Final Report for Grain Sorghum Commission (KGSC)

KGSC Contract Number:

Project Title: Use of grain sorghum as a novel carbohydrate source in high-value aquatic feed market and feasibility trials with tilapia and shrimp

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1. Executive Summary

Overfishing of wild fish stocks has led to a rapid increase in aquaculture production since 1990. Increasing global protein demands has led to the global consumption of fish increasing at an annual rate of 3% between 1961 and 2019 (FAO, 2022), almost double that of the annual world population growth (1.6%). Aquatic animals (farmed and caught) provide 17% of global animal protein, and over 3 billion people rely on fish for at least 20% of the daily protein intake (FAO, 2022). Farm raised fish currently account for 49% of total aquatic animal production (178 million tons).

Phase 1 of this research focused on grain sorghum as a sustainable ingredient in floating tilapia feed and studied process sustainability through grinding efficiency and energy inputs across particle sizes. Ground sorghum was successfully incorporated into nutritionally balanced diets formulated for tilapia and processed through a pilot-scale single-screw extrusion system to produce floating feed. Results were encouraging; 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). Energy requirements of the process increased as particle size decreased which led to impact on pellet quality aspects such as water absorption and durability. As particle size decreased, water absorption increased (202 to 377.8%) and was further impacted by grain source, with sorghum based diets having a lower water absorption than wheat based diets at the same particle size. Grain source also impacted pellet durability, with sorghum based diets having a higher PDI than wheat diets on average. Dietary sorghum inclusion was proven to be successful in a 12-week growth trial, while intensive grinding did not improve growth rates. Tilapia fed the sorghum based diet with a medium grind had the highest growth response, while fish fed the wheat based fine grind diet responded the worst. 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.

Phase 2 of the research focused on grain sorghum as a sustainable carbohydrate ingredient in shrimp feed and studied process sustainability through the effects of steam addition during preconditioning. Two Pacific White shrimp (or Whiteleg shrimp) diets (sorghum and wheat) were subjected to three levels of steam input (high, medium, low) in the preconditioner. Thermal energy (TE), bulk density, sectional expansion index (SEI), water absorption, sinking percentage, pellet durability, and water stability were measured. Thermal energy in wheat and sorghum-based diets decreased as steam inputs were reduced (236.8 to 113.4 kJ/hr, and 279.8 to 120.4 kJ/hr, respectively). Mechanical energy inputs did increase as thermal energy decreased, but overall process energy requirements decreased along with thermal energy inputs. Wet bulk densities were significantly different, with the low thermal energy sorghum diet having the highest density off the extruder (524 g/L). Sectional expansion index increased as thermal energy decreased, most likely due to increased mechanical energy inputs. Water absorption, sinking percentage, and pellet durability was not significantly affected by grain source or thermal energy. Water stability of the feeds ranged from 80% to 59%, with water stability levels decreasing as steam input decreased. Neither grain source nor steam addition significantly impacted growth rates of the Whiteleg shrimp during an *in vivo* animal feeding trial.

2. Technical Objectives

The overall project had the following 4 primary objectives.

a. Extrude high-quality floating and sinking feed for tilapia and shrimp, respectively, and determine the impact of processing parameters on energy use
b. Determine the physical impact of particle size on floating and sinking feed quality
c. Determine the impact of particle size of raw diets after processing into aquatic feed using extrusion on tilapia and shrimp through a growth study
d. Determine the nutritional impact of sorghum inclusion in aquatic feed diets on tilapia and shrimp through a growth study

3. Background

Aquaculture, broadly speaking, is defined as the rearing of aquatic animals for food purposes. Globally speaking, the production of aquatic animals in 2020 was 178 million tons, with 88 million (49%) produced through aquaculture. 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 all of the growth in recent years has come from aquaculture. Overfishing of our wild fish stocks has led to a rapid increase in aquaculture production in order 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 (FAO, 2022). 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%) (FAO, 2022). This increase is higher than that of all other animal protein foods, including meat and dairy products, which increased by approximately 2.1% annually (FAO, 2020). More recently, world production of aquatic animals grew at an average rate of 5.3% from 2001-2018 (FAO, 2020). Currently, over 3 billion people rely on fish for at least 20% of their daily protein intake, and that number can rise as high as 70% in some southeast Asian regions (FAO, 2022).

Feeds for aquaculture rely heavily on fishmeal for protein requirements and for its "large quantities of energy per unit weight" (Miles & Chapman, 2006), but due to high costs and limited supplies due to increasing demand and overfishing, replacing fishmeal with alternative 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 (Shiau & Peng, 1993). As most 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. 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 (Tacon & Metian, 2015).

Potential cost savings exist if commonly used grains 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 (Van Oosterom et al., 2021). 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 (Fresco, 2013).

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%) (Bach Knudsen and Munck, 1988). 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. Previous research done here at Kansas State has shown grain sorghum DDG's to be a viable ingredient in aquatic feed for shrimp. (Adedeji et al 2017). As stated, wheat is a common ingredient in aquaculture feeds for various reasons. These reasons can range from being the most readily available ingredient at hand, it's binding capability in extruded or pelleted products, and its status as a non-GMO grain. Discussions with feed producers indicate that the industry prefers darker colored feed and burgundy sorghum will naturally help with that.

Particle size 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, 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 review by Glencross et al. of ingredient strategies in the industry found that "most studies proved little indication of particle size for any of the ingredients use."

4. Results

Phase 1. Tilapia Feed Formulation, Processing and Growth Trials

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. All diets, both wheat and sorghum-based, were formulated to be nutritionally complete and balanced, with similar levels of crude protein and lipids according to the dietary requirements of Nile Tilapia. The formulas, with calculated crude protein levels at 36% and crude fat at 8% are shown in Table 1. Ingredients were purchased, and major ingredients were sampled and analyzed for percent dry matter, crude protein, fat, ash, and total dietary fiber.

Formulas were then adjusted to account for the true nutritional value of the major ingredients. Because of this, ingredient inclusion levels were slightly different in each formula to account for the nutritional differences between wheat and sorghum. 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 Cargill. The vitamin and mineral premixes were sourced from an aquatic nutrition company (Ziegler Bros, Inc., Gardners, PA, USA). All other ingredients were sourced through a local feed mill (Fairview Mills, Seneca, KS, USA).

Table 1. Diet formulation for floating feed for tilapia.

Tilapia – Floating Feed	
36% Crude Protein, 8% Total Lipids	
Ingredients	%
Menhaden Fishmeal	6.00
Soybean Meal	**44/44.60**
Corn Protein Concentrate	9.00
Menhaden Fish Oil (100% Top-Coat)	2.00
Soy Oil	**3.52/3.00**
Soy Lecithin	1.00
Ground Wheat/Sorghum	**32.32/32.24**
Mineral Premix	0.07
Vitamin Premix	0.04
Choline chloride	0.20
Rovimix Stay-C 35%	0.10
CaP-dibasic	1.75
Total	100.00

Whole grains (wheat and sorghum) were ground using a pilot scale hammer mill (Model D Comminuter, Fitzpatrick, Westwood MA, USA). Initial grind was doing 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 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 in order 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.

Sorghum required less energy to grind than wheat, particularly in the initial coarse break using the 1.65 mm screen. Fine grinding was a much more energy process in both sorghum and wheat, representing a significant cost for feed producers.

Table 2. Grinding energy usage and throughput.

	Energy use during grinding (kJ/kg)			
	0.61 mm	1.02 mm	1.27 mm	1.65 mm
Wheat	150.9936	61.30915	27.21022	74.06103
Sorghum	140.053	54.43892	24.71137	31.44604

	Throughput during grinding (lbs/hr)			
	0.61 mm	1.02 mm	1.27 mm	1.65 mm
Wheat	387.23	797.48	1663.45	971.38
Sorghum	339.13	750.51	1448.02	1863.01

Sorghum had much higher throughput in the initial coarse break, but after that, throughputs per hour were slightly higher in wheat.

Energy data was also collected for grinding of the mixed diet. Post-batch grinding is an important step in aquatic feed manufacturing, largely for the same reasons as stated previously.

Table 3. Grinding energy usage post-mixing.

	Energy use during post-batch grinding (kJ/kg)		
	0.61 mm	1.02 mm	1.27 mm
Tilapia - Wheat	202.2481	63.8115	24.6450
Tilapia - Sorghum	202.8244	64.9963	24.7319

There was minimal difference in the energy use during post-batch grinding, largely because of diet composition. Wheat and sorghum were incorporated into the complete diets at approximately 30% and were already ground during the previous grinding steps, resulting in minimal differences in energy use when grinding the complete diet.

During this study, it is important to note that we did not have a desired particle size for each treatment, merely that we wanted to differentiate between three distinct particle sizes. As a result of that, particle sizes between treatments varied, even through the same hammermill screen size. 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. These differences are largely attributable to do the raw grain itself. Energy use for whole wheat grinding was higher across all particle sizes, lending itself to a finer particle size, most likely resulting in a finer particle size for the mixed wheat diets. As a result of this, particle sizes are not directly comparable across all treatments. For example, our 'fine' grind for the sorghum-based diet resulted in a particle size of 270 microns. 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.

Table 4. Particle size distribution of ground diets.

Micron Size	TW 0.61 %	TW 1.02 %	TW 1.27 %	TS 0.61 %	TS 1.02 %	TS 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
AVG DGW	**203.00**	**266.00**	**334.00**	**270.00**	**303.00**	**335.00**

Specific mechanical energy (SME) is considered to be the main mechanism behind product expansion and the degree of product cook. SME values ranged from 358.8 to 267.6 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. 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. 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 SME than wheat-based diets. Notably, this runs counter to what we would expect based on our particle size analysis. 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, we would expect sorghum-based diets 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.

Conversely, Specific Thermal Energy (STE) went up in the preconditioner (signifying that more steam was absorbed) as the particle size increased. STE is more difficult to calculate than SME, but good estimations can be made using temperatures and moisture contents from samples collected throughout the process. This trend existed in wheat (208.4 to 275.7 kj/kg) and in sorghum (252.8 to 292.6). All grind sizes of sorghum had a slightly higher STE than the respective STE for wheat; fine: 252.8/208.4; medium: 278.5/254.6; coarse: 292.6/275.5. At first glance, this appears contradictory. Finer particle sizes should lead to a larger STE as there is more surface area to absorb steam, but that is not the case in our results. It is likely that the finer grind size was leading to a smaller bed depth in the preconditioner due to particle flow and interactions. Bed depth, or preconditioner fill, is an important component of preconditioning. The more fill in the preconditioner, the more material is able to capture and hold onto the steam injected into the chamber.

Overall, STE:SME ratios were similar between grind sizes. Increasing in particle size, wheat based diets had an STE/SME ratio of 0.6, 0.9, and 0.9. Sorghum based diets were 0.7, 1.0, and 1.1. As the diet particle size increased, SME increased, leading to a larger proportion of energy inputs taking place through mechanical energy in all treatments.

Overall, energy requirements for hammermill grinding is less for sorghum than for wheat; however, gelatinization energy requirements were higher in the Differential Scanning Calorimetry (DSC) for sorghum than wheat. It's probably that the higher amount of energy required for gelatinization is what SME and STE is higher during extrusion processing as well. Ultimately, overall energy use for both grain based feeds are similar. Total process energy use was lower for diets with a medium to coarse grind size in both diets.

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 gram, and the known volume is in liters. Generally speaking, as particle size increased, bulk density decreased. Finer particle sizes lead to greater expansion, greater pellet diameter, and a lower bulk density.

Table 5. Pellet diameter and bulk density.

	Pellet Diameter - Floating Feed		
	Fine	Medium	Coarse
Wheat	2.722	2.864	2.793
Sorghum	2.932	2.565	2.41

	OE Bulk Density - Floating Feed		
	Fine	Medium	Coarse
Wheat	444	423	430.5
Sorghum	367.25	435	452.5

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 – 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.

For the finished feed, several analysis tests were conducted to determine feed quality. Water absorption is an important metric, representing possible nutrient loss through leeching and increased pellet size as water is absorbed, causing possible feeding issues.

Table 6. Water absorption of pellets.

Floating Feeds - Water Absorption (% by weight)		
Grain Source	Wheat	334.433
	Sorghum	255.667
Particle Size	Fine	329.28
	Medium	297.55
	Coarse	258.32

While there was not a significant interaction between grain source and particle size, individual interactions did increase. Wheat based diets in general were more prone to water absorption than sorghum based diets, and as the particle size decreased, water absorption increased. It is likely that this is because of the increased surface area and expansion; more expanded pellets have more air pockets in the feed, allowing the water to penetrate the pellet more easily. Additionally, wheat starch is easier to access – it is not bound in the protein matrix like sorghum is. Because the starch is more 'free', more water is absorbed into the starch than in sorghum, leading to higher water absorption percentages.

Following along with water absorption, water stability is a significant metric to consider. The industry is divided, with varying definitions of what water stability means and how to test for it. We decided to conduct a water stability test where we measure the loss in mass after the feed is soaked for a set period of time. If a feed had 60% of it's pre-soak mass post soak and drying, then we defined it as 60% water stable.

Table 7. Water stability of pellets.

Water Stability (%)			
	Fine	Medium	Coarse
Wheat	82	82	82
Sorghum	82	80	79

No significant differences were found between diets. All diets were physically water stable to an acceptable degree after one hour of soak time.

Pellet durability index is another important indicator of feed quality. The generation of fines is a significant problem in the industry. Fines and dust greatly contribute to poor water quality, leading to increased costs and labor at the farm, as well as decreased health and potential mortality issues for the fish. 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. 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 amount of aquatic feeds are smaller than the screen size present, rendering the test unable to be used. Salmon farms in Norway have created a new method called the DORIS, but use of it is very regional, and it is largely unknown. For our PDI test, we decided to use the standard tumbler box method and modified it with the addition of several hexagonal nuts. 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. While small, this difference is statistically significant. More importantly, this difference appeared with the tumbler test, which has historically had trouble differentiating between aquatic feeds. 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.

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.

Growth studies were conducted at Auburn University under the supervision of Dr. Allen Davis. Diets were top coated with 2% fish oil before feeding. It was a 12 week growth trial, with 20 fish per treatment and 4 replicates. The growth study was conducted in a recirculating aquatic system (RAS). Average starting weights were 3.83 +/- 0.03 g, and they were batch-sorted (group weighted) to uniform size and randomly stocked in tanks. At the end of the 12-week trial, fish were group weighed to determine mean final biomass, final weight, survival, percent weight gain, and feed conversion ratio (FCR).

Significant differences were observed in final weight and weight gain for fish fed different extrusion sizes of sorghum (S0.61, S1.02, and S1.27) and wheat (W0.61, W1.02 and W1.27). 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, lowest fish growth performance was observed in fish fed the finest grind, S0.61 and W0.61. There were no significant differences in FCR and fish survival between treatments. Highest growth performance was observed in fish fed S1.02, and the lowest performance was observed in fish fed W0.61.

Table 8. 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 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

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

[2] PSE = Pooled standard error.

[3] One-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.

Figure 1.

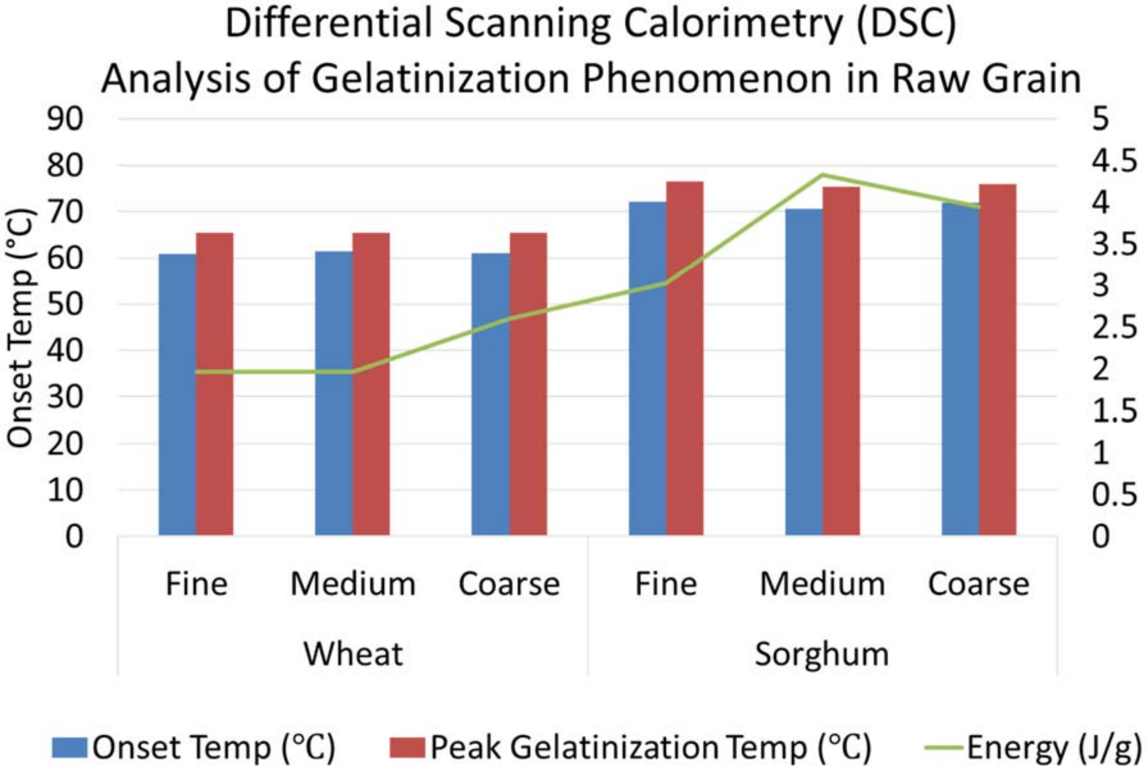


Figure 2.

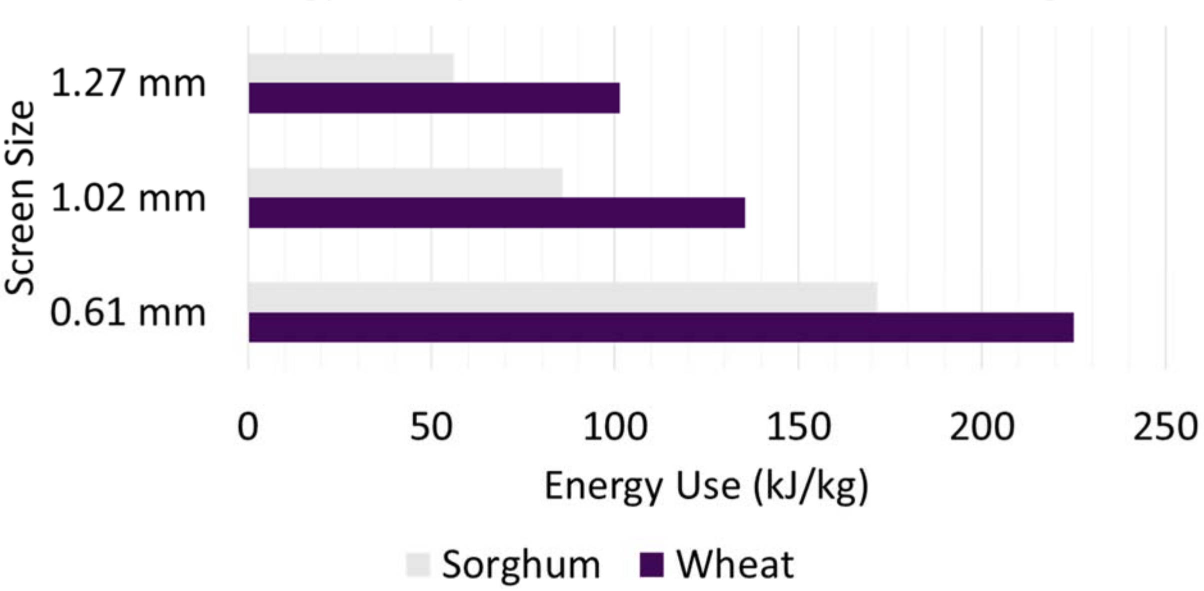


Figure 3.

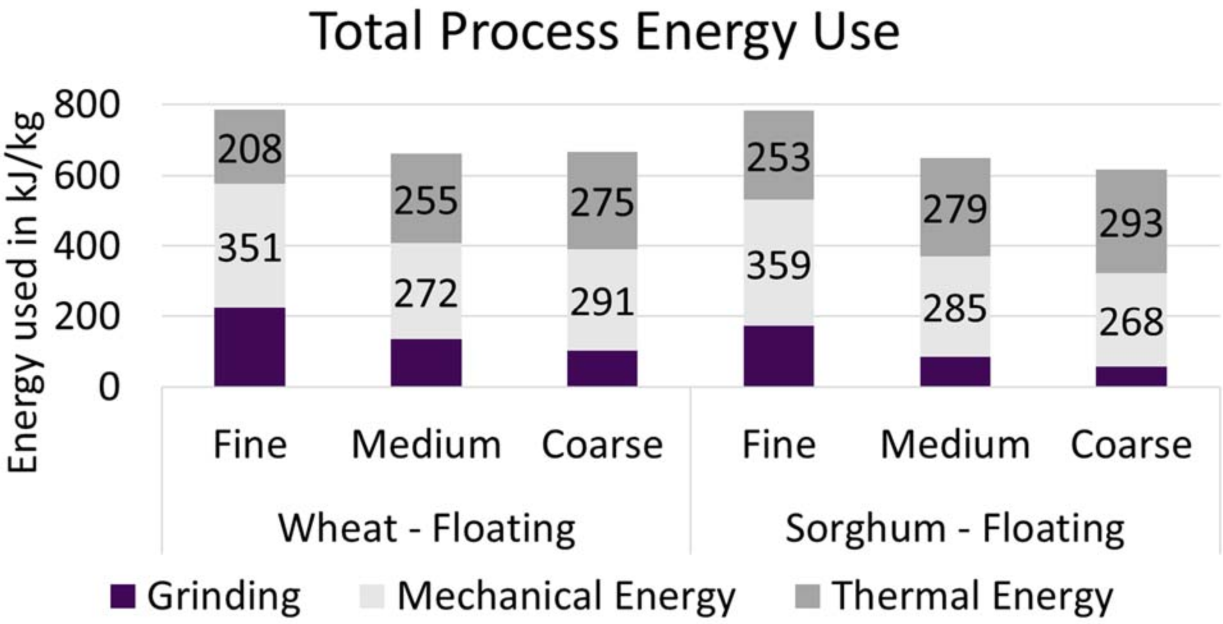


Figure 4.

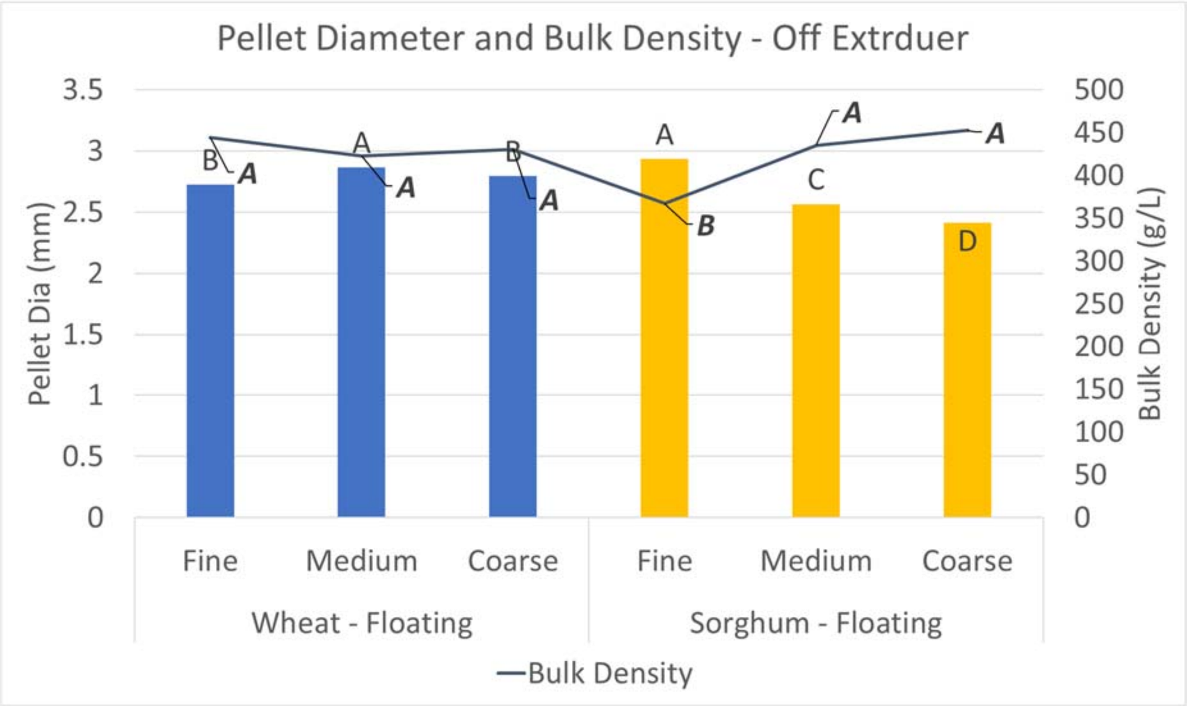


Figure 5.

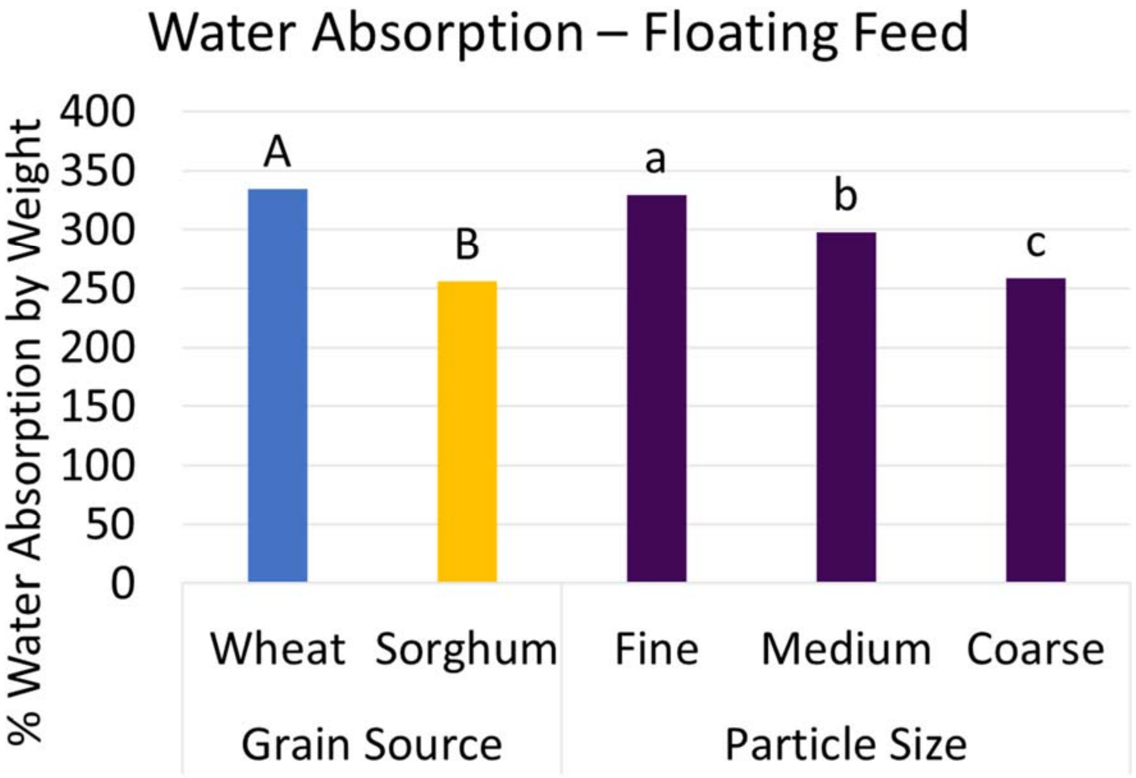


Figure 6.

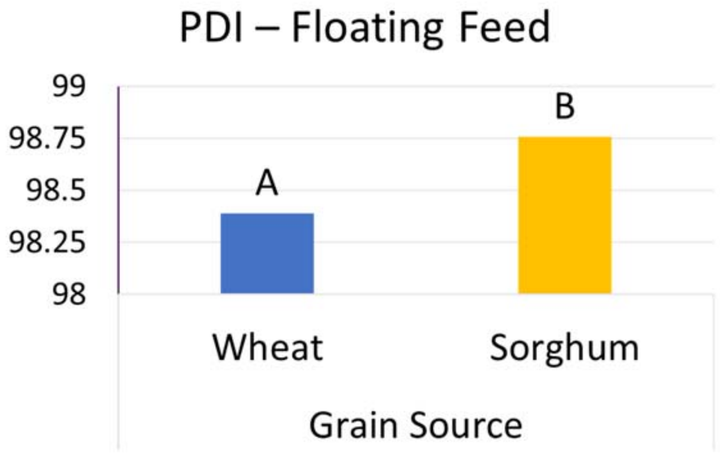
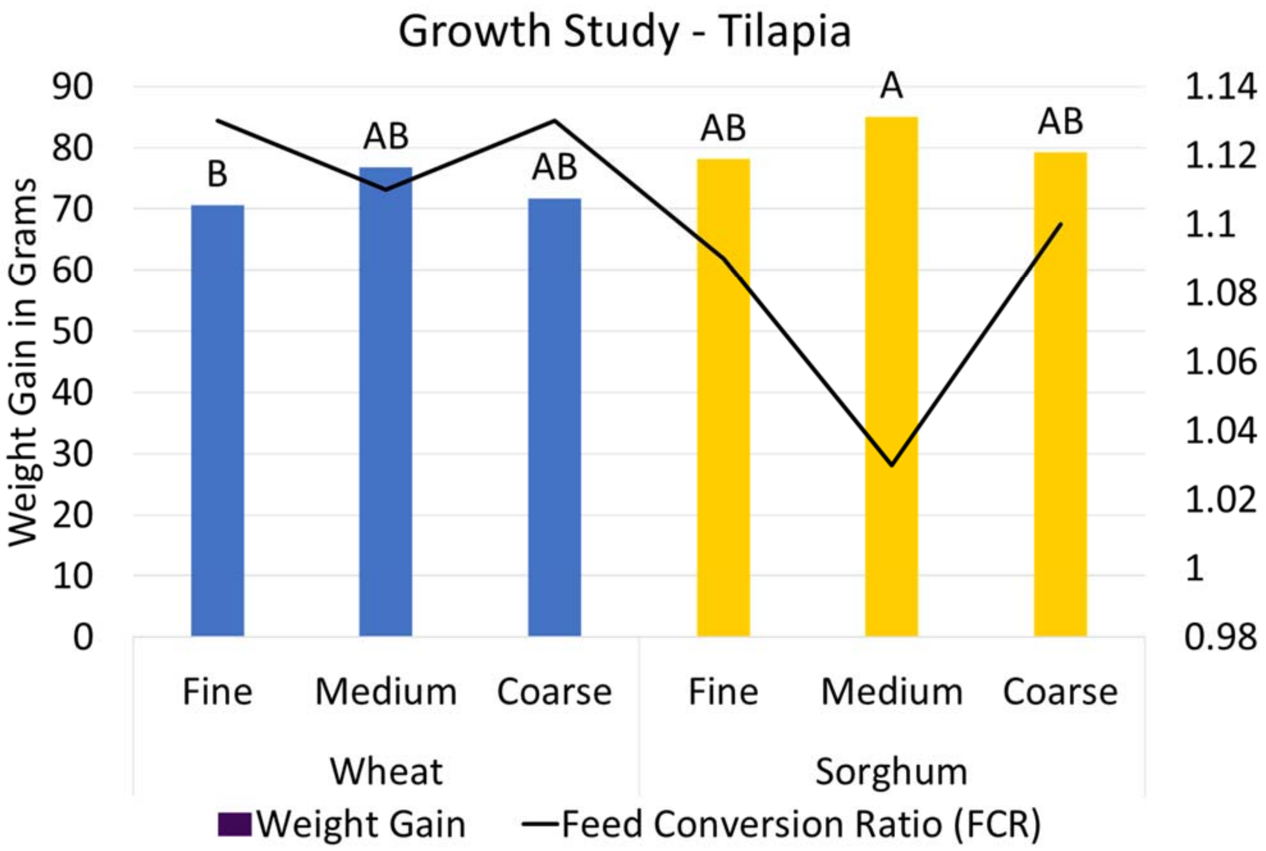


Figure 7.


Growth Study - Tilapia
Weight Gain in Grams
90
80
70
60
50
40
30
20
10
0
1.14
1.12
1.1
1.08
1.06
1.04
1.02
1
0.98
B
AB
AB
AB
A
AB
Fine
Medium
Coarse
Fine
Medium
Coarse
Wheat
Sorghum
Weight Gain
Feed Conversion Ratio (FCR)

Phase 2. Shrimp Feed Formulation, Processing and Growth Trials

Experimental Design

Two distinct diets were prepared for whiteleg shrimp: a wheat-based diet, and a sorghum-based diet (Table 9). Three treatments per diet were designed based on steam addition in the preconditioner: a high thermal energy, medium thermal energy, and low thermal energy treatment for a total of six diets. Diets were formulated to be 36% crude protein, 8% total fat.

Table 9. Whiteleg shrimp diets based on whole wheat (WW) and red sorghum (RS).

Complete Diet	WW	RS
Menhaden fishmeal	10	10
SE Soybean meal - 46	39	39.45
Corn Protein Concentrate	8	8
Menhaden fish oil (100% Top Coat)	2	2
Soy oil	3.02	2.65
Lecithin (soy)	1	1
Cholesterol - Added to top coat	0.12	0.12
Rice Bran	10	10
Whole wheat	23.2	0
Sorghum	0	23.12
Mineral premix Commercial	0.07	0.07
Vitamin premix Commercial	0.04	0.04
Choline chloride (0.2% all diets)	0.2	0.2
Rovimix Stay-C 35%	0.1	0.1
CaP-dibasic	1.75	1.75
Bentonite	1.5	1.5
Total	**100**	**100**

Extrusion Processing

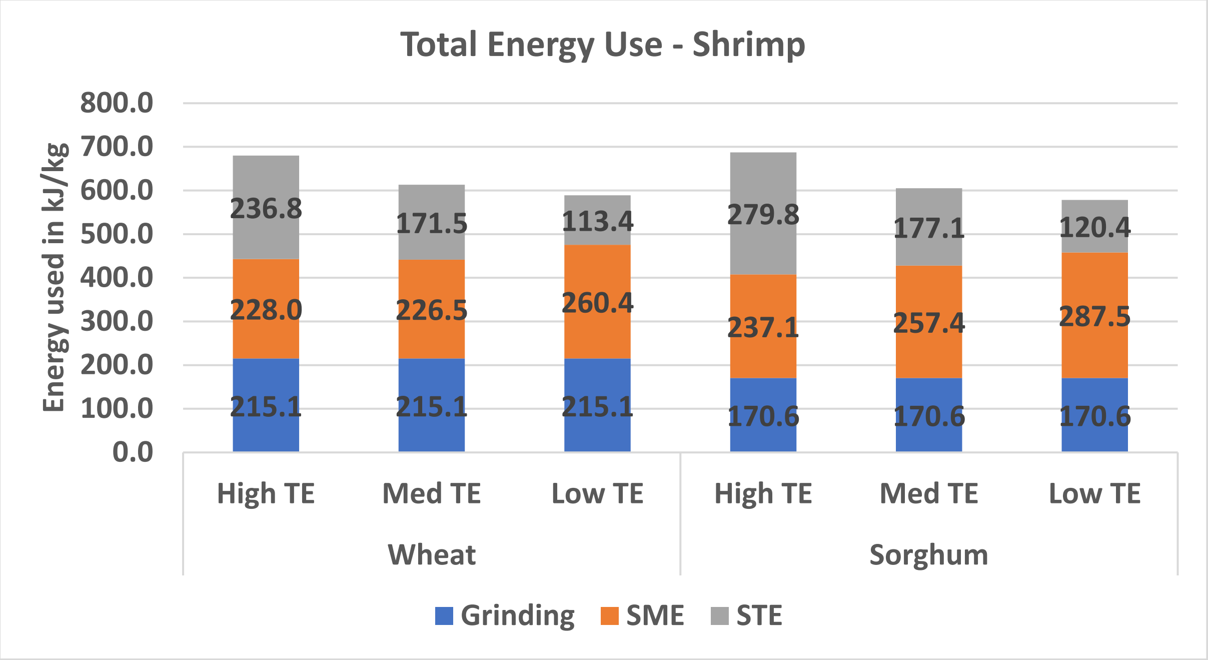
Diets were extruded on a Wenger X-20 single screw extruder with an attached preconditioner. During preconditioning, materials were heated with water and steam, which initiated the cooking process and softens the raw material by transitioning from the glassy to the rubbery state. This improves digestibility and gelatinization.

. **Table 10. Whiteleg shrimp feed extrusion process conditions.**

	Wheat			Sorghum		
	High TE	Med TE	Low TE	High TE	Med TE	Low TE
PC Water (kg/hr)	12.18	17.87	20.00	10.85	16.14	20.37
PC Steam (kg/hr)	13.51	9.27	5.85	16.35	10.18	7.01
PC Discharge Temp (°C)	96.68	80.00	58.37	96.33	79.29	59.68
PC Steam Loss (kg/hr)	**2.51**	**1.33**	**0.35**	**3.96**	**2.16**	**1.46**

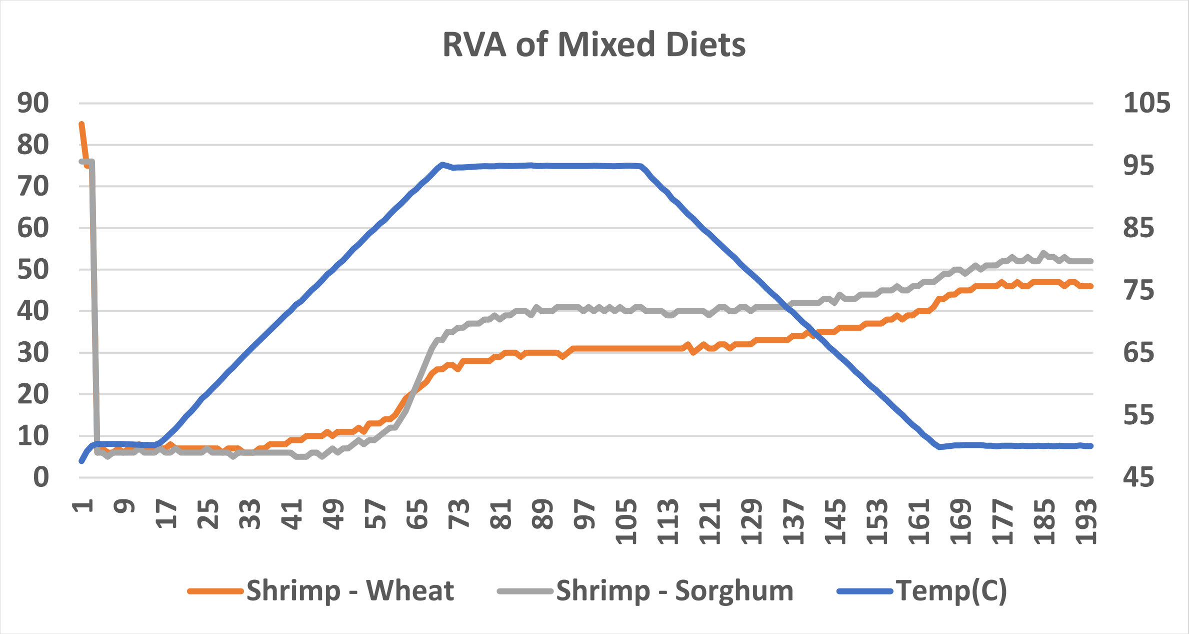
The amount of steam added during conditioning was controlled between treatments to lower/raise our downspout temperature, a good indication of how much thermal energy was added in (Table 10). Energy values for the extrusion process are below

Figure 8.



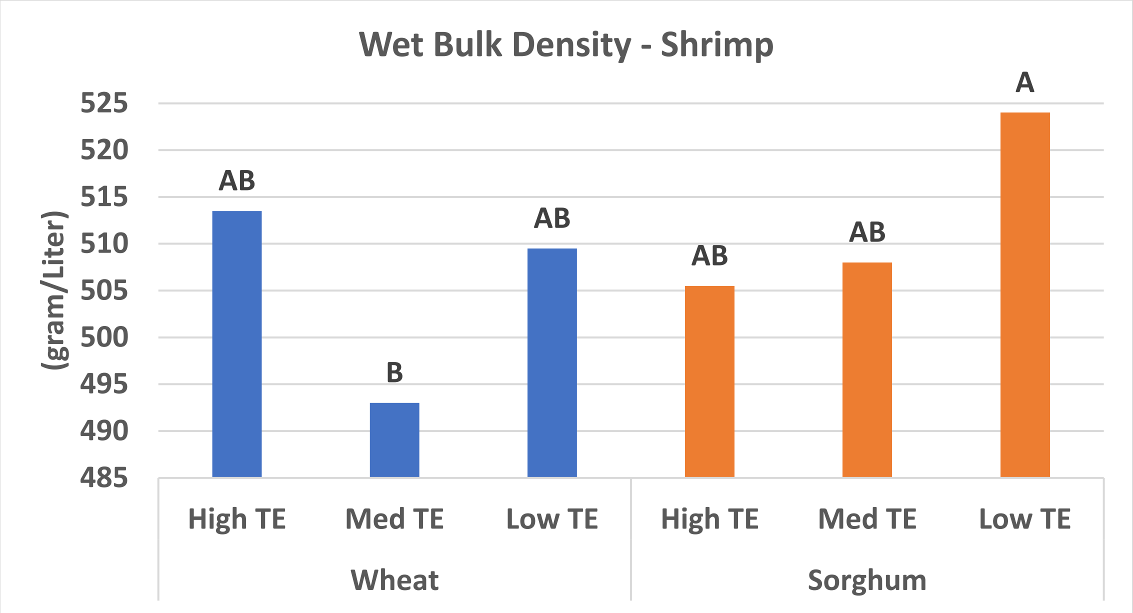
Grinding energy was the same within wheat/sorghum diets due to all diets being ground through a 1.02 mm hammermill screen (Figure 8). Specific thermal energy (STE) decreased as steam addition was reduced. As STE reduced, mechanical energy inputs (SME) increased due to the increased abrasiveness of the material.

Figure 9.



Wheat based diets hydrate and swell earlier in the rva curve, indicated less heat is needed for viscosity increases (Figure 9). Sorghum based diets reached a higher viscosity in total, but it required more time and heat.

Figure 10.



Off-extruder bulk density in wheat-based diets were different as thermal energy input decreased, but no trend was observed (Figure 10). Thermal energy addition in sorghum-based diets decreased bulk density due to increased expansion of the pellet.

Figure 11.

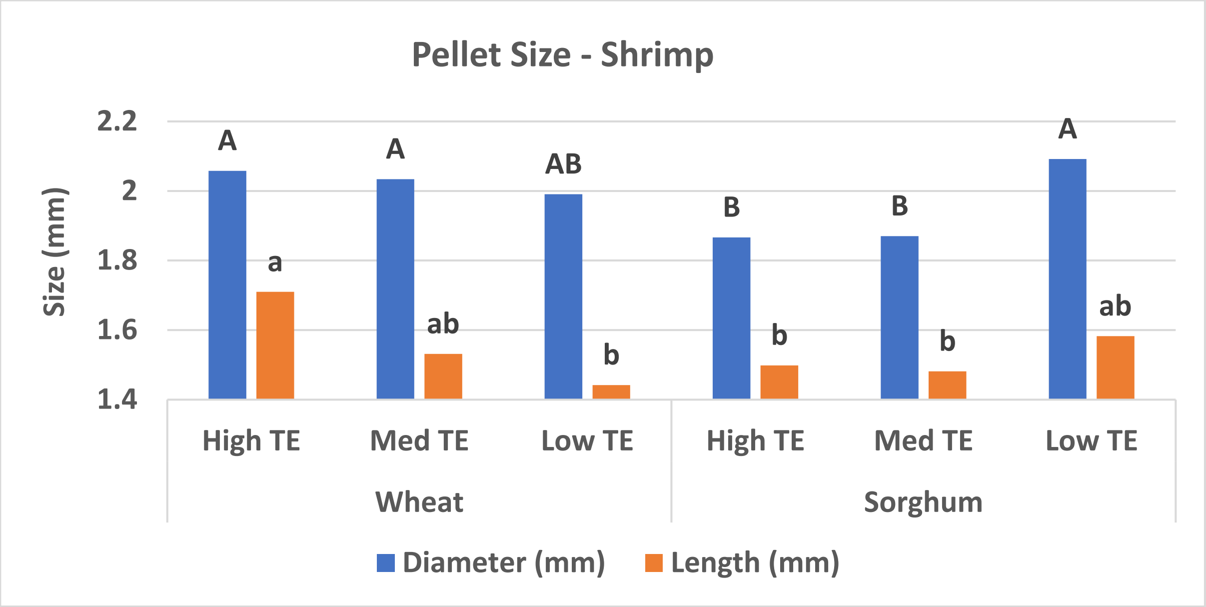
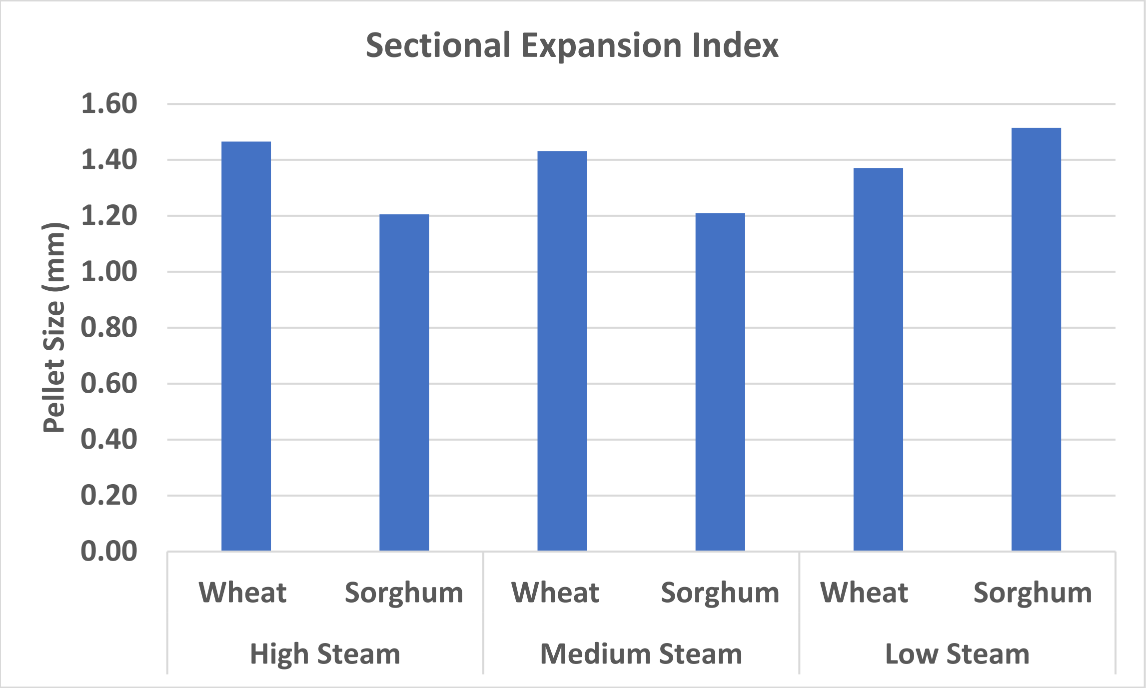


Figure 12.



As thermal energy decreased in wheat-based diets, diameter and length decreased, resulting in a decreased expansion index (ratio of expanded product to die diameter) (Figures 11 and 12). As thermal energy decreased in sorghum-based, expansion actually increased – this is due to SME values increasing in response to the decreased STE. No significant difference between

Figure 13.

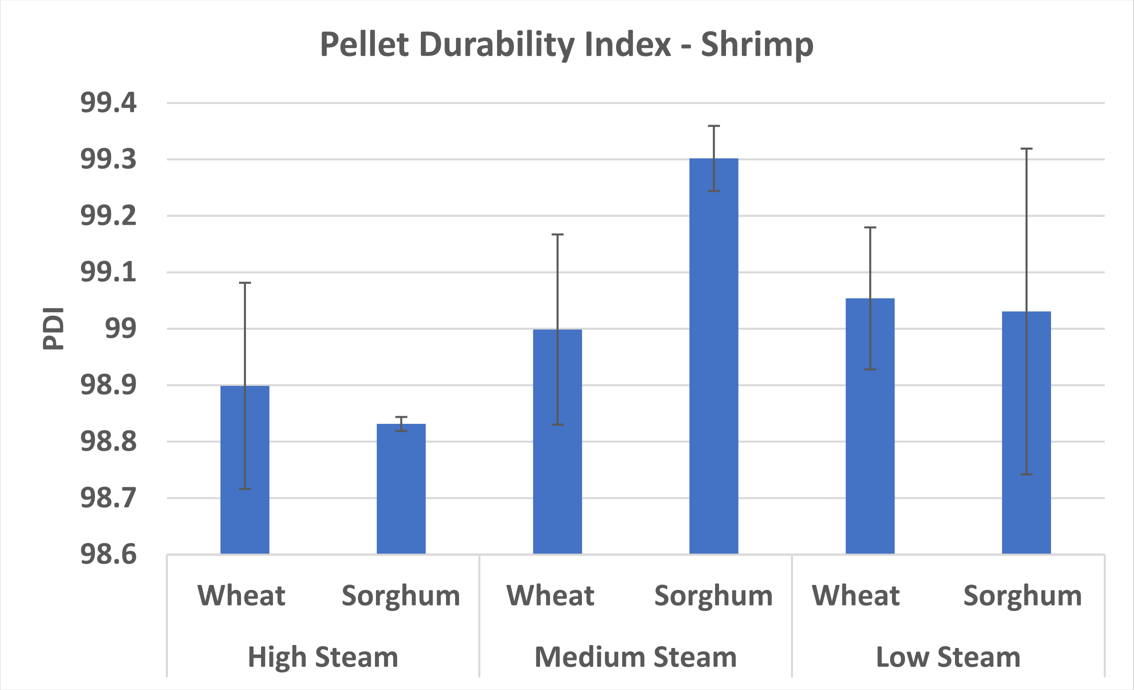
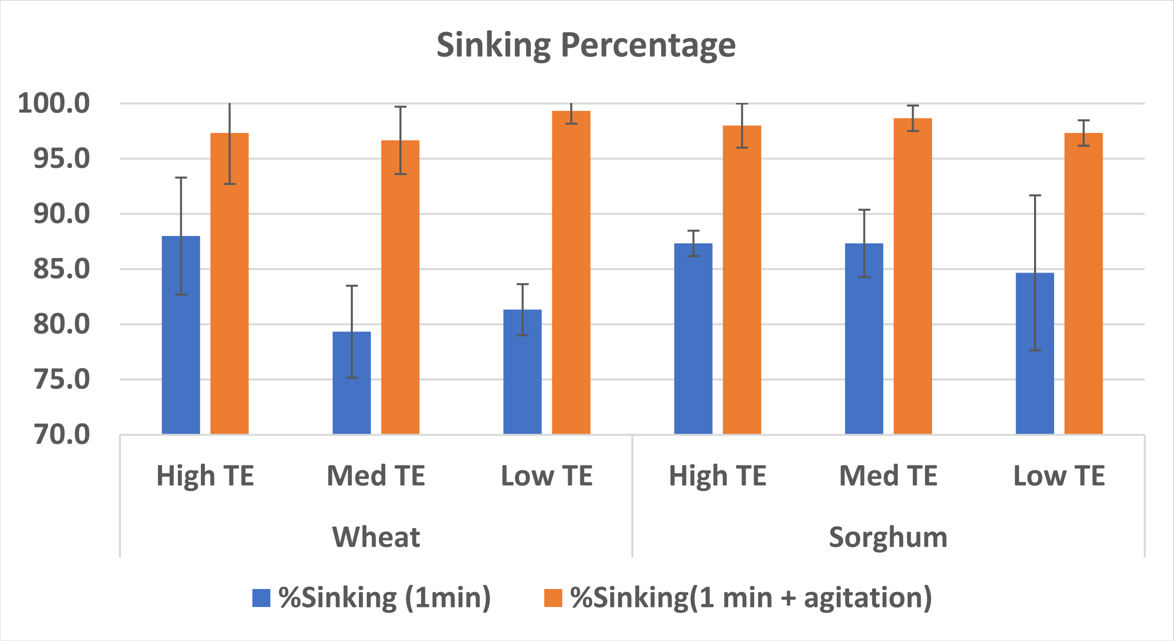


Figure 14.



grain type or thermal energy addition in pellet durability index (Figure 13). Pellet durability index (PDI) tests were performed using the tumble box method with the modification of two ½ hexagonal nuts. All PDI's were greater than 98.5%, which is typical for extruded products. No significant differences existed in sinking percentage of shrimp feed pellets (Figure 14). All diets were greater than 95% sinking with slight agitation after 1 minute; all diets were approximately 80% sinking or more after 1 minute.

Figure 15.

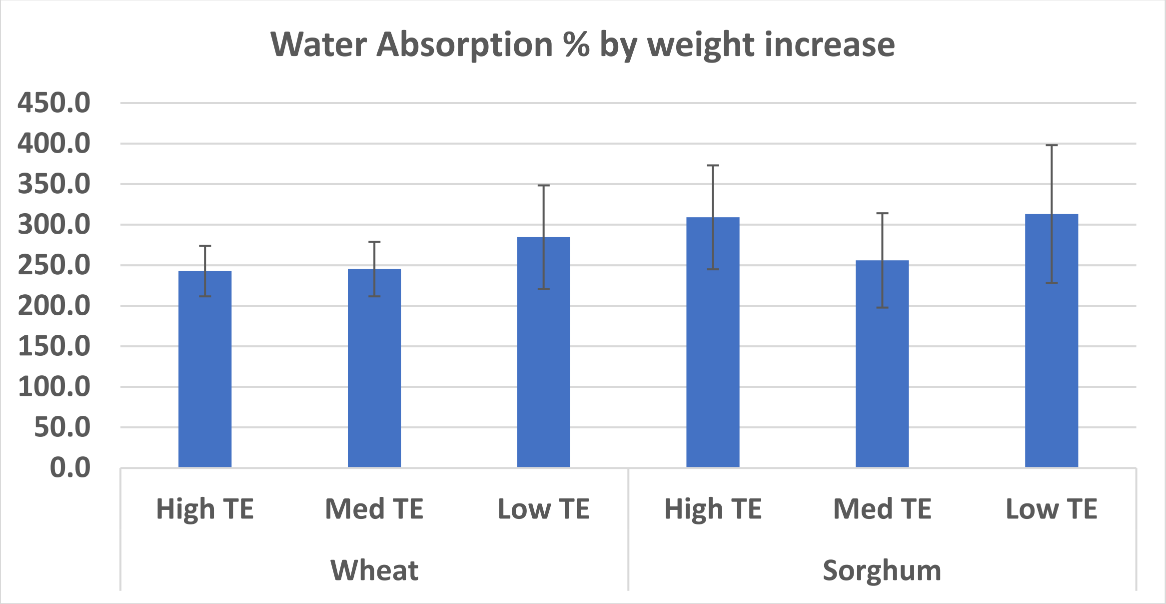
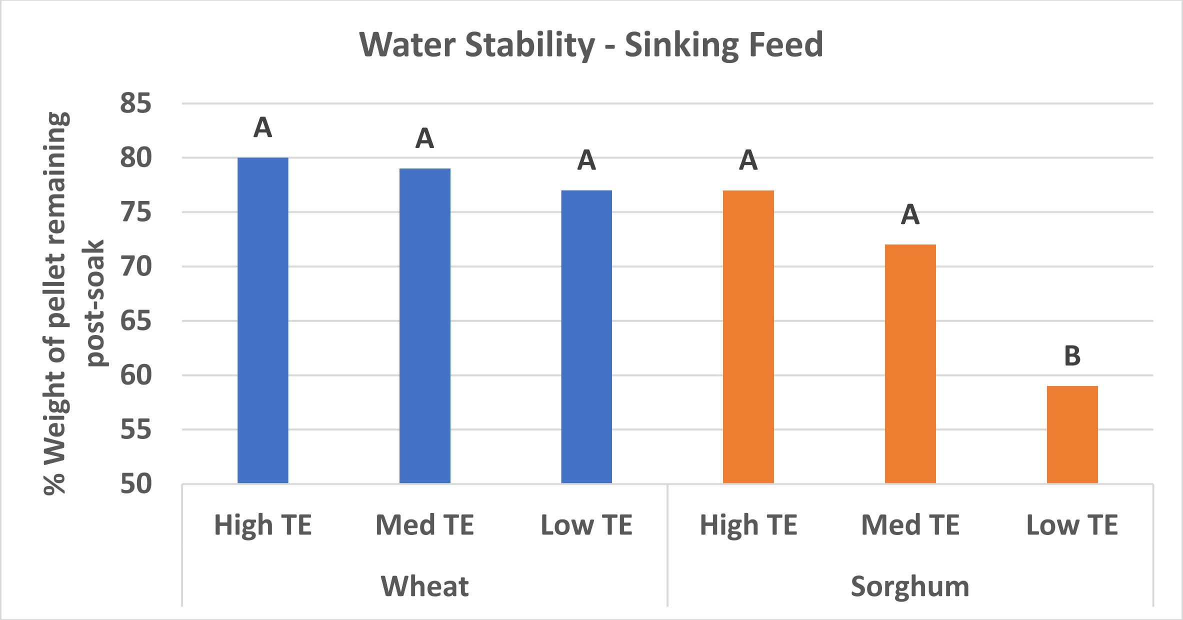


Figure 16.



No significant differences existed in water absorption (Figure 15). As thermal energy decreases, water stability slightly decreases in wheat diets, but not significantly (Figure 16). However, it does significantly decrease in sorghum diets, indicating that preconditioning is a vital step to ensure adequate binding and gelatinization of the pellet in sinking feeds. Decreased water stability results in increased water pollution and nutrient leakage into the water, potentially resulting in reduced growth rates or health outcomes.

In vivo feeding trials and growth study using Pacific White shrimp (Whiteleg shrimp) at Auburn University demonstrated no significant differences between treatments in shrimp growth rates or in survival percentage, indicating that sorghum is a viable alternative for wheat in sinking shrimp diets (Table 11). All diets had a better FCR than the commercial control diet.

Table 11. Response of white shrimp (mean initial weight 0.38g ± 0.02) fed diets containing sorghum and wheat within a 5-week period. Values represent means of six replicates.

Growth Parameters	Final Biomass (g)	Final Weight (g)	Weight Gain (g)	[a]Weight Gain (%)	[b]TGC	[c]FCR	Survival (%)
Commercial[1]	48.69	4.26	3.88	1018.70	1.30	1.88	76.67
Wheat_HT[2]	54.85	4.51	4.13	1079.97	1.39	1.70	81.11
Wheat_MT[2]	60.99	4.64	4.25	1106.13	1.43	1.56	87.78
Whear_LT[2]	53.46	4.66	4.28	1129.70	1.44	1.76	76.67
Sorghum_LT[2]	55.81	4.35	3.97	1043.25	1.33	1.68	85.56
Sorghum_MT[2]	52.38	4.43	4.06	1077.17	1.36	1.78	78.89
Sorghum_HT[2]	49.44	4.18	3.80	989.55	1.28	1.85	78.89
[d]PSE	3.930	0.206	0.204	55.021	0.068	0.116	4.675
p-value	0.393	0.590	0.576	0.576	0.593	0.555	0.536

[a] Weight gain= (final weight-initial weight)/initial weight × 100%

[b] TGC = = FBW^1/3-IBW^1/3)/Σ(Temp*days) *1000

[c] FCR=Feed conversion ratio = feed offered/ (final weight-initial weight)

[d] PSE = Pooled Standard Error

[1] N=4

[2] N=6

HT: high thermal energy

MT: medium thermal energy

LT: low thermal energy

5. Conclusions

Overall, these results are very promising for the future of sorghum in aquatic feeds. In the case of tilapia feed, overall process energy requirements are the same, but sorghum is significantly easier to grind than wheat, representing an opportunity to save time in the feed production process. Furthermore, sorghum had 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. Finally, dietary sorghum inclusion of approximately 30% did not negatively impact growth rates in juvenile tilapia. In fact, across all grind sizes, sorghum performed better than wheat diets, indicating that grain sorghum is a viable and promising alternative to wheat in tilapia diets.

In the case of shrimp feed, the overall process energy requirements decreased along with thermal energy inputs. However, water absorption, sinking percentage, and pellet durability was not significantly affected by grain source (wheat versus sorghum) or thermal energy. Water stability of the feeds ranged from 80% to 59%, with water stability levels decreasing as steam input (thermal energy) decreased. Neither grain source nor steam addition significantly impacted growth rates of Whiteleg shrimp, which indicated that sorghum is a viable alternative for wheat in sinking shrimp diets

6. Outcomes, Impact and Disseminations of Results

3 billion people consume 20% of their daily protein intake through seafood. By 2030, two-thirds of global seafood will be based on aquaculture, and production of aquatic animals such as shrimp and tilapia will double. This rapid growth is important to meet protein needs of a projected population of 8.6 billion. The role of processed aquatic feeds in aquaculture, with quality attributes such as nutrition, digestibility, water stability, durability, and cost-effectiveness is keeping pace with this trend. As the demand for sustainable processing methods and ingredients is increasing, this represents a significant opportunity. Grain sorghum is a very important crop in Kansas. It is one of the most sustainable of all cereals, requiring less water and other inputs to grow. Successful results such as the one in this study represent a significant opportunity to market sorghum to fish producers. Increased use of this crop in aquatic feed applications, which are higher value than other animal feeds, would bring economic benefit to Kansas growers and the Kansas sorghum market. ***Sorghum-based aquatic feed for shrimp and tilapia were found to be equal to wheat based aquatic feed in animal performance/ growth with some indication that overall process energy consumption for the former was also lower. The basic findings are being communicated to industry and has already helped in adoption of sorghum aquatic feed and US grain sorghum, for example in the Norwegian aquaculture industry.***

Technical Presentations

- T. Graff, D.A. Davis, S. Alavi. Grain sorghum as a sustainable ingredient in aquatic feed – grinding and processing energy studies. Poster presented at: Sorghum in the 21st Century, Global Sorghum Conference; Montpellier, France; 2023 June 5-9th
- T. Graff, D.A. Davis, S. Alavi. Grain sorghum as a sustainable ingredient in aquatic feed – grinding and processing energy studies. Oral presentation at the 14th Annual Grain Science Graduate Student Research Symposium; 2022 Nov 3rd

- T. Graff, D.A. Davis, S. Alavi. Grain sorghum as a sustainable ingredient in aquatic feed – grinding and processing energy studies. Poster presented at: Research and the State, Kansas State University; 2022 Oct 27. **Technical presentation selected as one of ten at K-State to move on and present at the Capitol Graduate Research Summit in Topeka, Kansas.**

- T. Graff, D.A. Davis, S. Alavi. Grain sorghum as a sustainable ingredient in aquatic feed – grinding and processing energy studies. Poster presented at: Capitol Graduate Research Summit; 2023 March 22; State Capitol Building, Topeka, Kansas.
- T. Graff. From Pond to Table: Meeting Global Animal Protein Demand through Sustainable Aquatic Feed Production. Three Minute Thesis Competition, Kansas State University; 2023 Feb 2. **Placed 1st in competiton heat and selected as one of ten people to present at the 3MT Finals at Kansas State University.**

Manuscripts: Two manuscripts are currently under preparation based on this project.

T. Graff, D.A. Davis, S. Alavi. *Impact of ingredient particle size and grain sorghum on physical feed quality and growth rates of nile tilapia.* Animal Feed Science and Technology. Under preparation.

T. Graff, D.A. Davis, S. Alavi. *Impact of different preconditioner steam inputs and grain sorghum on physical feed qualities and growth rates of whiteleg shrimp.* North American Journal of Aquaculture. Under preparation.

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