Proposal No. 2011_004_RC

The Andersons Research Grant Program

Project Title: Identification of factors related to sorghum protein quality

Principal Investigator(s)

Name	Institution/Agency/Other
Scott Bean	USDA-ARS CGAHR
Collaborators	
Tom Herald	USDA-ARS CGAHR
Jianming Yu	Agronomy Dept., Kansas State Univ.
Tesfaye Tesso	Agronomy Dept., Kansas State Univ.

(Attach an additional sheet if more space is needed.)

Project Contact (list one person to act as the primary contact):

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Period of Proposed Project Dates:

Beginning: Sept 2011 Ending: Aug 2013

Amount Requested (maximum $25,000 per year for two years):

Year 1: $24,825 Year 2: $24,900

Problem Identification and Related Research

Sorghum is one of the most drought resistant cereal grain crops and requires few inputs during growth. With increasing world populations and decreasing water supplies, sorghum represents an important crop for future human use. Sorghum is the third leading cereal grain in the U.S., behind wheat and maize. In recent years, however, sorghum production has declined in the U.S., with the total number of acres of sorghum harvested decreasing from ~12 million in the 1989 to ~ 6 million in 2009. Sorghum is an important crop in dry regions of the southern Great Plains, which contains many rural areas that are both culturally and economically dependent on agriculture. Continued use of sorghum for traditional uses such as animal feed and developing new uses that add value and enhance economic opportunities for the U.S. sorghum crop could play a critical role in the economic vitality of these regions.

Potential new uses of sorghum include bio-fuels, human foods, and new animal feed markets. The use of sorghum for ethanol production has steadily increased from 1-3% a few years ago to an estimated 30-35% in 2009. Production of wheat-free foods from sorghum for the celiac market may create substantial new markets for the sorghum industry. The wheat-free food market was estimated at $1.7 billion in 2007 and has experienced dramatic increases in value and the number of products available. There are ~3 million people in the U.S. with celiac disease and as much as 8% of the U.S. population may be eating wheat-free diets. Sorghum is a safe food for celiac patients and as such there is great potential in this market for sorghum based foods.

A limitation to the current utilization of sorghum and to the development of new uses of sorghum is protein quality, specifically with regards to protein digestibility. Two major findings have emerged from the research on sorghum proteins with regards to digestibility. First, the reduced digestibility of sorghum proteins is related to cross-linked protein complexes in both the raw grain and in cooked flour, with disulfide bonds the major type of crosslink (Duodu et al. 2003). Cooking appears to increase the amount of cross-linked proteins in sorghum to a greater degree than similar cereals such as maize (Hamaker and Bugusu 2003, Choi et al. 2008, Ezeogu et al 2008). When cooked, sorghum proteins form a web-like structure that encapsulates starch; this does not occur, in the closely related cereals maize and rice (Hamaker and Bugusu 2003). Protein cross-linking also differs between the vitreous and floury endosperm (Mazhar and Chandrashekar 1993, Ioerger et al. 2007). Second, reduced digestibility of sorghum is related to the protein body structure in the grain. In normal sorghum, the major class of the grain proteins (called kafirins) are found in spherical protein bodies. The outer edges of these protein bodies are composed of mainly β- and γ-kafirins which are high in cysteine and thought to be highly cross-linked into a digestion resistant shell (Shull et al. 1992). The interior of the protein bodies contain mainly α-kafirins, which are easily digestible, but protected by the outer highly cross-linked shell (Duodo et al. 2003).

Sorghum lines have been identified in mutant populations where the protein bodies are misshaped (Weaver et al 1998, Oria et al. 2000; Tesso et al. 2006). The exact reason for this is not known, but the γ-kafirins appear to be non-uniformly distributed around the outer edges of the protein bodies which may mean the protein cross-linking in the mutants is not as extensive as in wild type sorghums. 2011_004_RC

Protein cross-linking and protein body morphology have clearly been related to digestibility in sorghum, especially in mutant sorghum lines. However, several other factors have been hypothesized to play a role in digestibility of sorghum proteins including grain structure, phytate levels, phenolic compounds, protein body size, etc. (Duodu et al. 2003). Little research has been done on large, diverse sample sets to conclusively determine exactly what factors are related to sorghum protein digestibility. While the use of high digestible mutants appears promising to improve sorghum protein quality, these lines often have undesirable grain quality traits though progress has been made to address this issue (Tesso et al. 2006). Genetic engineering has also been used to improve the quality of sorghum proteins (da Silva et al. 2011). Future use of both mutant lines and genetically modified sorghum lines offers the potential to improve sorghum protein quality. Naturally occurring germplasm that has improved protein digestibility can be used now in traditional breeding programs however. Thus, identification of sorghum germplasm with improved protein digestibility in wild type sorghum populations is a viable method that can be used to increase the end-use quality of sorghum. Likewise, determining the factors that are directly related to protein digestibility in wild type sorghum populations will enable the screening of diverse sample sets to aid in identifying lines that can be used to increase the overall protein quality of sorghum.

Objectives

The objectives of this project fit NC 213 objective 1 “to characterize quality attributes...” The ultimate goal of this project is to provide the means to increase the protein quality of sorghum, a key factor determining the end-use quality of sorghum. This goal will be achieved by accomplishing the following specific objectives: 1) screening of a genetically diverse sorghum population (n~300) to identify germplasm with intrinsically high protein digestibility levels, and 2) determine the factors that govern digestibility in wild type sorghum lines.

Procedures

Objective 1

To provide the necessary variability in grain properties needed for this research, a genetically diverse population of sorghum samples (Casa et al. 2008) (hereafter referred to as the “sorghum diversity panel”) will be used as the initial sample population. The sorghum diversity panel was created to “span the genetic diversity of sorghum” and contains ~300 samples. Samples of the diversity panel grown in two

locations will be used. The diversity panel will be screened for digestibility using the method of Mertz et al. (1984). Based on preliminary screening of 50 samples of the diversity panel, we have found samples that range from ~30% to 70% digestibility indicating that suitable variability in digestibility exists within this sample set. Tannin containing samples will not be evaluated as tannins reduce digestibility via binding to kafirin proteins and inactivating digestive enzymes (the diversity panel has been screened for the presence of tannins; approximately 25% of the samples were found to contain tannins). Thus the presence of tannin containing samples would confound the results of this project.

Objective 2

Based on the initial screening of the diversity panel, 30-40 unique samples that span the range of digestibility values will be selected for additional research to determine what factors govern protein digestibility in "normal" sorghum lines.

Protein composition of the selected samples will be analyzed using multiple techniques. Overall protein composition will be measured using an "Osborne" type fractionation based on the method of Taylor et al. (1984) which will determine the amount of albumin/globulin, kafirin, and glutelin proteins. Briefly, albumin and globulins will be extracted from ground sorghum together as one class using 1.0 M NaCl. After a brief water wash to remove residual salt, "kafirin 1" or "soluble" kafirins will be extracted using 60% t-butanol. Kafirin 1 is composed of mainly monomeric kafirin proteins and small amounts of kafirins disulfide bonded into polymeric protein complexes (Ioerger et al. 2007). "Kafirin 2" will then be extracted with 60% t-butanol with sonication to extract the remaining kafirin proteins. Kafirin 2 is composed mainly of large polymeric complexes of kafirins, thought to be cross-linked together via disulfide bonds (Ioerger et al. 2007). The ratio of kafirin 1 to kafirin 2 provides information on the degree of cross-linking present in the kafirin proteins and information about the molecular weight distribution of the protein complexes. Similar extraction schemes are used to gain information about the molecular weight distribution of wheat protein complexes (Southan and MacRitchie 1999). The remaining residue will then be extracted with a Tris-borate pH 10 buffer containing 1% SDS and 2% β-mercaptoethanol to remove glutelin proteins. All samples from the above "Osborne" fractionation scheme will be separated by size exclusion chromatography (SEC) to determine the amount of protein present in each class. Kafirin 1 and 2 extracts will be further characterized by SEC coupled with multi-angle light scattering (MALS) to determine the M_w of the polymeric protein complexes present in these samples. This will allow us to correlate not only the amount of the polymeric proteins present, but also their size. As protein cross-linking is thought to play a key role in the morphology of sorghum protein bodies, this will make it possible to relate protein cross-linking to digestibility.

Composition of the kafirin 1 and kafirin 2 extracts will be measured using reversed-phase high performance liquid chromatography (RP-HPLC) (Bean et al. 2010)

and high performance capillary electrophoresis (HPCE) (Bean et al. 2000). Data from the RP-HPLC, HPCE, and SEC separations will be correlated (using simple correlation statistics) to protein digestibility to identify which proteins or protein complexes are key in determining digestibility.

At least two true replicates (not simply sub-samples) for each analysis will be conducted. Data will be analyzed using ANOVA and regression procedures to relate physical and biochemical properties to protein quality attributes.

In addition to the above protein analysis, several other factors that have been proposed to influence protein digestibility in sorghum will be evaluated. Physical grain traits will be measured using the single kernel characterization system (SKCS) with optimum settings for measuring grain hardness, weight, and diameter (Bean et al. 2006). The Folin-Ciocalteau assay will be employed to determine the total phenolic content of the selected samples to determine if the levels of phenolic compounds in sorghum influences protein digestibility. If significant correlations between phenolic content and digestibility are found, then the composition of the phenolic compounds will be determined using the HPLC method of Hahn et al. (1983). Phytate levels will be measured a commercially available colorimetric assay (Megazyme, Ireland).

Screening of the diversity panel and protein analysis as well overall project planning and data analysis will be the responsibility of Dr. Bean. Dr. Herald will be responsible for measuring phenolic content and composition. Dr's Tesso and Yu will provide the samples from the sorghum diversity panel as well as advice in selecting the subset of samples used for Objective 2 to insure that a wide genetic base is included in the subset.

Pitfalls and Limitations:

If attempts to correlate protein digestibility to kafirin composition and other grain properties are not successful, this would indicate that we are not searching for the right protein properties. If this occurs, we will repeat the experiments using isolated protein bodies of sorghum. It is possible that the cross-linking is related to either specific properties of the protein bodies themselves or by a protein in the protein matrix surrounding the protein bodies. Working with whole ground meal could then mask the effect of one of these components. Based on previous work by Taylor and Hamaker's groups we do not expect this, but if that turns out to be the case, working with isolated protein bodies may provide the necessary data. Another possibility is that cross-linking is not related to an inherent property of the proteins themselves, but rather to an endogenous small molecule such as glutathione. This has been researched as a factor in the cross-linking of wheat proteins for example. If working with kafirins or isolated protein bodies does prove our hypothesis to be false, we will use methods developed on wheat to begin investigating the possibility that other factors such as oxidants in the grain are responsible for the cross-linking.

Anticipated results, products, and impacts

This project will result in 1) identification of germplasm with intrinsically high levels of protein digestibility which can be used to breed sorghum for improved end-use quality; 2) determination of the factors that govern protein digestibility in sorghum which can then be used to help select sorghum lines with desired protein quality. Products from this proposal would include the protein digestibility data for the diversity panel which could be used by the sorghum breeding community/industry as well as scientific publications describing the relationships between grain traits, protein properties, and digestibility. The overall impact of this project would be the knowledge to improve sorghum end-use quality at the genetic level by improving protein digestibility of sorghum. Such improvements would increase the quality of sorghum for animal feed, bio-fuel production, and human nutrition. Improvements in sorghum protein quality would impact the animal feed industry immediately as well as the bio-fuels industry (protein quality has been linked to ethanol fermentation efficiency). In the longer term, improving sorghum protein quality would have major impact on developing countries that rely on sorghum as a basic food staple and could greatly impact US food aid programs.

Leveraging Resources

Results of this research project will be used in grant applications (e.g. NC 213 Anderson Team Grant, NSF-BREAD) seeking to complete association trait mapping of grain quality attributes and protein quality measured in this study. Genetic mapping of sorghum protein quality will speed the development of additional high digestible sorghum lines. Successful mapping of protein related traits will also pave the way to map other traits in sorghum related to starch properties as well as phenolic compounds.

Timetable

	Year 1												Year 2											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Objective 1																2011_004_RC								
Hiring of personnel																								
Preliminary sample preparation (n=900)																								
Screen diversity panel for digestibility (n=900)																								
Data analysis and sample selection for Objective 2																								
Progress report																								
Objective 2																								
Osborne fractionation and Kafirin Analysis																								
Determination of Phytate Levels																								
Determination of Phenolic compounds																								
Characterization of grain traits																								
Data analysis and manuscript preparation																								
Progress Report																								

Literature Cited

Bean, S. R., Lookhart, G. L., and Bietz, J. A. 2000. Acetonitrile as a buffer additive for the separation of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) storage proteins by HPCE. J. Agric. Food Chem. 48, 318-327.

Bean, S. R., Chung, O. K., Tuinstra, J. F., and Erpelding, J. 2006. Evaluation of the Single Kernel Characterization System (SKCS) for Measurement of Sorghum Grain Attributes. Cereal Chem. 83:108-113.

Bean, S. R., Ioerger, B. P., and Blackwell, D. L. 2010. Separation of kafirins on surface porous reversed phase-high performance liquid chromatography columns. J. Agric. Food Chem. 59: 85-91.

Casa, A.M., Pressoir, G., and Brown, P. J., Mitchell, S. E., Rooney, W. L., Tuinstra, M. R., Franks, C. D, and Kresovitch, S. 2008. Community resources and strategies for association mapping in sorghum. Crop Sci. 48: 30-40.

Choi, S. J., Woo, H. D., Ko, S. H., and Moon, T. W. 2008. Confocal laser scanning microscopy to investigate the effect of cooking and sodium bisulfate on in vitro digestibility of waxy sorghum flour. Cereal Chem. 85:65-69.

da Silva, L. S., Jung, R., Zhao, Z., Glassman, K., Taylor, J., and Taylor, J. 2011. Effect of suppressing the synthesis of different kafirin sub-classes on grain endosperm texture, protein body structure and protein nutritional quality in improved sorghum lines. J. Cereal Sci. 54:160-167.

Duodu, K.G., Taylor, J.R.N., Belton, P.S., and Hamaker, B.R. 2003. Factors affecting sorghum protein digestibility. J. Cereal Sci. 38:117-131.

Ezeogu, L. I., Duodu, K. G., Emmambux, N., and Taylor, J.R.N. 2008. Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. Cereal Chem. 85:397-402.

Hahn, D.H., Rooney, L.W., and Faubion, J.M., 1983. Sorghum phenolic acids, their HPLC separation and their relation to fungal resistance. Cereal Chemistry 60: 255–259.

Hamaker, B.R., and Bugusu, B. A., 2003. Overview: Sorghum proteins and food quality. Proceedings of AFRIPRO: Workshop on the proteins of sorghum and millets: Enhancing nutritional and functional properties for Africa. http://www.afripro.org.uk/ Last accessed 8/31/2011.

Ioerger, B., Bean S. R., Tuinstra, M. R., Pedersen, J. F., Erpelding, J., Lee, K., Herrman, T. 2007. Characterization of polymeric proteins from vitreous and floury sorghum endosperm. J. Agric. Food Chem. 55:10232-10239.

Mazhar, H., and Chandrashekar, A. 1993. Differences in kafirin composition during endosperm development and germination in sorghum cultivars of varying hardness. Cereal Chem. 70:667-671.

Mertz, E., Hassen, M., Whitten, C., Kirleis, A., Tu, L., and Axtell, J. 1984. Pepsin digestibility of proteins in sorghum and other major cereals. Proc. Natl. Acad. Sci. 81:1-2.

Shull, J.M, Watterson, J. J., and Kirleis, A.W. 1992. Purification and immunocytochemical localization of kafirins in *Sorghum bicolor* (L. Moench) endosperm. Protoplasma 171: 64-74.

Southan, M., and MacRitchie, F. 1999. Molecular weight distribution of wheat proteins. Cereal Chem. 76: 827-836.

Taylor, J., Schussler, L., and van der Walt, W. H. 1984. Fractionation of proteins from low-tannin sorghum grain. J. Agric. Food Chem. 32:149-154.

Tesso, T., Ejeta, G., Chandrashekar, A., Huang, C-P., Tandjung, A., Lewamy, M., Axtell, J.D., and Hamaker, B.R. A novel modified endosperm texture in a mutant high-protein digestibility/high-lysine grain sorghum (Sorghum bicolor (L.) Moench). Cereal Chem. 2006: 83, 194-201. 2011_004_RC

Weaver, C. A., Hamakar, B. R., and Axtell, J.D. 1998. Discovery of grain sorghum germplasm with high uncooked and cooked in vitro protein digestibilities. Cereal Chem. 75: 665-670.

CV
Scott Bean

EDUCATION:

- Ph.D. in Grain Science, Kansas State University, December 2011 2011_004_RC
  Dissertation Title: *Improving high performance capillary electrophoresis methods for characterizing the proteins of wheat, barley, oats, rice, maize, and sorghum.*

- MS in Grain Science, Kansas State University, May 1996
  Thesis Title: *Development of High Performance Capillary Electrophoresis for the Separation of Cereal Proteins.*

- BS in Biology, Kansas state University, May 1993

WORK EXPERIENCE:

- October 2001-present
- Research Chemist, USDA-ARS, CGAHR, Manhattan, KS
- Graduate Research Associate, Dept. of Grain Sci. and Industry, Kansas State University, August 1994-October 2001

HONORS AND AWARDS:

- Magel McMaster's Graduate Achievement Award, March 1998.
- USDA-ARS Early Career Scientist Award, 2008

PROFESSIONAL EXPERIENCE:

- Adjunct Professor, Kansas State University Agronomy Department, 2002-present
- Associate Editor for *Cereal Chemistry*, 2003-2009
- Member of Journal of Cereal Science Editorial Board, 2009-present
- Member of the scientific advisory board for the Celiac Sprue Association, 2009-present

GRANTS:

- NC 213 Anderson Team Grant "Factors governing Processing Suitability of Sorghum and Maize" $150,000/2 years. [Co-PI]
- USDA NIR "Sorghum as a Viable Renewal Resource for Biofuels and Biobased Products" $450, 000/3 years. [Co-PI]
- USDA-ARS HQ Post-doctoral research associate "Sorghum Endosperm Protein Cross-linking and digestibility" 2008. $100,000/2 years. [PI]
- KSU-Center for Sorghum Improvement "Relationship between grain mold, physiochemical kernel properties, and grain quality in sorghum" 2009. $30,000/1 year [Co-PI]

- United Sorghum Checkoff Program "Developing Healthy Foods from Special Sorghums" 2009. $159,000/1 year [Collaborator]
- Kansas Grain Sorghum Commission, "Effect of decortication on feed quality of sorghum DDGS" 2010. $20,000/1 year [PI]
- United Sorghum Checkoff Program, "Effect of heating on quality of sorghum DDGS." 2010. $20,000/1 year. [PI]
- Kansas Grain Sorghum Commission, "Developing sorghum flours with increased resistant starch content for health benefits." 2011. $38,000/1 year [collaborator]
- Kansas Grain Sorghum Commission, "Effect of starch content on the functional quality of sorghum." 2011. $12,364/1 year [Co-PI]

RELEVANT PUBLICATIONS:

Bean, S. R., Lookhart, G. L., and Bietz, J. A. 2000. Acetonitrile as a buffer additive for the separation of maize (Zea mays L.) and sorghum (Sorghum bicolor L.) storage proteins by HPCE. J. Agric. and Food Chem. 48, 318-327.

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Park, S. H., and Bean, S. R. 2003. Investigation and optimization of the factors influencing sorghum protein extraction. J. Ag. Food Chem. 51: 7050-7054.

Corredor, D. Y., Bean, S. R., Schober, T., and Wang, D. 2006. Effect of Decorticating Sorghum on Ethanol Production and Composition of DDGS. Cereal Chem. 83:17-21.

Bean, S. R., Chung, O. K., Tuinstra, J. F., and Erpelding, J. 2006. Evaluation of the Single Kernel Characterization System (SKCS) for Measurement of Sorghum Grain Attributes. Cereal Chem. 83:108-113.

Taylor, J., Schober, T., Bean, S. R. 2006. Non-traditional uses of sorghum and pearl millet. J. Cereal Sci. Journal of Cereal Science 44: 252-271.

Ioerger, B., Bean S. R., Tuinstra, M. R., Pedersen, J. F., Erpelding, J., Lee, K., Herrman, T. 2007. Characterization of polymeric proteins from vitreous and floury sorghum endosperm. J. Agric. Food Chem. 55:10232-10239.

Zhao, R. Bean, S. R., Ioerger, B. P., Wang, D., and Boyle, D. L. 2008. Impact of mashing on sorghum proteins and its relationship to ethanol fermentation. J. Agric. Food Chem. 56:946-953.

Bean, S. R., Ioerger, B. P., and Blackwell, D. L. 2010. Separation of kafirins on surface porous reversed-phase high-performance liquid chromatography. J. Agric. Food Chem. 59:85-91.

Current and pending

CURRENT & PENDING SUPPORT

Name:

Instructions:
Who completes this template: All individuals contributing to this research.
How this template is completed:

- Record information for active and pending projects, including this proposal.
- All current efforts to which individuals contributing to the research have committed a portion of their time must be listed, whether or not salary for the person involved is included in the budgets of the various projects.
- Provide analogous information for all proposed work which is being considered by, or which will be submitted in the near future to, other possible sponsors, including other programs.
- For concurrent projects, the percent of time committed must not exceed 100%.

NAME	SUPPORTING AGENCY/SPONSOR AND AGENCY ACTIVE AWARD/PENDING PROPOSAL NUMBER	TOTAL $ AMOUNT	EFFECTIVE AND EXPIRATION DATES	% OF TIME COMMITTED	TITLE OF PROJECT
	CURRENT:				
Jianming Yu Tesfaye Tesso	Kansas Grain Sorghum Commission	$52,000	3/1/11 - 9/30/11	20% Tesso 5% Yu	Screening Sorghum Germplasm for Abiotic Stress Tolerance and Biofuel Production
Jianming Yu Tesfaye Tesso	Kansas Grain Sorghum Commission	$60,000	3/1/11 - 9/30/11	15% Tesso 20% Yu	Genetic Analysis of Drought Tolerance in Grain Sorghum
Jianming Yu Tesfaye Tesso	Kansas Grain Sorghum Commission	$81,200	3/1/11 - 9/30/11	20% Tesso 5% Yu	Breeding Grain Sorghum for Improved Dryland Production
Jianming Yu	US DOE thru University of Nebraska 25-6222-0377-002	$139,704	9/15/09 - 9/14/12	20%	Characterization of Nitrogen Use Efficiency in Sweet Sorghum
Jianming Yu	NSF thru Cornell University 55838-8806	$737,737	9/1/11 - 8/31/14	20%	Genomic Analyses of Shoot Meristem Function in Maizes
Jianming Yu Tesfaye Tesso	DOE/USDA Plant Feedstock Genomics for Bioenergy	$800,000	9/1/11 - 8/31/14	15% Tesso 20% Yu	Sorghum Biomass Genomics and Phenomics
	PENDING:				
Bean	Kansas Grain Sorghum Commission	$30,000	9/1/11 - 9/30/12	10%	Assessment of Grain Quality Traits Related to Cold Tolerance in Sorghum
Bean	Kansas Grain Sorghum Commission	$38,000	9/1/11 - 9/30/12	10%	Developing Sorghum Flours with Increased Resistant Starch Content for Health Benefits
Jianming Yu Tesfaye Tesso	Kansas Grain Sorghum Commission	$52,000	9/1/11 - 9/30/12	20% Tesso 5% Yu	Screening Sorghum Germplasm for Abiotic Stress Tolerance and Biofuel Production
Jianming Yu Tesfaye Tesso	Kansas Grain Sorghum Commission	$100,000	9/1/11 - 9/30/12	15% Tesso 20% Yu	Genetic Analysis of Drought Tolerance in Grain Sorghum
Jianming Yu Tesfaye Tesso	Kansas Grain Sorghum Commission	$81,200	9/1/11 - 9/30/12	20% Tesso 5% Yu	Breeding Grain Sorghum for Improved Dryland Production
Bean	United Sorghum Checkoff Program	$30,000	9/1/11 - 9/30/12	10%	Grain Quality Evaluation of white-tan sorghum hybrids
Bean	NC 213 Anderson Grant	$49,725	9/1/11 - 8/31/13	20%	Identification of factors related to sorghum protein quality

Budget

ANDERSONS RESEARCH FUND - RESEARCH PROPOSAL BUDGET

Category	Year 1 Amt. requested from Andersons	Year 2 Amt. requested from Andersons	Total
Salaries and Wages*			
Post-Ph.D. research associate(s)			
Graduate assistant			
Stipend			
Tuition and fees			
Hourly wage			
Other (specify in Budget Narrative)	$12,750	$8811	
Total	$12,750	$8811	$21,561
Fringe Benefits			
Post-Ph.D. research associate(s)			
Graduate assistant			
Hourly wage			
Other	$1575	$1089	
Total	$1575	$1089	$2,664
Materials and Supplies	$10,000	$14,000	$24,000
Equipment (List individual pieces of equipment that are essential to the project in the Budget Narrative.)			
Travel	$500	$1000	$1,500
Publication charges			
Indirect costs**			
Total (Max. $25,000/yr from Andersons Research Grant Program)	$24,825	$24,900	$49,725

*Andersons funds cannot be used for faculty salaries, departmental space, or facilities.

**The Andersons Research Grant Program policy specifies that no indirect costs can be charged to this project.

Budget Narrative

For Year 1, salary ($12, 750) will be used to hire a temporary lab assistant (30 hr/week for 36 weeks) whose primary duty will be to screen the diversity panel for protein digestibility. The $10,000 requested for year 1 materials and supplies will be used to cover the actual cost of the protein digestibility assays (enzyme, buffer, tubes, and protein analysis). The $500 in travel requested will be used to attend the NC 213 annual conference.

For Year 2, salary ($8811) will be used to hire a temporary lab assistant (20hr/week for 36 weeks) to assist in the biochemical characterization of the samples and to run grain kernel characterization, phytate, and phenolic content assays. The $14,000 in materials and supplies will be used to purchase HPLC columns for protein and phenolic work, HPLC solvents, phytate assay kits, and routine lab supplies (gloves, tubes, reagents, etc). The $1,000 in travel requested will be used to attend the NC 213 annual meeting as well as to attend a national/international meeting such as AACC or ACS to present the findings of this research to a wider audience.