

***Human Health  
Flax Canada 2015  
Project Overview***

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**I. OPTIMIZING DIETARY N6/N3 FATTY ACIDS IN DISEASE - OXIDATIVE STRESS AND INFLAMMATION IN ADULTS AT RISK FOR TYPE 2 DIABETES**

**PRINCIPLE INVESTIGATOR:**

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The focus of this research is the potential beneficial effects of a diet high in monounsaturated fatty acids (oleic acid – OA) from canola oil and alpha linolenic acid (ALA) from flax oil in reducing markers of oxidative stress and inflammation in overweight subjects at risk for type 2 diabetes. The goal of this research is to develop and promote science-based recommendations for dietary fatty acid intakes that prevent or delay the onset of chronic diseases, such as diabetes and other immune and inflammatory disorders, through reducing the burden of oxidative stress and generation of inflammatory mediators. Our specific foci is the role of dietary fat composition in modulating cell membrane lipids, consequently impacting cell signalling, the generation of inflammatory mediators, and balance between nutrition and oxidative stress.

This research focuses on the importance of dietary OA and ALA as important modulators of membrane lipid compositions, oxidative stress and the generation of inflammatory mediators; key mediators in the early events leading to a pro-inflammatory state, development of insulin resistance, hyperglycemia, hyperlipidemia and the multiple events associated with diabetes, CVD and obesity.

A major limitation of current studies relating inflammatory mediators to the development of diabetes and CVD is the possibility of reverse causality, that is the markers of inflammation are not causally related to the development of diabetes or obesity, but rather diabetes and obesity develop and subsequently lead to upregulation of inflammatory mediators. This research aims to provide definitive evidence of causal relation through demonstrating that dietary intervention to correct aberrant fatty acid intakes will reduce inflammatory mediators. This will pave the way for longer term intervention to consider improved insulin sensitivity and delay in the onset of the manifestations and complications of type 2 diabetes.

The design employs a control diet resembling current dietary patterns with respect to saturated fat, monounsaturated, n-6 and n-3 fatty acids, with two modifications: first to reduce n-6 LA in and increase monounsaturated fat, and second to increase ALA and thus also decrease the LA/ALA ratio. A reduction in saturated fat is also inherent in proposed design. While consistent with current dietary recommendation, it could argued that any beneficial effects of the intervention are due to reducing saturated fats rather than reducing LA or the LA/ALA ratio. However, the measures of lipid peroxidation and n-6 fatty acid derived prostanoids directly address the effect of reducing LA. We predict

the decrease in LA and increase in monounsaturated fat will result in decreased markers of oxidative stress, while the additional increase in ALA will reduce markers of inflammation and pro-inflammatory cytokines, and ARA derived isoprostanes.

## **II. FLAXSEED EFFECTS ON PERIPHERAL ARTERIAL DISEASE AND DIABETES**

### **PRINCIPLE INVESTIGATOR:**

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It is becoming increasingly evident that cardiovascular disease is strongly influenced by dietary habits. Poor diets rich in, for example, trans fats, can have a significant negative impact upon clinical cardiovascular health. It is not surprising, therefore, that we are slowly beginning to realize that the corollary is also true: beneficial nutritional interventions can influence cardiovascular disease in a positive manner. A common dietary intervention used to alter cardiovascular disease today is the supplementation of the diet with omega-3 fatty acids. Fish are highly enriched in these fatty acids. Diets including fish reduce the severity of cardiovascular disease in both animal and human trials. However, dietary compliance for fish supplementation is a problem. The taste of fish oil is not generally well accepted. Thus, alternative sources of omega-3 fatty acids in the diet are welcome. One alternative source is flaxseed. It has a pleasant nutty flavor and is one of the richest plant sources of omega-3 fatty acids. Furthermore, flaxseed also contains lignans that are potent antioxidants and fibre that is known to be beneficial in controlling circulating cholesterol levels. Thus, it is possible that flaxseed supplementation may provide dramatic beneficial effects on cardiovascular health and be tolerated in the diet of the general public. Animal studies have shown dramatic anti-atherogenic, anti-arrhythmic and anti-inflammatory effects of dietary flaxseed. These studies have argued strongly for the initiation of a trial in a clinical population that may be helped by the cardiovascular effects of dietary flaxseed. This study represents the first trial of the influence of a flaxseed enriched diet on any aspect of cardiovascular disease in humans.

Our proposal is a two year, double blinded, placebo controlled study designed to examine and compare the effects of ground flaxseed supplementation in volunteers with claudication secondary to lower extremity atherosclerotic arterial disease (peripheral arterial disease). This patient population is ideal to receive the benefits of flaxseed. It suffers from accelerated atherosclerosis and a higher than normal incidence of arrhythmias, myocardial infarctions and stroke. Our study will be focused on the capacity of flaxseed to reduce clinical symptoms of the disease and increase exercise capacity in these patients.

Two general hypotheses will be tested in this proposal. First, we hypothesize that fewer primary and secondary clinical events (mortality, stroke, myocardial infarctions, angina, arrhythmias) will be observed in patients who are ingesting flaxseed in their diet. Secondly, we hypothesize that flaxseed supplementation of the diet will induce beneficial

effects on exercise performance, blood pressure and circulating lipid levels. This trial will generate data on the safety and cardiovascular efficacy of dietary flaxseed in patients with peripheral arterial disease (PAD). It will study the genomic response of this population of patients to this nutritional intervention. Ultimately, this study represents the first test of flaxseed as a cardioprotective nutritional agent in a patient population.

The general public is very interested in these types of trials. This study has a great potential to help patients suffering from PAD. Furthermore, it has the unusual potential to be rapidly translated into applications for the general public to provide health-related benefits to a large component of our public at a relatively inexpensive cost with dramatic changes in the quality of life of patients with coronary artery disease. There are also significant opportunities for economic spin-offs with large multi-national nutrition companies that may wish to develop such products in the future. We are uniquely situated at St Boniface General Hospital Research Centre to contribute to this project.

### **III. HIGH OLEIC CANOLA – FLAX AND HIGH OLEIC OIL BLEND AND EFFECTS ON CARDIOVASCULAR DISEASE AND ENERGETICS**

#### **PRINCIPLE INVESTIGATOR:**

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Canada Research Chair in Nutrition and Functional Foods

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#### *Alpha Linolenic Acid and Inflammation:*

Recent studies have focused upon alpha linolenic acid's (ALA) favorable effects on vascular inflammation and endothelial dysfunction. Increasing interest in the unique health attributes of ALA especially related to vascular endothelial activation have stimulated research into novel biomarkers of inflammation and endothelial cell function.

#### *Alpha Linolenic acid and Energy Metabolism:*

Isotopic tracer methodologies using various fatty acids in both animals and humans have revealed a pattern of selective oxidation of long chain unsaturated fatty acids over long chain saturated fatty acids. Measuring whole-body oxygen consumption in rats, Takeuchi and co-workers found that intakes of vegetable oils high in polyunsaturated fatty acids (PUFA), compared to intakes of animal fats high in saturated fatty acids (SFA), resulted in a greater contribution to the thermic effect of food (TEF). Using indirect calorimetry and the respiratory quotient to examine dietary lipid metabolism, subjects consuming a diet higher in PUFA compared to SFA had an increased contribution of fat oxidation to the thermic effect of food (TEF). Moreover, diets with higher P:S ratios have been associated with an increased level of whole-body oxidation.

Data from animal and human studies argue for a more rapid metabolic disposal of linolenic acid compared particularly with saturated forms of fat. As such, it can be suggested that plant oils rich in omega-3 fatty acids, particularly canola oil and flaxseed oil, would be oxidized more rapidly and result in less body fat accumulation than conventional oils such as tallow and dairy fats which are richer in saturated fat. However,

no systematic studies have explored these questions, particularly in the face of the current global epidemic of obesity. Using both whole body calorimetry and modern high resolution stable isotopic tracer approaches will allow the energetic tendencies of canola and flaxseed oil versus other dietary fats and oils to be investigated.

The proposed research project will demonstrate the positive effects of ALA from flax oil and oleic acid from canola oil on several biomarkers of inflammation and endothelial dysfunction. Research on canola oil has not specifically addressed the effects of its novel fatty acid profile on emerging measures of inflammation and endothelial function. This research is critical to ensure that canola oil benefits from the cutting edge science that is underway in lipids and human health.

In addition to canola oil as one control treatment, a canola/flax oil blend will also be assessed as an additional arm of the study. A blend with canola oil will improve the stability of flax, increase the overall ALA level of canola and capitalize on the low saturated fatty acid content of both oils. This research will generate data on the health benefits of ALA, as well as ALA in combination with oleic acid, through comparisons of the CVD effects of the canola/flax blend and a traditional canola oil with a “typical” Western style dietary fat composition. It is important to note that both of the treatments will be compared independently to the control diet.

#### **IV. FLAXSEED AND CARDIOVASCULAR RESPONSES TO STRESS**

##### **PRINCIPAL INVESTIGATOR:**

Sheila G. West, Ph. D.  
Associate Professor of Biobehavioral Health  
Director, Vascular Health Interventions Laboratory, Penn State University

##### **CO-INVESTIGATORS:**

Penny M. Kris-Etherton, Ph.D. R.D. Distinguished Professor of Nutritional Science Director, Metabolic Diet Study Center, Penn State	Tavis Campbell University of Calgary Department of Psychology
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The goal of this study is to show that saturated and polyunsaturated fatty acids have different effects on cardiovascular reactivity to acute stress. We will attempt to replicate a recently completed study showing that a meal containing saturated fat (a fast food breakfast) significantly increased cardiovascular reactivity to acute stress when compared to a low fat meal. Specifically, the high saturated fat meal was associated with larger blood pressure increases and greater vasoconstriction/lesser vasodilation in response to a battery of stressors including a speech task, two physical stressors, and a mental arithmetic task. Although these results are compelling, the previous study did not examine whether differences in lipid and insulin responses to the meals could explain this pattern of results. Perhaps more importantly, our recent work suggests that polyunsaturated fatty acids have neutral or beneficial effects on vascular reactivity. Therefore, the goal of the present study is to replicate the previous experiment, while adding blood draws and including an additional test meal containing flax as a primary fat source. We will enroll 20 healthy adults and test stress reactivity after 3 standardized meals in a randomized, crossover design. Measures (blood draws and cardiovascular parameters) will be collected under fasting conditions and again 2 hours after consumption of the meal.

The goal of the present study is to examine the effect of a single high-fat meal on resting hemodynamics and cardiovascular reactivity to acute stress. Reactivity will be measured in terms of changes in blood pressure (SBP and DBP), total peripheral resistance (TPR), heart rate (HR), stroke volume (SV), and cardiac output (CO). We hypothesize that healthy, normotensive individuals will exhibit significantly higher cardiovascular reactivity to a set of standard laboratory stress tasks following a meal high in saturated fat meal versus an isocaloric, low-fat meal. We believe that these differences in hemodynamic reactivity may be explained by a larger insulin response to the low fat/high carbohydrate meal. Furthermore, we hypothesize that the meal containing predominantly unsaturated fats from flaxseed will have an intermediate effect, although no previous studies have tested this hypothesis.

## **V. FLAXSEED – POTENTIAL QUALIFIED HEALTH CLAIM**

### **PRINCIPAL INVESTIGATOR:**

Guy H. Johnson, Ph.D.  
Johnson Nutrition Solutions  
8711 Swan Street  
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Dr Guy Johnson reviewed over 200 papers for ability to substantiate a health claim in the United States. An assessment of the clinical data related to flaxseed and effects on cardiovascular disease that is available was made. Final report on this assessment has been completed and approved by FC2015 Board. Recommendation made by Dr Johnson to FC2015 to conduct additional research in order to have more clinical data to support a QHC submission. Dr Johnson's final report provides specific recommendations for new required research. This research will be organized and funding permitting, will begin in fiscal April 2007-08. Depending upon the progress of clinical trials, the QHC submission to the FDA may itself take 3-5 years.

## **VI. GENERALLY RECOGNIZED AS SAFE –WHOLE AND MILLED FLAXSEED**

### **PRINCIPAL INVESTIGATOR:**

James T. Heimbach, Ph.D., F.A.C.N.  
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Dr Jim Heimbach commissioned to evaluate new data for a GRAS evaluation of whole and milled flaxseed. Project will expand and update a literature search that was conducted by FC2015 during Phase 1 (in 2005) for published metabolism, toxicology, and human clinical studies and other relevant data. JHEIMBACH LLC will identify and retrieve information relevant to a GRAS evaluation of whole and milled flaxseed including data with respect to the identity, proposed use (and use levels), intended

technical effect, production processes, specifications, analytical methods, metabolism, toxicology, and human clinical studies and other relevant data. Using this data, a comprehensive GRAS monograph is being prepared.

This research is a continuation of work undertaken by FC2015 during its first phase (2004-05) with the aim being to compile and publish data from a randomized controlled trial on the effects of ALA on prostate cancer risk. Data from a one year + feeding trial of ALA in the amount of 1.5-2.0g/day in a randomized design with a control group, and serial measurement of ALA and PSA over the year has been undertaken by Serge Renaud. He and his team, coordinated by Dominique Lanzmann, have studied 98 subjects, the majority of whom are men, in a one year study to assess the effects of ALA on atrial fibrillation (potential risk factor for cardiovascular disease).

The study successfully demonstrated that ALA reduced the occurrence of atrial fibrillation after electrocardioversion and their paper has been submitted for publication. Serum samples from all men was made available to the UT to complete PSA analysis on blood taken at time zero and 2 and 6 and 12 months after test (ALA) and control (conventional diets but no ALA oil or margarine).

## **VII. GLYCEMIC INDEX OF FLAX BASED INGREDIENTS AND FOODS**

### **In partnership with:**

Dr. Alexandra Jenkins, Senior Scientist  
Glycemic Index Laboratories  
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The Glycemic Index (GI) is a system of ranking the quality of carbohydrate in a food according to the immediate effect it has on blood glucose. Individual foods are compared to pure glucose, which has its Glycemic Index set at 100. For comparison, pearl barley has a value of 33, oranges are 49, and whole wheat bread is 71. The Glycemic Index was conceived in 1979 by Dr. David Jenkins, together with Dr. Thomas Wolever and Dr. Alexandra Jenkins. The GI breakthrough was the outcome of research conducted at both Oxford University and at the University of Toronto to determine which foods would be best for people with diabetes. Low GI foods are also recognized by consumers and promoted by health professionals for weight loss. GI labeling is used in the US to identify foods which have the potential to moderate blood glucose levels and weight through positive effects on insulin secretion and clearance by the body.

Preliminary studies indicate that addition of flax to a product may reduce the GI of the food. A study carried out by GI Labs, tested a bread which was formulated with and without flax, the bread with the flax had a 20% lower GI value than the regular bread. This resulted in a re-classification of the bread from a high GI food to a low GI food. Although these results are preliminary they are encouraging.

In this project, GI Labs will assess the GI of flax ingredients, and/or flax based food products as submitted by various flax companies.

## **VIII. DEVELOPING FLAX BASED FOODS FOR CLINICAL TRIALS**

### **PRINCIPLE INVESTIGATORS:**

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Specific foods are required for the two year clinical study at the Canadian Centre for Agri-food Research in Health and Medicine. Study participants will consume 13 different foods containing ground flaxseed including pasta, buns, muffins, bagels and bars containing 30 g (ca 2 T) of milled flax. The flaxseed and placebo (without flaxseed) muffin, buns, pasta formulations will be developed by the Canadian International Grains Institute (CIGI). The Food Development Centre (FDC) will develop the bars (flaxseed and placebo) and to produce both the bars and muffins on a large scale for the study.

## **IX. TECHNOLOGY TRANSFER ACTIVITIES: FLAX GENETICS FOR NUTRACEUTICAL APPLICATIONS**

### **PRINCIPLE INVESTIGATOR:**

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#### *Information Exchange Workshops*

The objectives of the Workshops overall are to share information developed to date in flax genomics and a research and development planning and priority setting exercise. Workshops will be held at the PBI in Saskatoon with the agenda being focused upon an update of the Flax NAPGENE project; breeding initiatives; intellectual property discussions, etc. Genome Prairie and FC2015 plan to host these workshops about every nine months. These sessions will provide a forum for scientific and other exchange of information between stakeholders involved in flax cultivar development including plant biotechnologists and the plant breeding community (public and private) as well as the industry, extension personnel and perhaps media. As a significant component of the meeting will be a discussion of ongoing and future directions in research.

#### *Communications Bulletins*

As part of the activities we hope to undertake will be regular email bulletins again with the objectives being information exchange and dissemination. A key component of the email Newsletter will be updates on developments in research and in regulations. Other

support materials such as fact sheets and technical transfer articles will be developed, funding permitting.

*Producer Extension Series*

Information generated from Genome Prairie and FC2015 activities will be disseminated to growers through Certified Crop Advisors. Computer presentations and Information Guides will be prepared for extension agents, industry representatives and flax producers. It is expected that this information will also be disseminated at field days, producer meetings and flax extension events. In addition, interactive workshops will be a vital part of extension programming efforts which will provide education on flax breeding and production to producers, extension personnel, industry representative, crop consultants and others needing such information.

***Animal Health  
Flax Canada 2015  
Project Overview***

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**I. FLAX FEEDING EFFECTS ON PORK QUALITY**

**PRINCIPLE INVESTIGATOR:**

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For successful introduction of the production and branding of pork containing omega-3 fatty acids in Western Canada, a 3-year research program has been established. Research objectives of the program are: (1) To determine and improve the exact amount of digestible fat and alpha-linolenic acid that is provided in processed flaxseed; (2) To determine the amount of ground flax seed needed in feed to cause a desired change in omega-3 fatty acid content in pork; (3) To ensure that ground flax seed does not reduce carcass quality and consistent pork omega-3 fatty acid content is reached; (4) To ensure that an increase in pork omega-3 fatty acid content does maintain other key pork quality indices for the consumer.

The study approach is to first establish a successful feeding program to consistently produce pork with a guaranteed omega-3 fatty acid content, and then to ensure that this pork across various meat cuts does not have reduced quality characteristics to allow successful branding to occur. As expected results, the most effective processing method for flax seed will be established, a successful feeding program to enrich pork with omega-3 fatty acids will be developed and concurrent studies evaluating product acceptability will ultimately lead to the development of knowledge necessary for the consistent production of pork with a verifiably enhanced fatty acid profile for the health-conscious consumer.

**II. Rumen Bypass – Enhancing Bypass of Bioactives to Meat and Dairy Products**

**PRINCIPLE INVESTIGATOR:**

Dr. Essi Evans  
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There is a great deal of interest in developing flax feed rations for beef and dairy cattle in order to improve the quality attributes of consumer products. A particular focus is omega-3 enrichment of meat and dairy products. In addition, product development opportunities exist for antioxidant enrichment in milk, specifically lignans, a key component of flax.

The greatest challenge in using an oilseed such as flax for purposes of improving the quality attributes of the consumer product however is increasing the amount of bioactives that reach the beginning of the small intestine (thus avoiding ruminal biohydrogenation) in order to increase blood absorption. The development of effective rumen bypass technologies is a significant priority for the industry.

Major activities have been completed:

1. Rumen Researcher Database
2. Literature review.
3. Industry Development Workshop for Rumen Bypass.
4. Establish an Expert Stakeholder Group to provide guidance on the project, discuss and make recommendations.

### **III. The Interaction of Rumensin in Feed and Flaxseed Hulls on Milk Components**

#### **PRINCIPLE INVESTIGATORS:**

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Rumensin (monensin sodium), which is an ionophore, has been used extensively in the diet of dairy cows, and effects on milk production and composition are well documented. Rumensin is also known to decrease in vitro ruminal biohydrogenation of PUFA and to increase in vitro total CLA concentration, thus suggesting that dietary supplementation of Rumensin could increase milk concentration of CLA and other PUFA.

Rumensin supplementation increased concentrations of *cis*9, *trans*11-18:2 (CLA) and decreased concentrations of saturated fatty acids in milk fat and that feeding ground flaxseed with Rumensin resulted in higher milk fat concentration of *trans*11-18:1 (precursor of CLA) than when feeding whole flaxseed with or without Rumensin or ground flaxseed without Rumensin. Therefore, Rumensin supplementation with a supply of omega 3 fatty acids contributes to modify milk composition for better human health. However, this has to be done without any detrimental effect on the transfer of antioxidants (mammalian lignans) in milk.

Therefore, feeding a combination of Rumensin and flaxseed hulls could increase concentrations of some PUFA in milk although feeding such a combination on the transfer of antioxidants in milk is unknown. The objective of this study is to evaluate the

interaction of Rumensin premix in feed with feeding flaxseed hulls on milk concentrations of:

- 1) antioxidants
- 2) bioactive lipids

#### **IV. FLAX OIL BASED AQUACULTURE FEED INGREDIENTS**

PRINCIPLE INVESTIGATORS:

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Recently, the price of fish oil and meal have risen dramatically to more than double their costs one year ago. This has resulted in increased interest in the aquaculture industry to replace fish oil in aquaculture diets. In a recent trip to Ecuador and Peru at the requests of the Canadian Embassies in those countries, 15 aquaculture feed companies were visited by the PI. All of them indicated an interest in using flaxseed oil to replace all or part of the fish oil they are currently using. The high omega-3 fatty acid content of flaxseed oil makes it a desirable oil for fish nutrition. However, its nutritional properties are not well understood by the aquafeed industry.

Objectives of the research were to:

- 1) To determine the oxidative stability of flaxseed oil, flaxseed oil stabilized with commercial antioxidants and encapsulated flaxseed oil compared to commercial fish oil before and after extrusion processing.
- 2) Measure the growth performance of rainbow trout fed flaxseed oil, flaxseed oil stabilized with commercial antioxidants, encapsulated flaxseed oil or fish oil.
- 3) Measure the fatty acid composition and sensory quality of filets from rainbow trout consuming the diets in Objective 2.

Results included:

- 1) The addition of antioxidants or encapsulation markedly increases the oxidative stability of flaxseed oil before and after processing into aquaculture feeds. This effect lasted for a 168 day period. This indicates that the addition of Vitamin E and BHT to flaxseed oil can reduce oxidation of flaxseed oil for long term storage and make it feasible to use this product in commercial feed mills.
- 2) Complete replacement of fish oil with flaxseed oil does not affect fish growth performance or physiology. The addition of antioxidants to flaxseed oil did reduce fish mortality. This further supports the use of flaxseed oil containing Vitamin E and BHT for use in aquaculture diets.
- 3) Fillets from fish feed flaxseed oil are equal in flavour, odour and overall consumer acceptability compared to fillets from fish fed fish oil. Previous studies on the use of other vegetable oils in aquaculture diets have seen decreases in the sensory quality of fish products. This indicates that flaxseed oil is a preferred vegetable oil replacement for fish oil in aquaculture diets.

***Plant Breeding and Agronomy  
Flax Canada 2015  
Project Overview***

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**I. Flax Top Managers Grower Survey**

**Principle Investigator:**

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The Flax Managers Grower Survey represented the second year of the project, with the initial phase being conducted in 2005/2006. The goal of the survey was to provide information on the production practices of top flax producers. The results of the survey highlight best case and worst case production agronomic practices in use by producers. Five hundred growers were interviewed regarding flax production practices and the results posted on the FC2015 website for use by flax producers.

**II. Producer Interactive Self-Assessment Tool**

**Principle Investigator:**

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Insightrix Research, Inc. has developed a web-based interactive self-assessment tool for flax producers that will assist flax producers in increasing yields and quality of their flax crops. In order to develop the tool, the best practices of the 'top managers' as identified in the Grower Survey were used. A web based tool can be accessed through the FC2015 web site.