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FINAL REPORT for Contract RG001-20

TITLE: Enhancing Sorghum Opportunities in Domestic and Export Aquafeed Sectors

Species: Rainbow Trout

Enhancing Sorghum Opportunities in Domestic and Export Aquafeed Sectors

Objective #1 - *Develop proximate analyses for the following sorghum varieties such as; commodity #2 or higher grade, tan and/or white and high protein sorghum.*

Since the project did not really start until fall of 2020, we discarded the sourced sorghum varieties from 2019, and freshly harvested sorghum was sourced from 2020 crops. The varieties we acquired were: Red/Bronze (from Richardson Seeds); Tan/White (from Richardson Seeds); and High-Pro from Mojo Seeds via Natural Products, Inc. (Iowa). All of these seeds were shipped to USDA/ARS Bozeman Fish Technology Center for storage and subsequent collaborative use.

Proximate analyses were conducted by several labs and with replicates. We analyzed both sorghum and feed amino acids compositions and protein content. Please see the attached file.

Table 1. Proximate composition of four sorghum varieties (% dry matter basis) (First analysis)

	Texas	**Red**	**White/Tan**	**Super Sack**
Dry matter	86.5	88.4	88.9	87.8
Crude protein	14.9	15.0	10.9	13.4
Crude fiber	1.6	1.0	1.6	1.6
Starch	74.8	64.2	70.3	63.8
Crude fat	3.16	3.93	3.94	4.01
Ash	2.85	2.55	2.58	2.53
Tannins	ND	0.014	0.000	0.000

- TX. Sorghum with larger protein and starch contents can be superior
- Protein data are from the first CVAS results

After conducting the first series of the analysis, we decided to send blind samples (two per each) to two different labs to make sure that we will get logic data regarding the Protein content.

Table 2. A comparison of sorghum protein content between two labs (CVAS and Spectrum) (Second analysis)

	CVAS 2nd test (%DM)	**CVAS 2nd test (% as received)**	**Spectrum (%DM)**	**Spectrum (% as received)**
Red sorghum	15.3	13.66	14.04	12.50
Red sorghum	14.8	13.23	14.11	12.56
White /Tan	11.5	10.29	11.16	9.94
White /Tan	11.2	10.01	11.37	10.12
Super Sack	14.8	12.26	13.97	12.44
Super Sack	14.4	12.77	14.6	13.00

Amino acid profile also was determined for different samples.

Table 3. Amino Acid content of four sorghum varieties (g/100 g protein of sorghum)

AA	**Texas**	**Red**	**White/Tan**	**Super Sack**
Cysteine	1.42	1.67	1.88	1.87
Methionine	0.99	1.67	1.80	1.51
Lysine	1.60	1.54	2.14	1.94
Alanine	7.70	9.77	9.76	9.43
Aspartic Acid	6.40	6.81	6.42	6.48
Glutamic Acid	18.04	21.98	21.40	21.89
Glycine	2.71	2.76	3.17	2.74
Isoleucine	4.13	3.98	3.77	4.03
Leucine	12.07	13.50	12.84	14.11
Proline	11.58	10.15	9.50	8.93
Threonine	3.14	3.15	3.08	2.88
Valine	4.68	4.50	5.05	4.97
Arginine	3.76	3.41	3.51	3.24
Histidine	1.60	1.61	2.05	1.94
Phenylalanine	3.08	4.50	4.71	5.11
Serine	4.19	4.31	4.45	4.39
Tyrosine	1.23	3.60	3.34	3.46
Tryptophan	11.70	1.09	1.11	1.08
Total amino acid	100	100	100	100

Objective #2 - *For the most promising varieties, conduct baseline digestibility coefficients in* ***rainbow trout.***

The sorghum grains were shipped to the USDA/ARS lab in Bozeman. For the apparent digestibility trials, rainbow trout (Trout lodge, WA, USA) were cultured at the Bozeman Fish Technology Center, Bozeman, MT. For this objective, Dr. Sealey manufactured the digestibility

trial diets and subsequently conducted the rainbow trout digestibility trials and analysis; with the following resultant data.

Table 4. ADCs for sorghum varieties determined in Rainbow Trout.

		Red Sorghum	White/Tan Sorghum	Supersack Sorghum
ADCs				
	Dry Matter	45.6a	37.9b	40.6ab
	Crude lipid	100a	82.7b	100a
	Crude protein	66.3	73.1	63.4
	Gross energy	58.0a	46.4b	54.1a
	Phosphorus	36.7	23.3	28.2

Objective #3 - Conduct baseline nutrition trials with **rainbow trout** utilizing optimal varieties from Objectives 1 and 2.

With completion of the finalized data from the rainbow trout sorghum varietal digestibility trials conducted at the USDA/ARS Bozeman lab, the rainbow trout research nutrition diets were manufactured by Dr. Sealey at USDA/ARS, and then shipped to the VSAREC to conduct the preliminary rainbow trout nutrition experiment. These experimental test diets incorporate sorghum at different inclusion rates including 5, 10, and 20%, along with a standard reference diet as the control. There were 3 replicates of each. Fish were randomly stocked in 300 liter tanks, ten fish per tank. Fish were weighed at stocking so that every tank of fish was within two percent plus/minus every other tank of fish. Fish were fed twice a day, and fish were group-weighed as a tank every two weeks to monitor growth. Fish feed was also weighed and recorded by tank number so that food conversion ratios could be determined by individual tanks. Water quality parameters of Dissolved Oxygen, and Temperature were collected and recorded daily. Total Ammonia, Nitrogen, Nitrites, Nitrates, pH, and Alkalinity were collected and recorded at once a week. The study ran for eight weeks, and at eight weeks fish were again group-weighed by tanks, with as well eight fish from each tank had individual weight and total length recorded. Five fish from each tank were frozen for whole body analysis and three fish were dissected to obtain viscera, liver and filet weights.

The rainbow trout sorghum grain nutrition trial was conducted and completed at the VSAREC on December 20, 2021. Upon completion of the trial, resultant data and samples were sent to Dr. Sealey at the USDA/ARS Bozeman lab for further analysis. Resultant RBT growth performance data.

Table 5. Composition of the practical-type control diet (% dry-weight) to which sorghum was substituted at four levels and fed to rainbow trout.

Ingredients	(% dry-weight)			
	S0	S5	S10	S20
Menhaden Fish Meal	*13.55*	*13.55*	*13.55*	*13.55*
Poultry by-product meal	*14.00*	*14.00*	*14.00*	*14.00*
Corn protein concentrate	*5.00*	*5.00*	*5.00*	*5.00*
Blood meal	*5.00*	*5.00*	*5.00*	*5.00*
Feather meal	*5.00*	*5.00*	*5.00*	*5.00*
Soybean meal	*13.00*	*13.00*	*13.00*	*13.00*
Red Sorghum	0.00	5.00	10.00	20.00
Wheat flour	20.00	15.26	10.54	1.06
Monocalcium phosphate	2.45	2.40	2.30	2.20
Lysine HCl	2.45	2.44	2.43	2.41
DL-Methionine	0.65	0.64	0.63	0.61
Threonine	0.67	0.66	0.66	0.64
Lecithin	*1.50*	*1.50*	*1.50*	*1.50*
Stay-C 35	*0.20*	*0.20*	*0.20*	*0.20*
Vitamin premix ARS 702	*1.00*	*1.00*	*1.00*	*1.00*
Choline chloride 50%	*1.00*	*1.00*	*1.00*	*1.00*
Yttrium oxide	*0.10*	*0.10*	*0.10*	*0.10*
TM ARS 1440	*0.10*	*0.10*	*0.10*	*0.10*
Astaxanthin	*0.08*	*0.08*	*0.08*	*0.08*
CaProprionate	*0.15*	*0.15*	*0.15*	*0.15*
Menhaden Fish Oil	7.55	7.46	7.38	7.20
Poultry Fat	6.55	6.46	6.38	6.20
Analyzed composition				
Crude protein (%)	47.5	48.3	49.3	49.4
Crude fat (%)				
Energy (cal/g)	*5261*	5276	5286	5303
Total P (%)				
Pellet Durability (% pellet loss ± sd)	*20.6±1*	*18.6±1*	*15.3±1*	*14.7±2*

Table 6. Growth performance and body condition indices of juvenile rainbow trout (59.1 ± 0.07 g) fed test diets containing sorghum for 8 weeks.

Inclusion level	Growth Performance				Condition Indices		
	Final Fish Wt (g) [1]	Increase (%)[2]	FCR (feed g/gain g)[3]	Intake (%)[4]	Visceral Index (%) [5]	Fillet Ratio (%)[5]	Hepatosomatic Index (%) [7]
0%	250a	321a	0.84	2.2	15.5	57.2	1.7
5%	241ab	309ab	0.82	2.1	14.6	57.7	1.7
10%	236ab	299ab	0.80	2.0	14.6	57.5	1.6
20%	228b	287b	0.81	2.1	14.0	57.4	1.8
Commercial	*206*	*249*	*1.02*	*2.5*	*14.0*	*56.7*	*1.8*
P value	*0.0088*	*0.0120*	*0.1800*	*0.1131*	*0.2259*	*0.9351*	*0.5845*
Pooled SEM	*8*	*9*	*0.02*	*0.02*	*0.3*	*0.3*	*0.03*

[1] Final tank weight (g) / number of fish in the tank.

[2] Percent increase (%) = (final weight – initial weight) x 100 / initial weight.

[3]FCR, feed conversion ratio = g feed consumed / g weight gained.

[4]Feed intake (%) = g dry feed consumed/average fish biomass (g) /culture days x 100.

[5]Visceral somatic index (%) = viscera mass X 100 / fish mass

[6] Fillet ratio (%) = fillet with rib mass X 100 / fish mass.

[7] Hepatosomatic index (%) = liver mass X 100 / fish mass.

[8] Data within a column followed by different letters are significantly different as determined by Students t test.

Note: "Initial results indicate the potential that wheat as an ingredient in flour form, may have provided a slight advantage as a carbohydrate source when compared to whole-grain sorghum. This observation is something that should be studied more precisely to validate if grain sorghum in flour form is nutritional equal to wheat in flour form or if whole-grain sorghum is nutritional equal to whole-grain wheat as a carbohydrate source in Rainbow trout diets".

Objective #4 - Conduct economic analysis of results from industrial context.

Based upon the current data generated in this study, it appears as a substitute carbohydrate source, sorghum can be incorporated into commercial RBT diets at about 10%. This will certainly become of greater interest as more producers potentially target production of non-GMO products. If RBT are used as a reference species for salmonids in general (*Salmo salar*), this would permit market extrapolation of sorghum grain to the larger salmon production sectors. This sector showed 2022 production levels of approximately 2.8 million tonnes (https://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1640993/) . If we

utilize a very conservative 1.1 FCR, at 10% dietary inclusion, that yields about 3.08 million tonnes of feed needed on an annual basis, or potentially 380,000 tonnes of sorghum per year.

Furthermore, this data helps validate the current ongoing initiatives to optimize and test novel sorghum protein concentrates in RBT as a salmonid reference species, tilapia as a warm water/freshwater omnivore, and pompano as a target warmwater marine species.

Outreach deliverables

Peer-Reviewed Publication:

> Zarei M, Amirkolaei AK, Trushenski JT, Sealey WM, Schwarz MH, Ovissipour R. Sorghum as a Potential Valuable Aquafeed Ingredient: Nutritional Quality and Digestibility. Agriculture. 2022 May 6;12(5):669.

United States Aquaculture Society:

Presentations: Zarei, M., M.H. Schwarz, R. Ovissipour. Extraction Yield, Amino Acid Composition, and Functional Properties of Protein Extracted From Sorghum by Alkaline and NaOH-Ethanol-Reducing Agents (NER) Methods. Aquaculture America 2022. (February 28, 2022) **Abstract Published**

> Significant inquiries and discussions were held throughout the 4-day conference on sorghum and Sorghum Protein Concentrate opportunities in aquafeeds.

Tradeshow Booth**:** The Virginia Seafood AREC hosted a booth at both Aquaculture America 2022 (San Diego, CA), and 2023 (New Orleans, LA), promoting the potential benefits and uses for sorghum in SmartFeeds for aquatic species. The USAS meetings draws thousands of producers and industry partners to examine the latest trends and technologies in aquaculture. Outreach materials highlighting sorghum developed and displayed at the event included a large poster and flyers with photos supplied by the United Sorghum Checkoff Program and an overview of the versatility of the crop, potential for new markets, and applications for developed SmartFeeds.

Virginia Ag Expo 2021 and 2022: The Virginia Seafood AREC highlighted work with controlled environment agriculture and the benefits and uses for sorghum in SmartFeeds at the Virginia Ag Expo, Virginia's largest agricultural exhibit. The expo draws thousands of producers and industry partners to examine the latest trends and technologies in agriculture. For the events, VSAREC staff coordinated with partners to advertise a line-up of scheduled speakers to draw producers to the tent. In a presentation led by Dr. Michael Schwarz, attendees were exposed to applications for CEA-sorghum for SmartFeeds for aquaculture. There was significant interest from farmers regarding this work, and potential for sorghum grain production expansion in VA, as well as from an aquafeed and food perspective.

Event promotion included a story on the Virginia Tech news site, Virginia Seafood AREC's newly redesigned website homepage, as well as the Facebook and Twitter accounts developed over the summer. Center social media posts alone garnered over 1,300 impressions or views on users' social media feeds.

Outreach materials highlighting sorghum developed and displayed at the event included a large poster and flyers with photos supplied by the United Sorghum Checkoff Program and an overview of the versatility of the crop, potential for new markets, and applications for developed SmartFeeds. The tent, co-hosted by the Seafood AREC was one of the largest displays organized at the expo, which event organizers estimate drew together 1,200 attendees.

Social Media: Sorghum grain promotion of this event included a story on the Virginia Tech news site, Virginia Seafood AREC's newly redesigned website homepage, as well as the Facebook and Twitter accounts developed over the summer. Center social media posts alone garnered over 1,300 impressions or views on users' social media feeds.

Virginia Aquaculture Conference: 2022 and 2023:

Presentation: Schwarz, M.H., R. Ovissipour, J. Trushenski, W. Sealey, J. van Senten, L. Duscher, M. Zarie, K. Rouse, S. Urick, E. McAlhaney, and T. Rose. 2022. United Sorghum Checkoff Program – SmartFeeds Programming. Virginia Aquaculture Conference. Newport News, VA. January 7-8, 2022.

VSAREC faculty/staff highlighted Sorghum in SmartFeeds in a tradeshow booth. Faculty used this opportunity to connect directly with the aquaculture industry to expose producers to new developments in technology and research for sorghum and SmartFeeds for aquaculture applications. Sorghum posters and flyers, and samples were demonstrated at a trade show booth.

Controlled Environment Agriculture Summit: October 25-26, 2022; AND September 19-20, 2023. VSAREC faculty/staff highlighted Sorghum in SmartFeeds in a tradeshow booth. In addition to significant Sorghum promotional materials, samples, displays, and fliers, we enjoyed significant interest in sorghum as a NON-GMO feed ingredient. Numerous attendees are now aware of the collaborative USCP work, and advised will be monitoring our website for updates and information.

In Addition:

Numerous presentations about the opportunities of sorghum as a value-add resource conserving ingredient in aquafeeds were given: Dr. Jesse Trushenski to the USCP board; Dr. Delbert Gatlin to the (June 2023 Export Sorghum event in Houston, TX to global sorghum buyers) Dr. Reza Ovissipour at a departmental seminar at the Virginia Tech Department of Food Science and Technology; and numerous outreach extension presentations by Dr. Schwarz with public and private aquaculture sector stakeholders.

Sealey, W.M., Zarei, M., Ovissipour, R., Urick, S., McAlhaney, E., Trushenski, J., Crafton, B. and Schwarz, M. (Invited) The Suitability of Sorghum for Rainbow Trout Feeds. USTF. September 2023.

Sealey, W.M., Zarei, M., Ovissipour, R., Urick, S., McAlhaney, E., Trushenski, J., Crafton, B. and Schwarz, M. Investigation of the Nutritional Value of Sorghum for Rainbow Trout. Fish Feeds and Nutrition Workshop July 2023.

Rouse, K., J. van Senten, M.H. Schwarz, R. Ovissipour. 2022. Virginia Seafood AREC Sorghum Poster. Virginia Agriculture Expo. August 5. (Poster)

Schwarz, M.H. 2021. Controlled Environmental Agriculture. CAIA Lightning talk. Nov 17. Heavy focus on SmartFeeds, Sorghum, and Sorghum Protein Concentrates.

Schwarz, M.H., J. van Senten, R. Ovissipour, R. Lane, K. Parraga-Estrada. 2021. Virginia Seafood Agricultural Research and Extension Center. VT Agriculture Experiment Station External Review. Virginia Beach, VA. October 25. Heavy focus on SmartFeeds and review of collaborative USCP programming with potential to enhance the Virginia agriculture sector.