ANNEXURE - I

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

PROFORMA FOR PREPARATION OF STATUS REPORT FOR PROPOSAL OF A NEW RESEARCH PROJECT (Refer for Guidelines ANNEXURE-XI(A))

1. Institute Name:

Central Institute for Research on Goats, Makhdoom, Farah, Mathura (U.P.)

2. Title of the project:

Effect of nutritional deficiency diseases on gene expression profiles in goats.

3. Type of research project: Basic/Applied/Extension/Farmer Participatory/Other (specify)

4. Genesis and rationale of the project:

Numerous studies in humans, animals, and cell cultures have demonstrated that macronutrients (e.g., fatty acids and proteins), micronutrients (e.g., vitamins), and naturally occurring bioreactive chemicals (e.g., phytochemicals such as flavonoids, carotenoids, coumarins, and phytosterols; and zoochemicals such as eicosapentaenoic acid and docosahexaenoic acid) regulate gene expression in diverse ways . In the changing scenario of ruminants' nutrition, the objective of nutrigenomics is to study the effects of diet on changes in gene expression or regulatory processes that may be associated with various biological processes related with animal health and production. There is very scarce information about the effect of diet on expression of genes related to diseases or productive or reproductive traits of livestock, especially in goats.

Nutrigenomics can be used to identify the specific markers to manipulate gene expression through use of nutrients or their combinations so as to improve productive as well as overall animal performance. It may prove be a path breaking tool through identification of pathways and candidate genes responsible for dietary induced diseases and ultimately reduction in production losses due to these diseases in animals. Employing the cutting-edge tools of genomics and microarray technologies, the present study is proposed to determine the gene expression pattern in copper, zinc and copper & zinc combined deficient goats compared to healthy and control animals, and also whether the corrective measures for nutrient deficiency can restore the gene expression pattern comparable to healthy and control goats.

5. Knowledge/Technology gaps and justification for taking up the present project:

In the changing scenario of ruminants' nutrition, the objective of nutrigenomics is to study the effects of diet on changes in gene expression or regulatory processes that may be associated with various biological processes related with animal health and production. There is very scarce information about the effect of diet on expression of genes related to diseases or productive or reproductive traits of livestock, especially in goats.

It may be possible to begin to understand the importance of the relationship between individual nutrients and the regulation of gene expression. In studies of steers under nutritional restriction due to intake of poor quality feeds, expression of specific genes associated with protein turnover, cytoskeletal remodeling, and metabolic homeostasis was clearly influenced by diet (Byrn et al., 2005). A study on diet induced gene expression showed that selenium deficiency altered protein synthesis at transcriptional level (Rao et al., 2001), which led to adverse effect like enhancement of stress through up-regulation of specific gene expression and signaling pathway. On the other hand genes responsible for detoxification mechanism and protection from oxidative damage were hampered, these consequences ultimately led to alteration of phenotypic expression of related symptoms of selenium deficiency. Diet-induced milk fat depression (MFD) represents an exciting example of nutrigenomics; specifically, an example where bioactive fatty acids produced as biohydrogenation intermediates during rumen fermentation act to down-regulate the expression of key lipogenic genes involved in milk fat synthesis. The first of these bioactive fatty acids identified was *trans*-10, *cis*-12 18:2 (conjugated

linoleic acid), and it has been the most extensive investigated. Multiple conjugated linoleic acid isomers have been observed to reduce milk fat synthesis in the cow. Diet-induced milk fat depression is a reduction in milk fat caused by specific bioactive fatty acids produced during ruminal biohydrogenation under some dietary conditions (Minihane, 2009; Bauman et al., 2011).

From the above example it is apparent that possibly nutrigenomics can be used to identify the specific markers to manipulate gene expression through use of nutrients or their combinations so as to improve productive as well as overall animal performance. Nutrigeonmics will be a path breaking tool through identification of pathways and candidate genes responsible for dietary induced diseases and ultimately reduction in production losses due to these diseases in animals. Therefore, the present study is planned with the following hypothesis and objectives.

6. Critical review of present status of the technology at national and international levels along with complete references:

Efforts to unveil the etiology of disease often recapitulate the nature versus nurture debate. But today's biologists concede that neither nature nor nurture alone can explain the molecular processes that ultimately govern human or animal health. The presence of a particular gene or mutation in most cases merely connotes a predisposition to a particular disease process. Whether that genetic potential will eventually manifest as a disease depends on a complex interplay between the genome and environmental and behavioral factors. This understanding has helped spawn numerous multidisciplinary gene-based approaches to the study of health and disease.

One such endeavor is nutrigenomics, the integration of genomic science with nutrition. Although genes are critical for determining function, nutrition modifies the extent to which different genes are expressed and thereby modulates whether individuals attain the potential established by their genetic background. Nutrigenomics therefore initially referred to the study of the effects of nutrients on the expression of an individual's genetic makeup. More recently, this definition has been broadened to encompass nutritional factors that protect the genome from damage. Ultimately, nutrigenomics is concerned with the impact of dietary components on the genome, the transcriptome (the sum total of all mRNAs) the proteome (the sum of all proteins), and the metabolome (the sum of all metabolites) (Mead, 2007).

Numerous studies in humans, animals, and cell cultures have demonstrated that macronutrients (e.g., fatty acids and proteins), micronutrients (e.g., vitamins), and naturally occurring bioreactive chemicals (e.g., phytochemicals such as flavonoids, carotenoids, coumarins, and phytosterols; and zoochemicals such as eicosapentaenoic acid and docosahexaenoic acid) regulate gene expression in diverse ways (Mead, 2007). Many of the micronutrients and bioreactive chemicals in foods are directly involved in metabolic reactions that determine everything from hormonal balances and immune competence to detoxification processes and the utilization of macronutrients for fuel and growth. Some of the biochemicals in foods (e.g., genistein and resveratrol) are ligands for transcription factors and thus directly alter gene expression. Others (e.g., choline) alter signal transduction pathways and chromatin structure, thus indirectly affecting gene expression (Glunde and Serkova, 2006).

Much of the nutrigenomic focus has been on single-nucleotide polymorphisms (SNPs), DNA sequence variations that account for 90% of all human genetic variation. SNPs that alter the function of "housekeeping genes" involved in the basic maintenance of the cell are assumed to alter the risk of developing a disease. Dietary factors may differentially alter the effect of one or more SNPs to increase or decrease disease risk.

An elegant example of a diet–SNP interaction involves the common C677T polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) gene (Hanson et al., 2001; Guieterez et al., 2003). This variant causes MTHFR enzyme activity to slow down. This result in reduced capacity to use folate (or folic acid) to convert homocysteine to methionine and thence to the *S*-adenosylmethionine required for the maintenance methylation of cytosine in DNA and control of gene expression, among many other reactions. But the same variant also may increase the form of folate that can be used to make thymidine, one of the bases in DNA, and to prevent mutagenic uracil from being incorporated instead. This shift in methylation status may explain why in a low-folate environment (for example, where there is low intake of folate-rich vegetables such as spinach and asparagus or a lack of supplemental folate) homozygous carriers of the C677T polymorphism may be more prone to developmental defects but at the same time could be protected against certain Cancers. The key point here is that the activity of the

reaction catalyzed by the *MTHFR* gene can be modified depending on the amount of two essential nutrients: folate, which is the substrate for *MTHFR*, and riboflavin, a cofactor of *MTHFR*. Therefore, the risks associated with *MTHFR* activity can be markedly modified, for better or for worse, depending on fortification and supplementation strategies.

There are several ways in which bioactive dietary components can alter the action of specific genes and their variants. A primary mechanism for modulating gene expression involves transcription factors (TFs) which bind to specific DNA sequences (or DNA elements). In some situations the extracellular molecule itself enters the nucleus and binds a receptor; the resultant complex serves as the TF. In other situations, the TF can bind one or more additional molecules to form the TF complex, which then binds to the DNA. The binding of the TF causes a conformational change in the DNA and promotes or inhibits transcription, as needed for the appropriate response to the environment.

Bioactive dietary components can indirectly influence the ability of TFs to bind to their DNA elements. For example, vitamin A modifies expression of several genes through the retinoic acid receptor TF. Polyunsaturated fatty acids (PUFAs) influence the ability of peroxisome proliferator activator receptor/retinoic acid receptor complexes to bind to their DNA elements. Flavonoids regulate various genes through three TFs: nuclear factor kappa-B (NF-kB), estrogen receptor, and activating protein-1. In each case, the bioactive dietary components influence gene expression and thereby alter genetic outcomes (Karlsen et al. 2007; Mead, 2007).

The dietary factors seem to have a profound effect on many aspects of the human health including ageing, inflammation, obesity, reproductive performance, cancer and other diseases at least partly, through interaction with the genome, which results in altered gene expression. Many diseases and disorders, including reproductive disorders are related to suboptimal nutrition in terms of essential nutrients, imbalance of macronutrients, or event toxic concentrations of certain food compounds. There are multietioetiological diseases which are due to interaction of different nutrients along with several genes (Mariman, 2006).

While the effects of nutrition on fertility are poorly understood, there are reports of some fertility associated gene expression patterns that are changed by supplementation of various dietary forms of selenium e.g. selenomethonine, sodium selanite and selenium yeast. In mice, using a basic 23,000-element murine microarray, the researchers found a dramatic change in gene expression of 2500 genes being influenced by selenium supplementation and at least 100 of these seem to be associated with reproductive function directly or indirectly (Dawson, 2006). It has been observed that a set of oxidative stress-associated genes and the genes involved in the thioredoxin electron carrier system also get readily influenced by selenium supplementation.

There is an emerging body of data supporting the remarkable effects nutrients can have on gene expression. It is now well known that nutritionally significant dietary metals such as zinc do affect the gene expression levels. Zinc interacts with DNA binding proteins forming the motifs known as zincfingers. The zinc fingers assume appropriate conformation and bind to DNA and thereby act as molecular switches affecting gene expression by blocking the transcription (Cousins, 1994). In addition to metals, some macroscopic nutrients like soluble and insoluble dietary fibres have been shown to affect gene expression e.g., colonic gene expression gets affected by the fibre (Cousins, 1996).

From the above example it is apparent that possibly nutrigenomics can be used to identify the specific markers to manipulate gene expression through use of nutrients or their combinations so as to improve productive as well as overall animal performance. Nutrigeonmics will be a path breaking tool through identification of pathways and candidate genes responsible for dietary induced diseases and ultimately reduction in production losses due to these diseases in animals. Therefore, the present study is undertaken to determine the gene expression pattern in copper, zinc and copper & zinc combined deficient goats compared to healthy and control animals, and also whether the corrective measures for nutrient deficiency can restore the gene expression pattern comparable to healthy and control goats.

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7. Brief note on Proprietary/Patent Perspective (for projects related to technology development)/Ethics/Animal Welfare/Bio Safety Issues

Successful completion of the research project providing assessment of gene expression profiles and molecular characterization of specific gene expression uopregulation or downregulation in Cu, Zn and Cu&Zn combined-deficient goats is likely to deliver reliable genomic markers and molecular profiling techniques and tools for evaluating practical nutrient requirements and diet formulation strategies in a relatively short time. Altered host gene expression following nutrient deficiency can be exploited as marker for facilitated/early diagnosis of the disease as well as marker for early prediction of the disease corrective (protective) measures. Analysis of differential molecular signatures of nutritionally deficient and healthy animals could contribute in our understanding about the molecular mechanism and pathways of micronutrient functions and utilization. Being a unique and pioneer nutrigenomics study in Indian goats, the resultant genomic markers and molecular profiling techniques and tools for evaluating practical nutrient requirements, diet formulation strategies, early nutrient deficiency diagnosis and early prediction of disease will have ample scope for patent filing and proprietary ownership of the tools/techniques evolved.

8. (a) Expected output

i. Nutrigenomics promise to provide the tools to understand the role of nutrition in gene expression; we will begin to appreciate the long-term effects of nutrition on animal diseases, reproductive and production performance.
ii. Gene expression studies will allow for the identification of pathways and candidate genes responsible for deficiency disease development and progression to overt sign.

iii. It will also be possible to examine the tissue programming that is triggered by nutrition in goats when transcriptomic, proteomic and metabolomics studies are taken up after successful gene expression profiling.
iv. More importantly, the more precise measures of nutrient effects afforded by more reliable genomic markers and molecular profiling techniques will provide new tools for evaluating practical nutrient requirements and diet formulation strategies in a relatively short time. This will lead to improved livestock productivity, improved sustainability and the production of more wholesome animal products.
v. Using system biology approach (microarray) to analyse the gene expression profiling (molecular signature) of host following nutritional deficiency. Altered host gene expression following nutrient deficiency can be exploited as marker for facilitated/early diagnosis of the disease as well as marker for early prediction of the disease corrective (protective) measures. Finally analysis of differential molecular signatures of nutritionally deficient and healthy animals could contribute in our understanding about the molecular mechanism and pathways of micronutrient functions and utilization.

(b) Clientele/Stake holders (including economic and socio aspects)

i. Goat farmers – Poor and marginal farmers rearing the goats for their livelihood.
ii. Goat rearing entrepreneurs – small and large commercial goat farmers.
iii. Disease diagnostic laboratories, especially nutritional disease diagnosis

Signatures:

Project Leader: (RVS Pawaiya)

Co-PIs: (UB Chaudhary) (Nitika Sharma) (Shivasharnappa N)

Comments and signature:

[Head of Division]

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

RESEARCH PROJECT PROPOSAL PROFORMA FOR INITIATION OF A RESEARCH PROJECT (RPP - I)

(Refer for Guidelines ANNEXURE-XI (B))

1. **Institute Project Code** (to be provided by PME Cell):

2. **Project Title: Effect of nutritional deficiency diseases on gene expression profiles in goats.**

3. **Key Words:** Gene expression profile, goat, nutritional deficiency, nutrigenomics, pathology, selenium, Vitamin E, zinc.

4. (a) Name of the Lead Institute: **CIRG, Makhdoom, P.O. Farah, Mathura (U.P.)**

(b) Name of Division/ Regional Center/ Section: **Division of Animal Health**

5. (a) Name of the Collaborating Institute(s), if any

(b) Name of Division/ Regional Center/ Section of Collaborating Institute(s)

6. Project Team (Name(s) and designation of PI, CC-PI and all project Co-PIs, with time proposed to be spent)

S. No.	Name, designation and Institute	Status in the project (PI/ CC-PI/ Co-PI)	Time to be spent (%)	Work components to be assigned to individual scientist
1.	RVS Pawaiya, Principal Scientist (Vet Pathology)	Principal Investigator	30	Procurement of consumables/ biochemical/ labwares/ equipments etc., planning and designing of experimentation and implementation supervision, periodical examination of animals for the development of any deficiency lesions/ clinical signs, collection of biosamples from the experimental animals. Molecular and genomic studies and microarray studies and outsourcing of the services wherever required.
2.	UB Chaudhary Principal Scientist & Head, NFRPT (Animal Nutrition)	Co-PI	25	Procurement of consumables/ equipments etc., to provide and facilitate experimental animals of required age and sex groups, with proper housing, feeding and managemental care, formulation of Cu, Zn and Cu&Zn combined-deficient as well as –sufficient and balanced diets for the experimentation and feeding the same in precise manner for the successful running of the study. Molecular and microarray studies.
3.	Nitika Sharma, Scientist (Vet Medicine)	Co-PI	25	Collection of biosamples & AAS studies. Processing of the biosamples for the molecular biological studies and to help undertake the molecular biological and genomic studies and microarray studies.
4.	Shivasharnappa N, Scientist (Vet Pathology)	Co-PI	25	Periodical examination of animals for the development of any deficiency lesions/ clinical signs, collection of biosamples from the experimental animals. Carrying out pathological studies. Processing of biosamples, Molecular and genomic studies and microarray studies.

7. **Priority Area to which the project belongs:**
(If not already in the priority area, give justification)

Flagship programme on nutrigenomics and immunogenomics.

8. **Project Duration:** Date of Start: October, 2012 **Likely Date of Completion:** September, 2016 and for the 4 years duration.

9. **(a) Objectives:**

1. Analysis of gene expression profiling of nutrient-deficient (Zn, Cu and Zn&Cu combined - deficient) goats.
2. Identification and characterization of genes specifically affected (upregulation and downregulation) by nutritional deficiency in goats.
3. To determine whether corrective measures to nutrient deficiency can restore the gene expression pattern comparable to healthy and control goats.

(b) Practical utility:

Nutrigenomics promise to provide the tools to understand the role of nutrition in gene expression; we will begin to appreciate the long-term effects of nutrition on animal diseases, reproductive and production performance. Gene expression studies will allow for the identification of pathways and candidate genes responsible for deficiency disease development and progression to overt sign.

It will also be possible to examine the tissue programming that is triggered by nutrition in goats when transcriptomic, proteomic and metabolomics studies are taken up after successful gene expression profiling. It will help in understanding and maneuvering the dietary manipulations and nutritional strategies as key tools for deficiency disease prevention and influencing goat production. Nutrition and genetic makeup both strongly influence the reproductive performance of milch animals. This is particularly important during the transition period and early lactation, when the animal is particularly sensitive to nutritional imbalances. Nutrigenomics may provide new tools that can be used to more clearly understand how nutritional management can be applied to address disease, performance and productivity in animals.

More importantly, the more precise measures of nutrient effects afforded by more reliable genomic markers and molecular profiling techniques will provide new tools for evaluating practical nutrient requirements and diet formulation strategies in a relatively short time. This will lead to improved livestock productivity, improved sustainability and the production of more wholesome animal products.

Using system biology approach (microarray) to analyse the gene expression profiling (molecular signature) of host following nutritional deficiency. Altered host gene expression following nutrient deficiency can be exploited as marker for facilitated/early diagnosis of the disease as well as marker for early prediction of the disease corrective (protective) measures. Finally analysis of differential molecular signatures of nutritionally deficient and healthy animals could contribute in our understanding about the molecular mechanism and pathways of micronutrient functions and utilization.

10. Activities and outputs details:

Objective wise	Activity	Month & Year of		Output monitorable target(s)	% to be carried out in different years				Scientist(s) responsible
		Start	Completion		1	2	3	4	
1. Analysis of gene expression	Procurement of consumables/ biochemical/	Nov., 2012	March, 2015	Procured biochemical, labwares,	20	25	25	30	RVS Pawaiya & UB Chaudhary

profiling of nutrient-deficient (Zn, Cu and Zn&Cu combined - deficient) goats.	labwares/ equipment etc., planning and designing of experimentation, animal allocation for experiment			equipment & design of experiment, availability and division in groups of animals for experiment					
	Housing, feeding and managemental care of expl animals; Formulation and preparation of Zn, Cu and Zn&Cu combined - deficient and – sufficient diets and balanced diets for various animal groups,	Nov., 2012	June, 2013	Expl. animals managed, diets formulated and prepared	20	25	25	30	UB Chaudhary, Nitika Sharma & N Shivasharnappa
	Examination of animals for the development of any deficiency lesions/ clinical signs, collection of biosamples	Aug., 2013	Sep, 2016	Lesion, clinical signs observed	20	25	25	30	RVS Pawaiya & UB Chaudhary & Nitika Sharma & N Shivasharnappa
	Processing of the biosamples and to undertake the molecular biological and genomic studies and microarray studies.	Nov., 2013	Sep, 2016	Biosamples processed and results	20	25	25	30	RVS Pawaiya & UB Chaudhary Nitika Sharma & N Shivasharnappa
	Tissue Cu & Zn analysis	Nov., 2013	Sep, 2016	Tissue analysed and results	20	25	25	30	RVS Pawaiya & UB Chaudhary & N Shivasharnappa
	Analysis of gene expression profiling of nutrient-deficient (Cu, Zn and Cu&Zn deficient) goats	Nov., 2013	Sep, 2016	Results of analysis	20	25	25	30	RVS Pawaiya & UB Chaudhary
2. Identification and characterization of genes specifically affected (up-regulation and down-regulation) by nutritional deficiency in goats.	DNA isolation, purification, and Gene sequencing	Aug., 2013	Sep, 2016	Results	20	25	25	30	RVS Pawaiya & N Shivasharnappa
	Outsourcing services for gene sequencing	Aug., 2013	Sep, 2016	Results	20	25	25	30	RVS Pawaiya & N Shivasharnappa & UB Chaudhary
	Gene sequence alignment using specific software	Nov., 2013	Sep, 2016	Results	20	25	25	30	RVS Pawaiya & N Shivasharnappa
	Compilation and documentation of results	Nov., 2013	Sep, 2016		20	25	25	30	RVS Pawaiya & UB Chaudhary & Nitika Sharma & N Shivasharnappa
3. Determining whether corrective	Comparison of gene expression profiles of nutrient	Nov., 2013	Sep, 2016	Results	20	25	25	30	RVS Pawaiya & N Shivasharnappa & UB Chaudhary

measures to nutrient deficiency can restore the gene expression pattern comparable to healthy and control goats.	deficient goats and recovered goats								
	Comparison of gene expression profiles of nutrient deficient goats and healthy control goats	Dec., 2013	Sep, 2016	Results	20	25	25	30	RVS Pawaiya & N Shivasharnappa & UB Chaudhary
	Compilation and documentation of results	Dec., 2014	Sep, 2016		20	25	25	30	RVS Pawaiya & N Shivasharnappa & UB Chaudhary

11. Technical Programme (brief):

Experimental animals: Healthy goats of about 3-6 months will be taken for the experimentation and kept for a period of 15 days under observation for acclimatization and any health problem. Meanwhile, deworming and vaccination for specific infectious diseases, if required, will be done.

Experimental design and research interventions: The animals will be divided in four groups comprising of both sexes equally. After calculating and formulating the requirements of balanced ration and micronutrients (zinc and copper), the treatments will be given as below.

Group-I (A): Copper-deficient
Group-II (B): Zinc-deficient
Group-III (C): Copper & Zinc combined-deficient
Group-IV (D): Healthy control with balanced ration

The duration of this experimentation will be till the appearance of overt clinical signs of specific deficiency diseases in treated groups. Thereafter, the animals of deficiency-treated groups will be provided with non-deficient diets in order to correct the deficiency and restore the normal health.

Observations:

1. The experimentation will be carried out in the experimental animal shed following standard hygiene and rearing management.
2. The duration of experimentation will be till the appearance of overt clinical signs of specific deficiency diseases in treated groups and then, after deficiency corrective measures, till the restoration of normal health in animals.
3. Biomaterials including blood, serum and different organ tissues will be collected at 15 days interval and preserved appropriately for both histopathology and molecular studies.
4. The micronutrient levels of Cu and Zn will be monitored in blood serum and tissues of different organs at 30 days intervals.

Tissue Zn & Cu analysis: Total Cu and Zn in serum and tissues will be analyzed by atomic absorption spectrophotmeter following the methods described previously (Maas et al., 1992).

Analysis of gene expression profiling of nutrient-deficient (Zn & Cu deficient) goats:

Microarray analysis of genes: Total RNA will be extracted and subjected to cDNA synthesis and labeling with a two colour (Cy5-dCTP and Cy3-dCTP) labeling microarray quick labeling kit (Agilent Technologies). Finally the specimens will be hybridized with Caprine Designed MicroArray (Census service; http://www.sipo.gov.cn) as per the instruction of manufacturers or any alternatively available procedure the time of experimentation (eg. RNA sequencing etc.). Arrays will be scanned (PerkinElmer, Waltham, MI) at 5-mm resolution and raw files will be generated. Featured extracted data will be analyzed using GeneSpringGx v 11.0 software (Agilent).

Quantitative RT-PCR (qRT-PCR): To confirm the microarray data for the selected genes qRT-PCR will be performed.

Data analysis: Detailed protocols for data analysis of Affymetrix microarrays and extensive documentation of the sensitivity and quantitative aspects of the method have been described (Lee et al., 1999; Rao et al., 2001) and will be used for data analysis.

(a) Material: Biochemicals, molecular biological reagents, body fluid samples, tissue samples etc.

(b) Techniques/Methodology: The gene profile will be determined using microarray and/or RNA sequencing methods and confirmation with qRT-PCR procedures.

(c) Instrumentation: Centrifuge, refrigerator, deep freezer (-20^{o}C, -40^{o}C, -80^{o}C etc.), ultra centrifuge, tissue processor, tissue stainer, slide coverslipper, hot plate, tissue float bath, tissue embedding center, thermocycler, real-time PCR, incubator, hot air oven, image analysis and sequence alignment software programmes etc.

(d) Special material: Chemicals and biochemicals, molecular biology kits, molecular diagnostic kits, ELISA kits, etc.

(e) Analytical tools: SAS, DNA Star and other related molecular and statistical analytical tools.

12. Financial Implications (` in Lakhs)

(A) Financed by the institute

12.1 Manpower (Salaries / Wages)

S. No.	Staff Category	Man months	Cost
1.	Scientific	32.4	-
2.	Technical	18	-
3.	Supporting	18	-
4.	SRFs/RAs (2 RAs; each @28000.00/m+HRA)	72	22.20
5.	Contractual (2 persons; each @10000.00/m)	72	7.20
	Total	**212.4**	**29.40**

12.2 Research/Recurring Contingency

S. No.	Item	Yr (1)	Yr (2)	Yr (3)	Total
1.	Consumables	10.00	12.00	8.00	**30.00**
2.	Travel	1.50	1.50	1.50	**4.50**
3.	Field Preparation/ Planting/ Harvesting (Man-days/costs)	-	-	-	**-**
4.	Inter-cultivation & Dressing (Man-days/costs)	-	-	-	**-**

5.	Animal/Green house/Computer Systems/Machinery Maintenance	2.00	1.00	1.00	**4.00**
6.	Outsourcing of gene primers/ array/ sequencing etc.	10.00	20.00	15.00	**45.00**
7.	Miscellaneous (Other costs)	5.00	5.00	4.00	**14.00**
	Total (Recurring)	**28.50**	**39.50**	**29.50**	**97.50**

Justification: The project will involve biochemicals/ reagents/ kits for performing molecular biological studies and also outsourcing of microarray facility for gene expression profiling studies. In addition, dedicated manpowers in the form of RAs and contractual workers will be required to perform the research work and other molecular techniques in a precisely and also to maintain and take care of laboratory animals.

12.3 Non-recurring (Equipment)

S. No.	Item	Yr (1)	Yr (2)	Yr (3)	Total	Justification
1.	2D Gel Electrophoresis with power supply and accessories	6.00	-	-	**6.00**	For DNA and RNA separation
2.	Vacuum evaporator	3.00	-	-	**3.00**	For DNA and RNA separation
3.	Cooling and heating water bath	5.00	-	-	**5.00**	For PCR analysis of samples and DNA and RNA separation
4.	Heating blocks (1.5 ml)	3.00	-	-	**3.00**	For PCR analysis of samples and DNA and RNA separation
5.	RT PCR Machine	20.00	-	-	**20.00**	For quantification of genome
6.	Refrigerated table-top centrifuge	5.00	-	-	**5.00**	For PCR analysis of samples and DNA and RNA separation
7.	Hot Plate	0.75	-	-	**0.75**	For carrying out histopathological work
8.	Paraffin Wax dispenser	1.25	-	-	**1.25**	For carrying out histopathological work
9.	Paraffin block moulds, cassettes, capsules etc.	0.50	-	-	**0.50**	For carrying out histopathological work
10.	Tissue float bath	0.75	-	-	**0.75**	For carrying out histopathological work
11.	Refrigerator	1.00	-	-	**1.00**	For storage of reagents and samples.
12.	Deep Freezer (-40^{o}C)	2.50	-	-	**2.50**	For storage of bio-chemicals & biosamples.
13.	Miscellaneous (petty items)	5.00	5.00	5.00	**15.00**	For research purpose
	Total (Non-recurring)	**53.75**	**5.00**	**5.00**	**63.75**	

Justification: The project will involve primarily molecular biological work including genomics and microarray techniques, requiring above listed equipment for successful accomplishment of the project.

12.4 Any Other Special Facility required (including cost)

Nil

12.5 Grand Total (12.1 to 12.4)

Item	Yr (1)	Yr (2)	Yr (3)	**Total**
12.1 - Manpower (Salaries / Wages)	9.80	9.80	9.80	**29.40**
12.2 - Research/Recurring Contingency	28.50	39.50	29.50	**97.50**
12.3 - Non-recurring (Equipment)	53.75	5.00	5.00	**63.75**
Grand Total	**92.05**	**54.30**	**44.30**	**190.65**

(B) Financed by an organization other than the Institute (if applicable)

(i) Name of Financing Organization
(ii) Total Budget of the Project
(iii) Budget details

S. No.	Item	Year(1)	Year(2)	Year (3)…	Total
1	Recurring Contingency				
	Travelling Allowance				
	Workshops				
	Contractual Services/ Salaries				
	Operational Cost				
	Consumables				
2	Non - Recurring Contingency				
	Equipment				
	Furniture				
	Vehicle				
	Others (Miscellaneous)				
3	HRD Component				
	Training				
	Consultancy				
4	Works (i) New (ii) Renovation				
5	Institutional Charges				

13. Expected Output:

Nutrigenomics promise to provide the tools to understand the role of nutrition in gene expression; we will begin to appreciate the long-term effects of nutrition on animal diseases, reproductive and production performance. Gene expression studies will allow for the identification of pathways and candidate genes responsible for deficiency disease development and progression to overt sign. It will also be possible to examine the tissue programming that is triggered by nutrition in goats when transcriptomic, proteomic and metabolomics studies are taken up after successful gene expression profiling. More importantly, the more precise measures of nutrient effects afforded by more reliable genomic markers and molecular profiling techniques will provide new tools for evaluating practical nutrient requirements and diet formulation strategies in a relatively short time. This will lead to improved livestock productivity, improved sustainability and the production of more wholesome animal products. Using system biology approach (microarray) to analyze the gene expression profiling (molecular signature) of host, altered host gene expression following nutrient deficiency can be exploited as marker for facilitated/early diagnosis of the disease as well as marker for early prediction of the disease

corrective (protective) measures. Finally analysis of differential molecular signatures of nutritionally deficient and healthy animals could contribute in our understanding about the molecular mechanism and pathways of micronutrient functions and utilization.

14. Expected Benefits in Economic Terms:

The research project involves application of cutting-edge molecular tools and technologies for improvement of goat health. Successful completion of the research project providing assessment of gene expression profiles and molecular characterization of specific gene expression uopregulation or downregulation in Cu, Zn and Cu&Zn combined -deficient goats is likely to deliver reliable genomic markers and molecular profiling techniques and tools for evaluating practical nutrient requirements and diet formulation strategies in a relatively short time. Altered host gene expression following nutrient deficiency can be exploited as marker for facilitated/early diagnosis of the disease as well as marker for early prediction of the disease corrective (protective) measures. Analysis of differential molecular signatures of nutritionally deficient and healthy animals could contribute in our understanding about the molecular mechanism and pathways of micronutrient functions and utilization. This will lead to improved livestock productivity, improved sustainability and the production of more wholesome animal products.

15. Risk Analysis:

The project envisages the improvement of goat health and production through cutting-edge technological intervention, wherein analysis of gene expression profile of nutrient-deficient (Se, Vit-E & Zn deficient) goats and identification of genes affected by nutritional deficiency in goats will be carried out. The work will need meticulous implementation of the technical programme in precise and time bound manner with all the required amenities placed in time to undertake the experimentation and outsourcing microarray and sequencing services. Any constraints with regard to the above amenities may adversely affect the outcome of the project and the very purpose of this study (i.e. to apply cutting-edge molecular tools and technologies for the improvement of goat health) may be marred in the absence of sufficient resources required to perform the activities of the studies.

16. DECLARATION: This is to certify that

- the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out in the Institute on the subject.
- the same project has not been submitted to any other agency(ies) for financial support (if already submitted identify Project & Agency)
- the Investigator/co-investigator have been fully consulted in the development of project and have fully undertaken their responsibility to carry out the programme as per the technical programme.

Signature

Project Leader: (RVS Pawaiya)

Co-PIs: (UB Chaudhary) (Nitika Sharma) (Shivasharnappa N)

17. Signature of HoD

18. Signature of JD (R)/ Director