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Genotype X Environment Interactions and Its Impact on Use of Medicinal Plants

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Abstract: There is a paradigm shift from cure to prevention when it comes to human health. We want to live a healthy life and prevent sickness using substances other than pharmaceuticals. The plant-based nutraceutical products or Natural Health Products (NHPs) as they are some times referred to are the most important groups that have the potential to fit the bill. However, these products are sold without proper science based information in spite of the fact that most researchers acknowledge the need for such information. Evidence-based scientific studies to support health and nutraceutical claims related to the use of medicinal plants and their extracts have to be undertaken. It is only through critical research efforts that we can provide strong endorsements for medicinal plant use and ensure consumer confidence in the industry. Much of the research published on the medicinal value of plants does not take into account variability generated from genetic differences among plants and their interaction with the environment. Research should be directed towards properly identifying plants with known medicinal properties which have been grown in environments that are conducive to consistent production of the active agents attributed to the plants. Production of dependable medicinal plant products can only be attained if we pay close attention to these research-based principles. This article was written with main goals: 1) To discuss the above points in greater detail with examples; and 2) to highlight life time accomplishments and significant contributions of a well respected nutritionist Dr. T. K. Basu and his collaboration in development of fenugreek as a NHP. We believe that collaboration among clinical and agricultural researchers is essential to make the NHPs utilized to its potential and the plants (parts such as seed, foliage or roots) should be developed to the extent that they can be used directly to take advantage of the synergistic effect of the chemical constituents.

Keywords: Fenugreek, genotypes, nutraceutical, variabilities, clinical trials, functional food, echinacea.

INTRODUCTION

Many species of plants are used for medicinal purposes [1]. Fenugreek (*Trigonella foenum-graecum* L.) is one of the most geographically-widespread species having multiple medicinal properties, and this will be considered as the model plant for describing the impact of genotype X environmental interaction in medicinal plants. This is an annual legume plant native to the Mediterranean region or south-east Asia [1]. It was cultivated widely in parts of central Europe, Africa, China and in Morocco, and largely in Egypt and in India, and has recently been introduced to North America and Australia [2]. Fenugreek has autogamous white flowers, ovate trifoliate leaves, branched stems, straight or slightly-curved pods, golden yellow seeds and nodule bearing roots. Low water requirement and its dry land adaptation are responsible for increased interest in this crop for cultivation in temperate climates [3].

Fenugreek has a long history of medical uses in Indian and Chinese medicine. It has been used for treating mouth ulcers and chapped lips, cure of baldness, alleviation of abdominal and abscesses pain, treating arthritis, dropsy, heart disease, spleen and liver enlargement, kidney ailments among several others. Fenugreek has been reported for its medicinal and nutraceutical properties due to presence of antimicrobial compounds in the shoot system of the plant [4, 5]. The chemical composition of fenugreek seeds mimics milk in its ratio between proteins and amino acids contents [6]. It contains several alkaloids among which Trigonelline is an important one. In recent years researchers have discovered several other medicinal properties of fenugreek. They include anti-diabetic, hypercholesterolemic, antioxidant, anti-neoplastic properties. The seeds and to a lesser extent the leaves contain three very important phytochemicals namely complex carbohydrates (eg. galactomannan), steroidal sapogenins (eg. diosgenin) and amino acids (eg. 4-hydroxy isoleucine) that are known for their medicinal properties. The fenugreek galactomanan is unique due to the ~1:1 - 1.2:1 ratio of galactose to mannose (G:M) molecules [7]. This polysaccharide appears to be beneficial in controlling type 2 diabetes in animals [8, 9] as well as in humans [8, 10]. This high ratio of galactose substitution helps galactomannans to absorb water resulting in reduced glucose absorption within the digestive tract [10]. Diosgenin is often used as a

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raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone [10, 11]. The steroidal sapogenins are also effective for the treatment of hypercholesterolemia [12]. The amino acid isoleucine is a precursor of 4-hydroxyisoleucine which is known to regulate the secretion of insulin in animals [13, 14].

The past decade has witnessed tremendous revival in the interest and use of medicinal plant products like "nutraceuticals" in which phytochemical constituents can have long-term health promoting or medicinal qualities especially in North America. Surveys of plant medicinal usage have shown an increase from just about 3% of the population in 1991 to over 37% in 1998 in America [15]. The past decade has also witnessed intense interest in "nutraceuticals" in which phytochemical constituents can have long-term health promoting or medicinal qualities. After the release of first North American forage cultivar “Tristar”, interest of nutraceutical and functional food industries on fenugreek has increased several folds due to presence of valuable phytochemicals. For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry and pharmacognosy. At present, a major concern with the use of phytomedicines regards the maintenance of consistent quality and quantity of medicinal components in plants. Only recently variations in phytomedicinal content due to differential effect of environment and impact of secondary metabolism in the plant material have been recognized as significant factors [16]. Legume crops such as *T. foenum-graecum*, important in food and medicine, were rarely grown outside their native habitat. Cultivation of *T. foenum-graecum* has spread to areas far removed from its native habitat due to traditional selective breeding practices worldwide. Well-defined cultivars adapted to local environments are developed for specific areas. These cultivars are genetically diverse in nature and contribute significantly to the variation in chemical composition and biological efficacy of the plant products. It is clear that understanding how environmental factors affect phytomedicinal properties will be of great importance to optimizing recovery of phytomedicinal properties of plants. Detailed studies are required to examine the extent to which the chemical composition varies in populations adapted to varying habitats. This review discusses the critical aspects of the variability observed in clinical trials involving fenugreek and other plant species and takes into consideration the enormous contributions of internationally recognized nutritional biochemist Dr. Tapan Basu and his research in elucidating this point.

VARIABILITY AND INCONSISTENT RESULTS INVOLVING PHYTOCHEMICALS

A very important aspect of scientific research is error control and the clinical trials are no exception to this rule. However, one of the most common observations pertaining to clinical trials and laboratory based investigations on plant based phytochemicals is the inconsistency. When one laboratory reports significant effect of a plant product on animal and human health, another refutes the results. This is also true for pharmacognostic studies nutraceutical and functional food properties of products or chemicals of plant origin. This may be due to use of non-standard protocol among laboratories, inefficient and improper experimental designs and inappropriate statistical analyses of data. Even if standard protocol is used and appropriate experimental design and analyses are used there would still be variability observed in plant based material. This is because characteristics of plant material can vary depending on the genotype of the plant, environment under which they are grown and the interaction between the previous two factors. The clinicians and functional food researchers do not pay attention to these aspects and consequently conflicting results are obtained making people suspicious about the plant based products. For example fenugreek genotypes grown in India and those that are adapted to Canada are different. They do not look the same and so should not be expected to produce same level of phytochemicals. In addition to this difference plants also react to the environment they grow in. Same genotype grown in two different environments may not grow the same way or produce similar phytochemicals. This is referred to by plant geneticists as Genotype X Environment (GE) interaction effect. Effects of GE are observed regularly for agronomic traits in most crops and so are of grate importance for plant breeders when developing cultivars. When the GE effect of a trait is strong the breeder has two choices 1) develop cultivars with adaptation to each environment (location) with a view to maximize output or 2) develop a cultivar with wide adaptation sacrificing output in individual environment (location). For example, in the presence of a strong GE interaction a fenugreek genotype selected for high yield under Southern Alberta irrigated condition may not the most productive across the entire prairies. On the other hand a genotype that produces well on an average over a number of locations in the entire prairie may not produce the highest yield under southern Alberta irrigated condition. The breeder has to choose what is important for the purpose and so may have to consider aspects other than the trait expression when developing cultivar. We have observed strong GE interaction for the three phytochemical levels in fenugreek genotypes that were selected for forage production in western Canada. For optimum level of all chemicals we may have to resort to inter-crossing of few lines and selection for optimum levels of all in one genotype. Alternatively, the breeders can develop separate cultivars with each important chemical constituent at high levels.

Variation in important chemical constituents in fenugreek seed have been studied earlier. These studies have firmly established that fenugreek genotypes of diverse origin when grown in western Canada locations vary in agronomic and yield parameters [1, 17-19]. These studies also observed strong GE effect on the production of important phytochemicals. A genotype that produces a very high level of a phytochemical at one location may not produce the same level of the phytochemical in another. Our experience suggests that a fenugreek genotype may produce different level of phytochemicals over years when grown in western Canada. Even the rank order of the genotypes in all location or years may not be similar due to GE interaction effect. It is possible that some genotypes would fluctuate less than others and if our goal is to produce phytochemicals at a consistent level we may have to select genotypes with least fluctuation among years and locations even if the chemical constituent level is low. If different seed lots are used (with unknown genotypes and growing conditions) for clinical trails chances would be high to get completely different results. It is important to note that production of phytochemicals are controlled by minor genes or polygenes and so are strongly influenced by environment [20].

One of the most convincing evidence of the variability in phytochemical production among fenugreek genotypes due to GE effect has been reported by Taylor *et al.* (2002) [19]. The researchers studied ten fenugreek genotypes originating from Iran, Ethiopia, Greece, Pakistan, Afghanistan, Spain, Morocco and Canada at three different locations in three different provinces of Canada (Lethbridge in Alberta, Saskatoon in Saskatchewan and Agassiz in British Columbia) over a period of two years for identifying any variations in their diosgenin content. The authors reported highly significant genotype, genotype X year and genotype X location interactions in their investigations on the diosgenin contents detected in the seed of different accessions. Seed diosgenin concentration detected by the authors in an extended part of the same study reported similar fluctuations when grown in another Canadian province of Manitoba. In a previous study by Fazli and Hardman (1968) [11] from United Kingdom, a variation in diosgenin content (0.8-2.2%, w/w) among 52 fenugreek accessions from 18 different countries was reported. The authors concluded that they detected a trend of higher diosgenin content in the seeds form African and European genotypes compared to those form middle-east and South Asian ones. In India, Sharma and Kamal (1982) [21] reported variation in seed diosgenin content (0.33-1.9%) from genotypes representing different biogeographical regions of India as a direct example of genotype X environment interaction.

In a similar 2005 study conducted in collaboration with University of Alberta lipid biotechnologist Dr. Randall Weselake we detected wide fluctuations in seed oil contents in 14 fenugreek genotypes grown under greenhouse conditions at Lethbridge Research Centre (LRC), Agriculture and Agri-Food Canada (AAFC) indicating genotypic variability for this trait [2].

A number of researchers have suggested anti-microbial properties of fenugreek (as reviewed in Petropoulos 2002) [7]. Bhatti *et al.* (1996) [22] demonstrated wide spectrum anti-bacterial (both Gram positives and negatives) using both aqueous and ethanolic extracts of fenugreek seed from Pakistan. However, in India, De *et al.* (1999) [23] did not find any anti-fungal or anti-bacterial effects of ethanolic extracts of fenugreek. In our study [18], using both aquatic and ethanolic seed extracts of 75 fenugreek accessions representing germplasms from Asia, Africa, North America and Europe and grown in western Canada we did not observe significant anti-bacterial (both for gram positive and gram negative bacteria) or anti-fungal (few yeast species) properties. We suggest that significant GE effect may be responsible for this observation from fenugreek seed grown in a very different environment [6, 7, 22]. Conflicting phytochemical production attributable to GE interaction effect have also been documented in case of Australian sweet lupin [24], the Nigerian cowpea [25], the Hawaiian kava [26], and capsicum [27]. Durton (2006) [28] also identified strong GE effect in Chinese star anise (*Illicium verum* f Hook), prompting Roche Pharmaceutical Company to extract shikimic acid (a secondary plant metabolite) from a specific line rather than pursuing purification from commercially available feed-grade anise.

Fenugreek cultivars in our germplasm collection have not been subjected to extensive breeding programs and exhibit wide variabilities in habit, morphology, maturity duration, biomass, seed yield and in the contents of chemical constituents such as complex carbohydrate (galactomannan), sapogenin (diosgenin), oil, protein, amino acid and fatty acids, several antimicrobial compounds etc [17, 18, 29, 30]. Studies on fenugreek reported from other countries provide further critical evidences in this regard [2]. In Australia, McCormick *et al.* (1998) [31] detected significant variations in growth habit and floral initiations of 207 fenugreek accessions representing 20 different countries. Huang and Liang (2000) [29] reported varied concentrations of phytochemicals (flavone glycosides) such as orientin (0.008-0.025%) and vitexin (0.002-0.018%) in fenugreek grown at four different locations in China. Our research with multi-location trials with 73 world accessions of fenugreek grown over a number of years and locations in western Canada also indicated wide variation in seed and forage yield [32].

This very significant aspect has been ignored by clinical nutritionists and food scientists. Conflicting results obtained from phytochemical based clinical trials were invariably explained as due to differential experimental designs and methodology. Dr. Basu was the first clinical nutritionist who recognized the important flaw in the trials after in-depth review of our research results. Paying attention to the genetic variability among plants of a species has been a consistent aspect of our collaborative projects involving fenugreek [1, 33-37]. We have been very much interested in observing how locally adapted fenugreek genotypes could impact specific nutritional, biochemical and clinical outcomes of studies on animal models. Interesting results from our collaborations are discussed below.

DR. T. K. BASU'S RESEARCH PROFILE AND OUR COMMON RESEARCH INTEREST

Dr. Basu's earlier research had focused on the metabolism of vitamins, particularly retinol, ascorbic acid and thiamine. He had studied the relationship of these vitamins with malignant disease. Highlights of these works include: 1) Establishment of a link between biochemical evidence of secondary deficiency of vitamin A due to its metabolic unavailability and cancers of the epithelial cell origin, such as bronchus, lung and endometrium in humans [38-42]; 2) Determination of an important association between breast cancer; especially with skeletal metastases and ascorbic acid deficiency as well as providing mechanistic beneficial effects of ascorbic acid supplement to these patients [43, 44];. and 3) Investigation of the thiamin antagonistic effect of widely used cytotoxic agent 5-fluorouraci on malignant diseases [45-47]. Dr. Basu's research on various aspects of ascorbic acid is well known, in particular, his extensive reports against its Mega dose usage have been considered to be fundamental and have received much attention [39, 44, 48-50]. Dr. Basu's recent research investigation on diabetes is another major break through in the contemporary nutritional research realm. He was been the first researcher to report that type 1 diabetes (T1D) is associated with impaired metabolic availability of vitamin A due to its decreased hepatic hydrolysis as well as the synthesis of its carrier protein and that this secondary deficiency of the vitamin is linked to diabetes-related retinopathy [48, 51-59]. The subnormal vitamin A status in poorly controlled diabetic subject does not seem to respond to vitamin A supplementation but rather it increases its load in the liver leading to hepatoxicity [60]. According to Dr Basu "*….The treatment with insulin reverses the abnormal serum vitamin A homeostasis……From nutritional standpoint, however, supplementation with zinc (a trace element, required for the synthesis of retinol-binding protein) appears to be important in preventing metabolic abnormality of vitamin A in T1D*"(personal communication).

We have come a long way from vitamin era to functional food era. Dr. Basu's research has also moved with the time. Thus over the last 15 years or so, his research focused more and more on phytochemicals with particular emphasis on antioxidants, dietary fiber, and other plant factors in health and disease. Clinical efficacy of Natural Health Products (NHPs) of plant origin is often limited and variable. This is likely due to the fact that the plant extract used in the preparation of a medication are not standardized. The roots, flowers, stems, leaves, and seeds of a plant could be used in a product either in isolation or collectively and called an 'extract'. According to Dr Basu "*The chemical constituents, however, can differ quantitatively and qualitatively between different parts and some times different species of a plant. There also exist multiple biologically active components in a plant that work synergistically…… It is essential that these components are present in a product in their optimal amounts and proportions for the best effective clinical outcome. Because of these complexities, a clinical study design for a natural health product, meeting all criteria, becomes a challenge……*" (personal communication).

Dr. Basu's research addressed these complex issues and provided critical evaluations of the validity of their clinical efficacy claims, and described an approach to a randomized, double-blind placebo-controlled clinical trial, which were designed taking into account the complexities that are involved in NHPs. The plants (and their products) that he investigated include germinated wheat [61]; rhubarb (*Rheum rhaponticum* L.) [62-69]; Echinacea (*Echinacea purpurea* L. Moench) [67, 70-73]; North American ginseng (*Panax quinque folium* L.) [61, 74, 75] and fenugreek (*T. foenum-graecum* L.) [33, 34, 36, 37]. The lead authors association with Dr Basu's research started because of the common interest in fenugreek. The lead author was interested in developing fenugreek cultivars for western Canada and Dr. Basu wanted to look at the nutraceutical qualities of fenugreek grown in western Canada. Our lab has focused on the improvement of agronomic and nutraceutical traits in fenugreek for western Canada [1, 33, 76], developing an agronomic package for production in this area [30, 32, 35, 77-81] and possible pest profile for this crop [82, 83] for fast adoption of the crop to the Prairie conditions. Dr. Basu's research team, on the other hand, generated fundamental data on varied aspects of nutritional perspectives of fenugreek phytochemicals such as *in vitro* cholesterol and bile acid binding capacity of saponins extracted from locally grown fenugreek [34], inhibition of *in vitro* intestinal glucose uptake by galactomannan from Canadian grown fenugreek seed in genetically lean and obese laboratory rats [37], modifying effect of the same phytochemical on the glycemic and lipidemic status in laboratory rats [36] and several other related research projects. Many of the reports and publications dealing with the nutritional and clinical aspects of Canada grown fenugreek seeds on animal models are in press at the time of preparation of this manuscript (Tapan Basu, personal communication).

CONTEMPORARY RESEARCH INVOLVING CLINICAL TRAILS BY DR. TAPAN BASU

As a research leader in nutritional biochemistry and clinical nutrition, Dr. Basu has been critical about the quality of contemporary research in these areas. In a fairly recent publication (Basu and Basu 2007) while discussing about variations in results obtained by different research groups with respect to Echinacea plant, Dr. Basu commented, "*…….literature supporting its clinical efficiency is often difficult to interpret because of a) extensive variation in Echinacea extracts and their route of administration, b) study design not meeting all criteria and c) failure of products utilized in studies to be standardized*." Through several examples available from primary literature Dr. Basu suggested that "*….. These results underscore the importance of the species of Echinacea used during experimentation, the method of extraction used and the route of administration employed. Furthermore, each Echinacea preparation came from different plant parts, which, may, in turn, have altered the concentrations of potential active components from the herb. It is also possible that shortcomings in these studies are reflection of the fact that Echinacea does not have any immunomodulatory effect in healthy individuals. ……..However, most studies are subject to unsatisfactory methodological quality, including interpretation of results*." These observations are similar to our observations with fenugreek and several other research groups on different crop species conducted across the world.

Another critical observation made by Dr. Basu [70] has been the over simplification of results on clinical studies on Echinacea by different reviewers and research groups. Dr. Basu indicated that most researchers have treated Echinacea extract used in clinical studies as a drug, but truly speaking it is NHP that has many factors "*that often act synergistically*." According to Dr. Basu this fact has been greatly ignored by many prominent researchers and this adds a fundamental flaw to research results and interpretations. Most researchers used experimental designs suitable for drug evaluation rather than NHPs and that may have significantly impacted the results obtained and interpretations made out of it. Dr. Basu professes that one of the most important challenges while working with NHPs is that they lack any standardized test as such and hence the products vary significantly from batch to batch making it extremely difficult to generate consistent clinical results. He commented "*…..it seems that the reviewers* (of meta-analysis) *paid too much emphasis to accuracy of the study design when a bigger issue lies with standardization of the preparations….As a consequence the clinical outcomes of these preparations are often contradictory. Meta-analysis of these studies sends only a confusing message*." Dr. Basu strongly recommends that it is very important that the natural plant extracts are chemically defined and standardized before venturing into abrupt clinical trials to assess their effectiveness. It is important to systematically investigate dose-response correlations to precisely and scientifically determine the immunomodulatory potential of medicinal plants such as Echinacea. Such seminal observations are important in clinical trials; and provide a new perspective about the reasons for observing widely divergent and contradictory results published in the literature from time to time.

Dr. Basu's life-time research work has earned him respect of his colleagues. His contribution in the area of micronutrients and phytochemicals are truly exemplary and he has been well recognized by his peers through awards and major positions in societies dealing with human nutrition. Currently Dr. Basu is a Professor Emeritus of Nutritional Biochemistry at the Department of Agricultural, Food & Nutritional Science, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta. He continues to provide guidance and directions to the students and industry in the field of Nutrition and Food Science in North America. He is also the Executive Director of International College of Nutrition which he helped establish and is one of the three founder members of the Canadian Chapter of this organization. Dr. Basu's novel research approach has made a true impact in our understanding of the role of NHPs on health and disease. Dr. Basu has also been a dedicated teacher and has trained several graduate students and post-doctoral fellows over his long and accomplished career. For his teaching excellence he has received Outstanding Teacher Award many times during his career in University of Alberta. Truly Dr. Basu is a "star" when it comes to teaching and researcher who has laid the basic foundation of modern Nutritional Sciences and indeed deserves to be regarded as one of the major players in shaping the "*Clinical Practices and Fundamental Research in Nutritional Sciences*" in Canada.

INITIATION OF NEW CLINICAL TRIALS USING NHPS

The two main lipids in blood are cholesterol and triglyceride. They are carried in lipoproteins, globular particles that also contain proteins known as apoproteins. In serum, the densest (and smallest) family of particles mainly of high apoproteins, high cholesterol and very low triglyceride is called high-density lipoproteins (HDL). Somewhat less dense are the low-density lipoproteins (LDL). In fasting serum, most of the cholesterol is carried on LDL. Least dense are the very-low-density lipoproteins (VLDL), consisting mainly of high triglycerides, which is synthesized in and released from the liver. HDL particles are made in the liver and intestine and appear to facilitate the transfer of apoproteins among lipoproteins. The higher the LDL cholesterol, the greater is the risk of atherosclerotic and coronary heart disease. Diabetes and alcohol use, obesity, hepatic diseases including cirrhosis, obstructive jaundice and hypothyroidism are commonly associated with high lipid level [84-86]. These LDL and HDL can be measured in human or other animal subjects with accuracy and ease. The NHPs with known genetic and production source can be used to understand its impact on these two blood lipids.

Community health study and investigation in metabolic syndrome of poor nutrition, dyslipidemia, hepatic derangement and associated cardiovascular risk factors are currently of immense importance [87]. In concurrence with technological advancement, occupational and dietary life-styles in all ages of both men and women irrespective of racial and ethnic differences are rapidly changing [88]. Habitual changes of life-style of people in both urban and rural settings are also of no great difference like before [89] in India as in other parts of the world. Clinical manifestation of early age of onset of arteriosclerosis, ischemic heart, CAD along with hepatic derangements and dyslipidemia are the most common health disorders prevalent in every society. Data and information on these manifestations from developed countries such as USA, Canada, Australia and Europe are now available [89, 90]. But data from comparatively less developed and developing nations in Asia including India are still lacking [91]. Moreover, survey records and information are less available from rural sectors than urban areas and the least from the tribal communities [92, 93].

Drawing inspirations from Dr. Basu's research work we hope to identify important correlation among the study populations and identify and also substantially justify through controlled experiments the important nutraceutical properties of fenugreek and its protective, pharmacognostic attributes in marinating good health. Along Dr. Basu's line of research we expect to detect whether including fenugreek as a functional food and nutraceutical in the diet could possibly reduce blood glucose levels and improves the blood lipid profiles towards healthy sustainable life.

CONCLUSIONS AND FUTURE DIRECTIONS

Current estimates suggest that the Canadian nutraceutical and functional food industry has the potential to develop into a 50 billion US $ strong industry in the not so distant future (as reviewed in Basu *et al*. 2007) [35]. In view of its multiple uses, fenugreek needs to be widely cultivated in areas where climatic conditions favour its growth. In this regard, the research contribution of biochemical nutritionists such as Dr. Tapan Basu is indeed invaluable. His legendary research findings and detailed clinical investigations in important plant species such as wheat, rhubarb, North America ginseng, echinacea and most recently fenugreek will continue to boost the confidence of the consumers on one hand and benefit the crop producers on the other. Canadian government and producers need to develop the market for these NHPs and also agronomic and breeding technology for successfully producing the crops with consistent quality. Our joint research initiatives have helped improve understanding about these crops and have set the stage for improved utilization of NHPs. It is especially important when genotype x environment interaction plays a significant role in determining the quality of the NHPs. We sincerely thank Dr. Basu and his enormous achievements in shaping this collaboration that hopes to bridge the long standing gap between agriculture and nutritionists and encourage new partnership involving a wide range of stakeholders. We believe such collaborations have the potential to foster a healthier nutraceuticals industry in Canada in near future.

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ABBREVIATIONS

AAFC	=	Agriculture and Agri-Food Canada
AARI	=	Alberta Agriculture Research Institute
EU	=	European Union
GE effect	=	Genotype X Environment effect
HDL	=	High-density lipoproteins
LDL	=	Low-density lipoproteins
LRC	=	Lethbridge Research Centre
NHPs	=	Natural Health Products
T1D	=	Type 1 diabetes
VLDL	=	Very-low-density lipoproteins

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