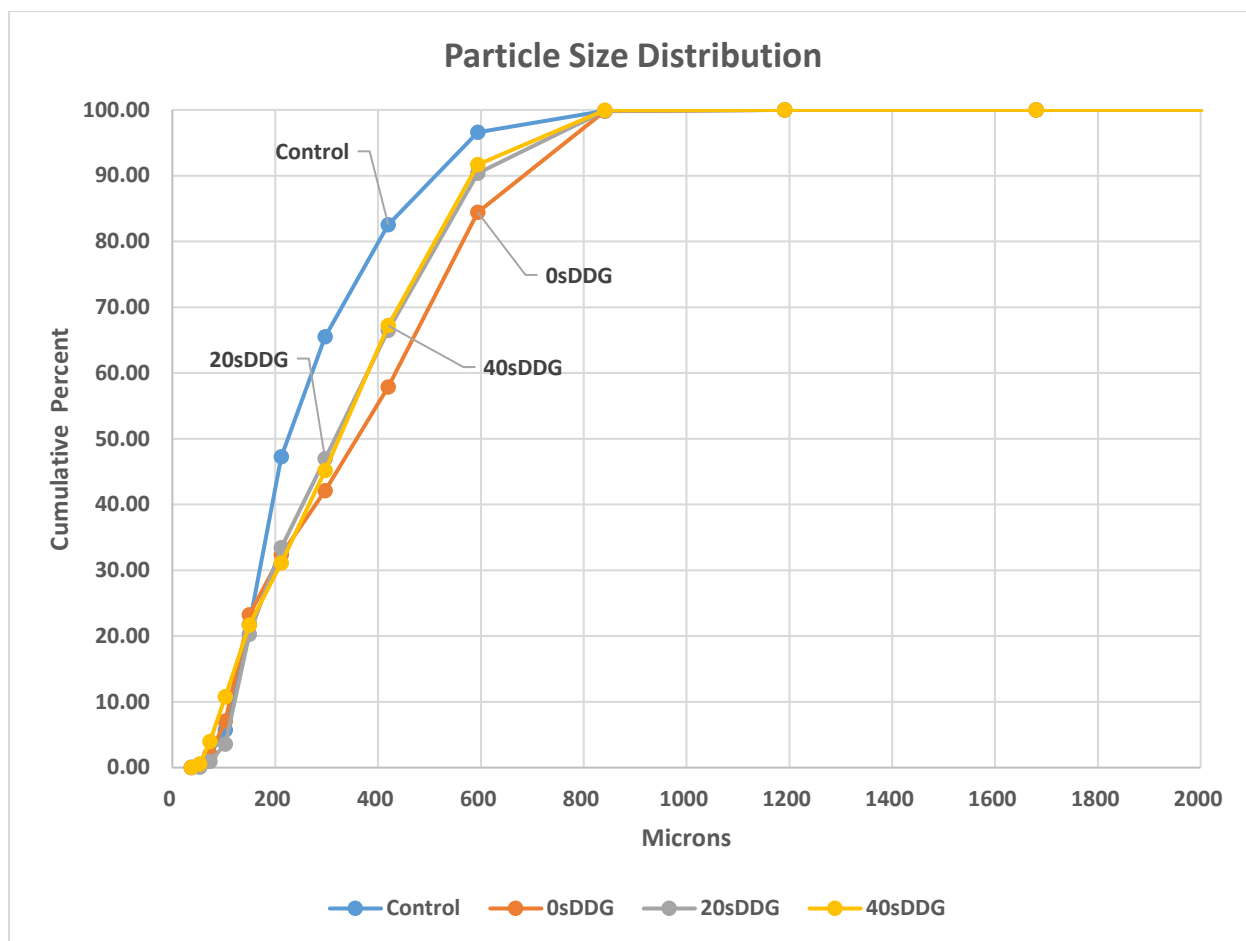


Figure 8.2: Cumulative Particle Size Distribution of Ground Diets

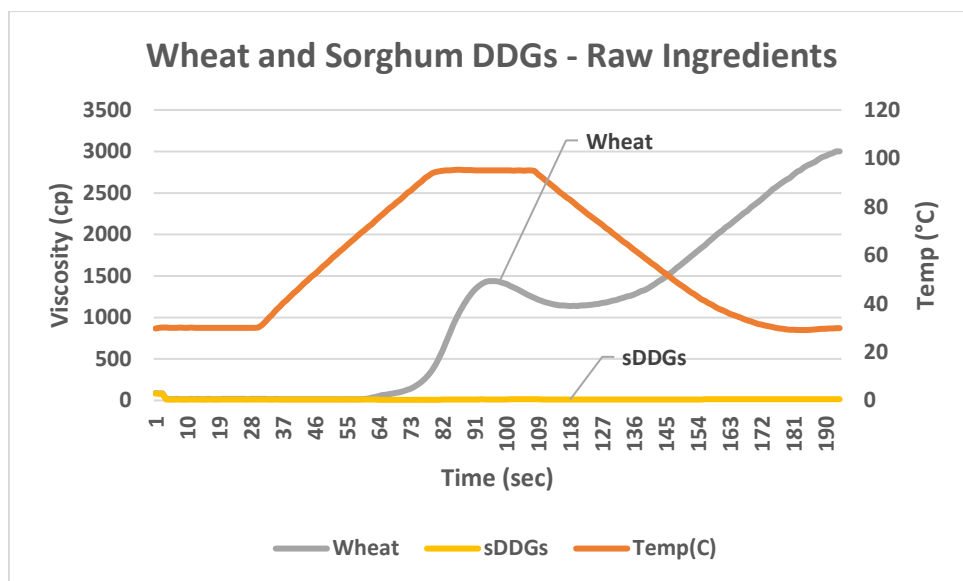
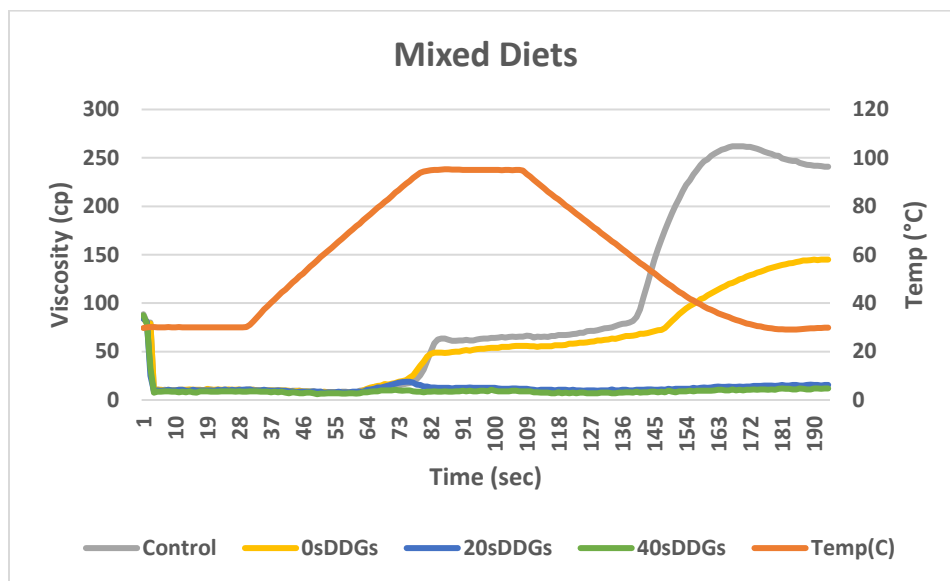


Figure 8.3: Average of RVA Curves for Raw Ingredients

(a)



(b)

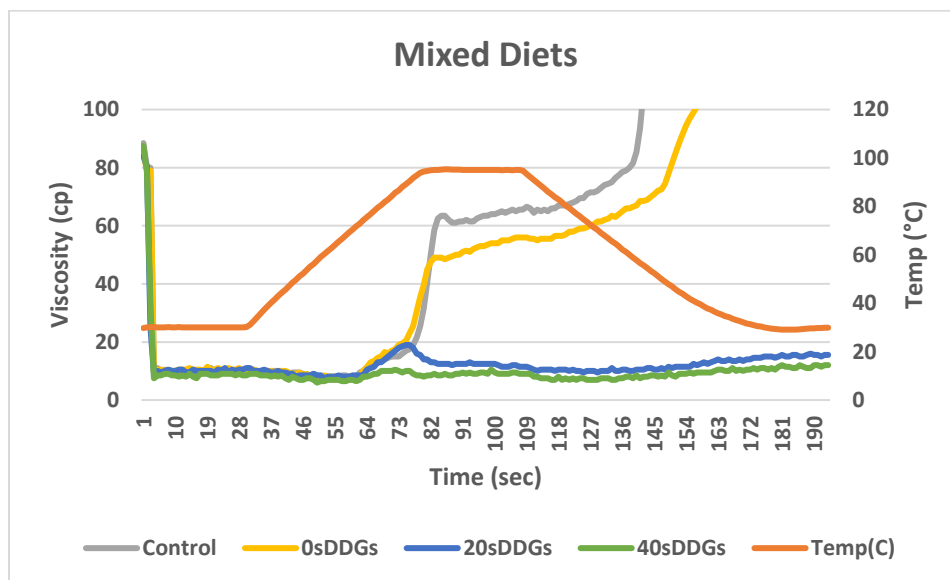


Figure 8.4: RVA Curves for Mixed Diets

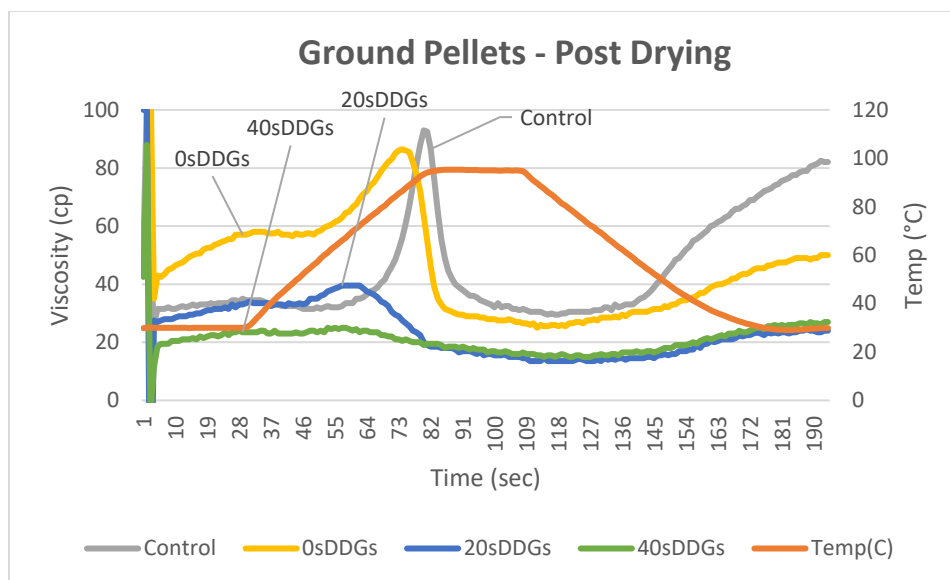


Figure 8.5: RVA Curves for Extruded Pellets with Different Protein Sources

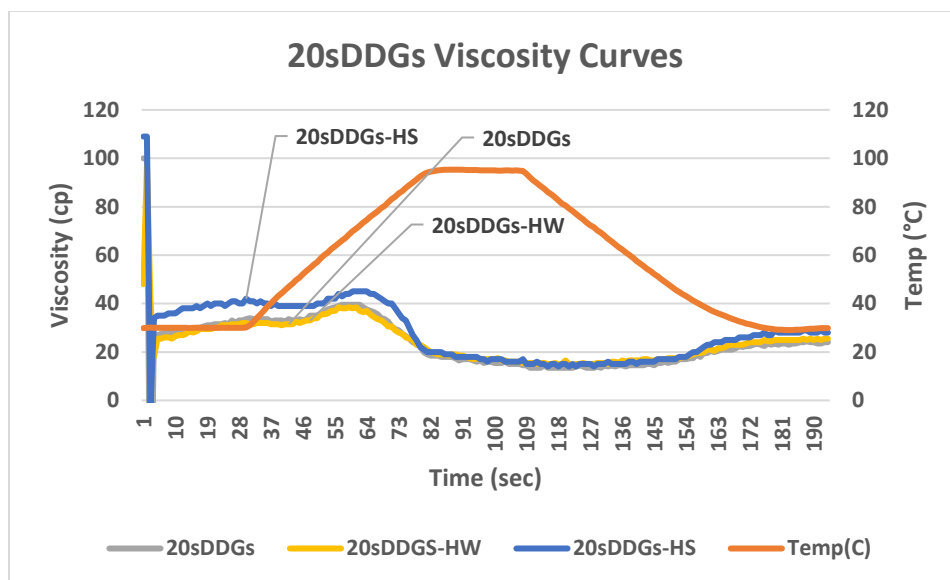


Figure 8.6: RVA Curves for PP-20ddgs Pellets with Different Preconditioning Inputs

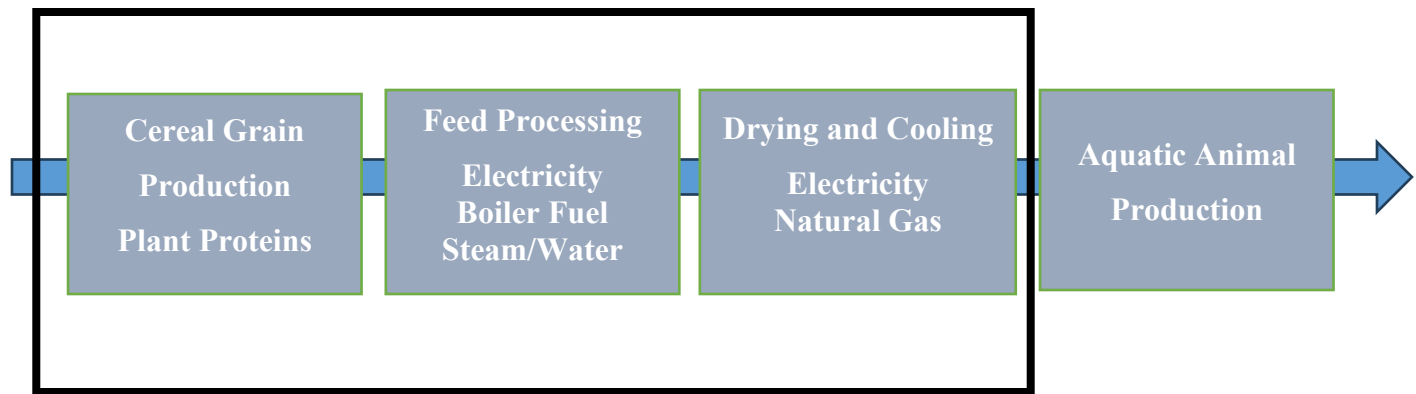


Figure 8.7: System Boundary for LCA

Table 8.1: Dietary formulations

Formula	Fishmeal	PP-0ddg	PP-20ddg	PP-40ddg
Fishmeal	38	6	6	6
Soybean Meal	0	44	34	21.5
Sorghum DDG's	0	0	20	40
Corn Protein Concentrate	9	9	9	9
Soy Oil	3.52	3.52	3.52	3.52
Soy Lecithin	1	1	1	1
Wheat	45.67	31.82	21.32	13.82
Mineral Premix	0.07	0.07	0.07	0.07
Vitamin Premix	0.04	0.04	0.04	0.04
Choline Chloride	0.2	0.2	0.2	0.2
Rovimix Stay C	0.1	0.1	0.1	0.1
Cap-dibasic	1.75	1.75	1.75	1.75
Fish oil	0.65	2.5	3	3
Total	100	100	100	100

Table 8.2: Energy use during mixing and grinding

Diet/Grain	Mixing (kJ/kg)	Grain Grinding (kJ/kg)	Post Batch Grinding (kJ/kg)	Total Energy (kJ/kg)
Fishmeal	1.230 ± 0.018a	70.366	55.104 ± 5.134a	126.700
PP-0ddg	1.257 ± 0.014ab	70.366	91.053 ± 0.554b	162.676
PP-20ddg	1.227 ± 0.019a	70.366	90.275 ± 3.879b	161.867
PP-40ddg	1.291 ± 0.024b	70.366	90.464 ± 2.572b	162.120
PSE	0.011		2.034	
P-value	0.0076		<0.0001	

Table 8.3: Energy use during extrusion processing

Diet	STE (kJ/kg)	PC Temp °C	SME (kJ/kg)	TSE (kJ/kg)	Die Temp (°C)	PC Steam Loss (%)
Fishmeal	221.7	89.5	232.4	454.1	115	13
PP-0ddgs	182.8	86	279	461.8	118	35
PP-20ddgs	208.2	89	256.8	465	115	27
PP-40ddg	239	89	213.8	452.8	108	6
PP-20ddgs- 60/40	208.2	89	256.8	465	115	27
PP-20ddgs- 66/33	210.6	78	271.9	482.5	111.5	7
PP-20ddgs- 23/77	366.1	99	204.5	570.6	117	41

Table 8.4: Bulk Density of Extruded Feed

Diet	Wet Bulk Density (g/L)	Dry Bulk Density (g/L)
Fishmeal	424.75 ± 7.42b	391.50 ± 5.66c
PP-0ddgs	443.00 ± 16.97b	420.00 ± 4.24b
PP-20ddgs	462.50 ± 14.85ab	436.25 ± 7.78b
PP-40ddg	499.50 ± 2.12a	487.00 ± 4.95a
PSE	8.43	3.25
P-value	0.013	0.0001
PP-20ddgs-60/40	462.50 ± 14.85	436.25 ± 7.78
PP-20ddgs-66/33	477.50 ± 3.54	438.00 ± 5.65
PP-20ddgs-23/77	450.00 ± 1.41	455.50 ± 13.44
PSE	6.26	6.09
P-value	0.1151	0.189

Table 8.5: Expansion Characteristics of Extruded Feed

Diet	SEI	Specific Length (cm/g)	Piece Density (g/cm ³)
Fishmeal	2.64 ± 0.55a	15.70 ± 2.12d	0.81 ± 0.16b
PP-0ddgs	1.86 ± 0.41b	21.87 ± 5.77c	0.83 ± 0.13b
PP-20ddgs	1.39 ± 0.25c	27.41 ± 4.37b	0.87 ± 0.16b
PP-40ddg	0.95 ± 0.24d	34.12 ± 4.64a	1.04 ± 0.20a
PSE	0.09	9.9	0.04
P-value	<0.0001	<0.0001	0.0001
PP-20ddgs-60/40	1.39 ± 0.25	27.41 ± 4.37ab	0.87 ± 0.16
PP-20ddgs-66/33	1.37 ± 0.21	29.17 ± 4.46a	0.82 ± 0.10
PP-20ddgs-23/77	1.48 ± 0.28	24.93 ± 5.77b	0.91 ± 0.14
PSE	0.06	10.97	0.03
P-value	0.325	0.0288	0.1229

Table 8.6: Floating Percentage of Extruded Pellets

	Minutes					
	5	10	15	20	25	30
Fishmeal	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
PP-0ddg	91 ± 4.2a	90 ± 2.8a	90 ± 2.8a	90 ± 2.8a	90 ± 2.8a	90 ± 2.8a
PP-20ddg	61 ± 7.1b	58 ± 5.7b	57 ± 7.1b	57 ± 7.1b	55 ± 9.9b	52 ± 8.5b
PP-40ddg	4 ± 2.8c	3 ± 1.4c	0 ± 0.0c	0 ± 0.0c	0 ± 0.0c	0 ± 0.0c
PSE	3.08	2.29	2.69	2.69	3.64	3.16
P-Value	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
PP-20ddgs-60/40	61 ± 7.1a	58 ± 5.7a	57 ± 7.1a	57 ± 7.1a	55 ± 9.9a	52 ± 8.5a
PP-20ddgs-66/33	74 ± 2.8a	64 ± 0.0a	63 ± 1.4a	59 ± 1.4a	59 ± 1.4a	56 ± 2.8a
PP-20ddgs-23/77	15 ± 4.2b	13 ± 4.2b	12 ± 2.8b	12 ± 2.8b	12 ± 2.8b	11 ± 1.4b
PSE	3.56	2.89	3.16	3.16	4.24	3.7
P-Value	0.0027	0.002	0.0026	0.003	0.0075	0.0057

Table 8.7: Pellet Durability Index

Diet	PDI%
Fishmeal	83.86 ± 2.50b
PP-0ddgs	88.21 ± 0.27ab
PP-20ddgs	90.71 ± 0.56a
PP-40ddg	91.10 ± 0.84a
PSE	0.956
P-value	0.0178
PP-20ddgs-60/40	90.71 ± 0.56a
PP-20ddgs-66/33	91.46 ± 0.35a
PP-20ddgs-23/77	84.57 ± 0.64b
PSE	0.374
P-value	0.0019

Table 8.8: Water Stability of Extruded Pellets

	Water Absorption%	Water Stability %
Fishmeal	295.63 ± 6.73b	89.16 ± 0.39a
PP-0ddgs	321.65 ± 5.00a	87.95 ± 0.36a
PP-20ddgs	284.99 ± 3.00b	85.28 ± 1.92a
PP-40ddg	283.20 ± 3.54b	79.18 ± 0.06b
PSE	3.39	0.7
P-value	0.004	0.0019
PP-20ddgs-60/40	284.99 ± 3.00b	85.28 ± 1.92
PP-20ddgs-66/33	262.67 ± 4.58c	86.14 ± 0.49
PP-20ddgs-23/77	310.89 ± 1.53a	83.71 ± 1.25
PSE		
P-value	0.0016	0.3281

Table 8.9: Dryer Input and Output Energy Streams (kJ/kg)

Dryer Input Streams				Dryer Output Streams			
Diet	Qgas	Qdia	Qwprod	Qdea	Qdprod	Qloss	Dryer Efficiency (%)
Fishmeal	24370.22	1305.60	108.74	3644.70	100.43	22039.44	14.52
PP-0ddgs	24567.48	1239.87	110.89	3539.40	100.27	22278.57	14.04
PP-20ddgs	24896.76	1380.15	120.61	3943.11	109.67	22344.73	15.35
PP-40ddg	27562.76	1264.32	113.81	3556.82	104.58	25279.50	12.65
PP-20ddgs-60/40	24896.76	1380.15	120.61	3943.11	109.67	22344.73	15.35
PP-20ddgs-66/33	27901.53	1374.91	119.93	3804.98	109.89	25481.51	13.32
PP-20ddgs-23/77	24896.76	1392.50	108.70	3713.45	103.43	22581.07	14.46

Table 8.10: Cooler Input and Output Energy Streams (kJ/kg)

Cooler Input Streams			Cooler Output Streams		
Diet	Qcia	Qdprod	Qcea	Qcprod	Output/Input (%)
Fishmeal	2176.01	100.43	2421.46	44.84	108.34
PP-0ddgs	2066.45	100.27	2550.44	52.66	120.14
PP-20ddgs	2300.25	109.67	3075.23	48.08	129.6
PP-40ddg	2107.21	104.58	2437.65	49.82	112.46
PP-20ddgs-60/40	2300.25	109.67	3075.23	48.08	129.6
PP-20ddgs-66/33	2291.52	109.89	3088.47	47.84	130.6
PP-20ddgs-23/77	2320.84	103.43	2734.34	45.62	114.67

Table 8.11: Moisture Content of Extruded Product throughout Drying and Cooling

Diet	Off-Extruder MC%	Off-Spreader MC%	Off-dryer MC (%)	Off-Cooler MC%	Dryer Moisture Loss (kg/kg)	Cooler Moisture Loss (kg/kg)
Fishmeal	25.33	24.82	3.05	3.87	0.27	-0.01
PP-0ddgs	25.02	24.66	3.77	4.13	0.26	0.00
PP-20ddgs	25.98	25.55	3.47	3.28	0.28	0.00
PP-40ddg	28.02	27.85	4.01	4.27	0.31	0.00
PP-20ddgs- 60/40	25.98	25.55	3.47	3.28	0.28	0.00
PP-20ddgs- 66/33	26.64	26.01	4.23	3.05	0.28	0.01
PP-20ddgs- 23/77	21.44	19.47	2.28	2.2	0.20	0.00

Table 8.12: Product Temperature during Drying

Diet	Product Temperature	
	Off Spreader (°C)	Off Dryer (°C)
Fishmeal	38.6	59.5
PP-0ddgs	39.65	59
PP-20ddgs	40.15	61.05
PP-40ddg	37.3	58.4
PP-20ddgs-60/40	40.15	61.05
PP-20ddgs-66/33	39.7	60.4
PP-20ddgs-23/77	39.1	58.75

Table 8.13: Comparison of different methods for calculating dryer efficiency

Diet	SEC (MJ/kg water)	Efficiency % (Latent Heat)	Efficiency % (Heat Loss)	Efficiency % (Real/Calculated Feed Rate)
Fishmeal	89.42	2.53	14.52	4.17
PP-0ddgs	94.39	2.39	14.04	3.97
PP-20ddgs	89.24	2.53	15.35	4.20
PP-40ddgs	88.44	2.56	12.65	4.27
PP-20ddgs-60/40	89.24	2.53	15.35	4.20
PP-20ddgs-66/33	99.96	2.26	13.32	3.76
PP-20ddgs-23/77	125.51	1.80	14.46	3.05

Table 8.14: Efficiency of the overall Drying and Cooling System using Thermal and Mechanical Energy

Diet	Overall Efficiency (%)
Fishmeal	2.53
PP-0ddgs	2.39
PP-20ddgs	2.53
PP-40ddgs	2.56
PP-20ddgs-60/40	2.39
PP-20ddgs-66/33	2.26
PP-20ddgs-23/77	1.80

Table 8.15: Energy Input Data for LCA per Kilogram of Raw Material

Diet	Grinding and Mixing (kJ/kg)	Specific Mechanical Energy (kJ/kg)	Boiler Fuel (m3/kg)	Dryer - Natural Gas (CF/kg)	Dryer and Cooler Electricity (kJ/kg)
Fishmeal	126.70	255.47	0.0097	17.80	0.05
PP-0ddgs	162.68	300.98	0.0096	19.42	0.05
PP-20ddgs	161.87	278.99	0.0102	18.72	0.05
PP-40ddg	162.12	236.43	0.0100	19.48	0.05
PP-20ddgs- 60/40	161.87	278.99	0.0102	18.72	0.05
PP-20ddgs- 66/33	161.87	294.59	0.0089	20.16	0.05
PP-20ddgs- 23/77	161.87	226.94	0.0203	20.25	0.05

Table 8.16: LCA Results for sDDGs-Based Diets with Physical Allocation

Impact category	Reference unit	PP-20ddgs	PP-40ddg
Acidification	kg SO2 eq	2.80	2.62
Eutrophication	kg N eq	0.23	0.33
Freshwater ecotoxicity	CTUeco	779.67	1535.02
Global warming	kg CO2 eq	1513.30	1587.86
Human health - particulate matter	PM 2.5 eq	0.08	0.08
Smog formation	kg O3 eq	68.88	58.50

Table 8.17: LCA Results for Diets with Different Preconditioning Intensities and Physical Allocation

Impact category	Reference unit	PP-20ddgs-60/40	PP-20ddgs-66/33	PP-20ddgs-23/77
Acidification	kg SO ₂ eq	2.80	2.37	2.34
Eutrophication	kg N eq	0.23	0.21	0.21
Freshwater ecotoxicity	CTUeco	779.67	775.61	775.82
Global warming	kg CO ₂ eq	1513.30	1404.33	1426.40
Human health - particulate matter	PM 2.5 eq	0.08	0.07	0.07
Smog formation	kg O ₃ eq	68.88	55.31	56.03

Chapter 9 - Conclusions

The overarching goal of this dissertation was to comprehensively assess the impact of various raw ingredient choices, their physical characteristics, and different extrusion processing parameters on the efficiency of production, physical quality, nutritional performance, and environmental sustainability of extruded aquafeeds. This research focused on key aquaculture species: Nile tilapia, Pacific white shrimp, and rainbow trout. A central theme was the investigation of sorghum and its derivatives as sustainable alternatives to traditional ingredients like wheat and fishmeal.

The studies systematically explored factors such as ingredient particle size, thermal and mechanical energy inputs during extrusion and drying, and variations in grain type and composition. The aim was to identify optimal processing conditions that maximize pellet quality, feed digestibility, and animal growth performance, while simultaneously minimizing feed costs and environmental impacts. By integrating processing trials with growth and digestibility assessments, energy balance analyses, and cradle-to-gate life cycle assessments, this research provides a holistic understanding of how feed formulation and manufacturing choices interact across the entire production chain.

Across multiple chapters, this research demonstrated how ingredient choice and processing variables interact to influence feed quality and extrusion efficiency. Chapter 2 established that sorghum, regardless of grind size, enhanced physical pellet qualities such as durability and reduced water absorption in floating tilapia feeds, often outperforming wheat. Chapter 3 showed that optimizing preconditioner thermal energy is essential for sorghum-based shrimp feeds, as lower thermal inputs reduced extrusion energy but led to

suboptimal starch gelatinization and water stability, especially in sorghum, which required more heat to reach ideal functional properties due to its dense protein matrix.

Chapter 5 extended the processing analysis to include waxy and non-waxy sorghum variants in rainbow trout feeds. Though all sorghum types supported acceptable physical pellet traits, variations in starch structure and grain hardness influenced extrusion dynamics and feed intake, with low amylose varieties showing potential for further optimization. Chapter 8 revealed that increasing sDDG inclusion significantly altered extrusion behavior, with impacts on pellet expansion, floatability, and durability.

Adjustments to preconditioning showed promise in mitigating some of the processing challenges posed by high sDDG levels, reinforcing the importance of tuning processing parameters when incorporating alternative proteins. For all aquatic species researched (tilapia, shrimp, and trout), sorghum-based diets consistently supported comparable, and sometimes superior, animal performance relative to wheat-based controls. Chapter 2 found that although particle size influenced pellet physicality, it had little effect on tilapia growth, indicating that coarser grinds may be used to lower energy costs without compromising performance. Final weights were often higher on sorghum diets despite similar feed conversion ratios, and apparent protein digestibility remained unaffected, affirming sorghum's nutritional adequacy.

In Chapter 4, Pacific white shrimp fed sorghum-based feeds exhibited no reduction in growth or survival, even when preconditioning steam was reduced, suggesting energy savings can be realized without sacrificing performance. While wheat treatments showed slightly better digestibility, particularly under high thermal processing, growth metrics were

statistically similar. Chapter 5 reinforced these findings in rainbow trout, where red sorghum supported growth and digestibility on par with wheat. Though low amylose sorghum grains led to increased feed intake and different tissue yields, their mineral digestibility and growth potential remained high, making them promising options.

Drying conditions played a critical role in determining pellet integrity and energy use, as shown in Chapters 6 and 8. Chapter 6 demonstrated that increased feed rates (simulating deeper dryer beds) in sorghum-based feeds led to greater moisture removal but also slightly higher final pellet moisture, implying possible airflow limitations at higher throughputs. For wheat-based diets, elevated drying temperatures effectively lowered pellet moisture content but significantly decreased thermal efficiency due to higher gas consumption and exhaust losses. Shifts in bulk density and durability revealed a complex interplay between drying intensity and product shrinkage, emphasizing the need for tight control of dryer settings. In Chapter 8, rising sDDG inclusion further complicated drying behavior. Changes in pellet composition affected drying efficiency and consistency, requiring careful adjustment of preconditioning and drying parameters to maintain product quality. The thermal behavior of sDDG-enriched diets highlighted the interaction between ingredient composition and post-extrusion processing, reinforcing the need for tailored drying strategies when alternative proteins are used.

Chapters 7 and 8 emphasized the environmental implications of ingredient selection and processing strategy. Chapter 7's cradle-to-gate LCA found that sorghum-based feeds offered a generally lower environmental burden than wheat across impact categories such as global warming potential, freshwater ecotoxicity, and eutrophication. However, energy-

intensive steps like fine grinding and high thermal extrusion settings could offset some of these gains. The study highlighted the potential to improve environmental outcomes by optimizing processing parameters alongside ingredient selection.

Chapter 8 extended the LCA analysis to diets containing sorghum distillers dried grains.

The study emphasized the dual nature of DDGs: while they enhance sustainability by utilizing by-products, they also demand careful consideration in formulation. By adjusting preconditioning and extrusion parameters, the study showed that some environmental costs can be mitigated, supporting the case for balanced, efficiency-driven feed design in sustainable aquaculture systems.