

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

RESEARCH PROJECT PROFORMA FOR MONITORING ANNUAL PROGRESS (RPP- II)

(Refer for Guidelines ANNEXURE-XI (E))

1. Institute Project Code: ANSC CIRG SI 2012 006 00217
2. Project Title: **Effect of nutritional deficiency diseases on gene expression profiles in goats.**
3. Reporting Period: **2013-14**
4. Project Duration: 4 Years; Date of Start – **October, 2012** Likely Date of Completion – **September, 2016**
5. Project Team (Name(s) and designation of PI, CC-PI and all project Co-PIs, (with time spent for the project) if any additions/deletions

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 (PI)	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.	N Shivasharnappa, Scientist (Vet Pathology)	Co-PI (June, 2013 onwards)	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.

6. (a) Activities and outputs earmarked for the year (as per activities schedule given in RPP-I)

Objective wise	Activity	Scientist(s) responsible	% of activity envisaged to be completed as per RPP-I	% achieved as targeted
1. Analysis of gene expression profiling of nutrient-deficient	Procurement of consumables/ biochemical/ labwares/ equipment etc., planning and designing of experimentation, animal allocation for	RVS Pawaiya & UB Chaudhary	20	70

(Zn, Cu and Zn&Cu combined - deficient) goats.	experiment			
	Housing, feeding and managerial 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,	UB Chaudhary, Nitika Sharma & N Shivasharnappa	20	100
	Examination of animals for the development of any deficiency lesions/ clinical signs, collection of biosamples	RVS Pawaiya & UB Chaudhary & Nitika Sharma & N Shivasharnappa	20	100
	Processing of the biosamples and to undertake the molecular biological and genomic studies and microarray studies.	RVS Pawaiya & UB Chaudhary Nitika Sharma & N Shivasharnappa	20	70
	Tissue Cu & Zn analysis	RVS Pawaiya & UB Chaudhary & N Shivasharnappa	20	50
	Analysis of gene expression profiling of nutrient-deficient (Cu, Zn and Cu&Zn deficient) goats	RVS Pawaiya & UB Chaudhary	20	0
2. Identification and characterization of genes specifically affected (up-regulation and down-regulation) by nutritional deficiency in goats.	DNA isolation, purification, and Gene sequencing	RVS Pawaiya & N Shivasharnappa	20	20
	Outsourcing services for gene sequencing	RVS Pawaiya & N Shivasharnappa & UB Chaudhary	20	0
	Gene sequence alignment using specific software	RVS Pawaiya & N Shivasharnappa	20	0
	Compilation and documentation of results	RVS Pawaiya & UB Chaudhary & Nitika Sharma & N Shivasharnappa	20	0
3. Determining whether corrective measures to nutrient deficiency can restore the gene expression pattern comparable to healthy and control goats.	Comparison of gene expression profiles of nutrient deficient goats and recovered goats	RVS Pawaiya & N Shivasharnappa & UB Chaudhary	20	0
	Comparison of gene expression profiles of nutrient deficient goats and healthy control goats	RVS Pawaiya & N Shivasharnappa & UB Chaudhary	20	0
	Compilation and documentation of results	RVS Pawaiya & N Shivasharnappa & UB Chaudhary	20	0

(b) If shortfall/addition, reasons for the same and how to catch up with the intended activities

Microarray analysis services could not be procured due to fund crunch. The Director is requested to kindly provide sufficient additional funds for procuring microarray analysis services.

7. Annual Progress Report (research results and achievements in bullets)

Low Cu and Zn feed formulation:

Sl. No.	Ingredients	Quantity (g/kg)
1.	Maize	542.50
2.	Starch	200.00
3.	Casein	230.00
4.	Mineral mixture	20.00
5.	Vitamin premix	5.00
6.	Sodium bicarbonate	2.50

Cu = 4.54 mg/kg

Zn = 21.25 mg/kg

Normal Cu & Zn requirement levels:

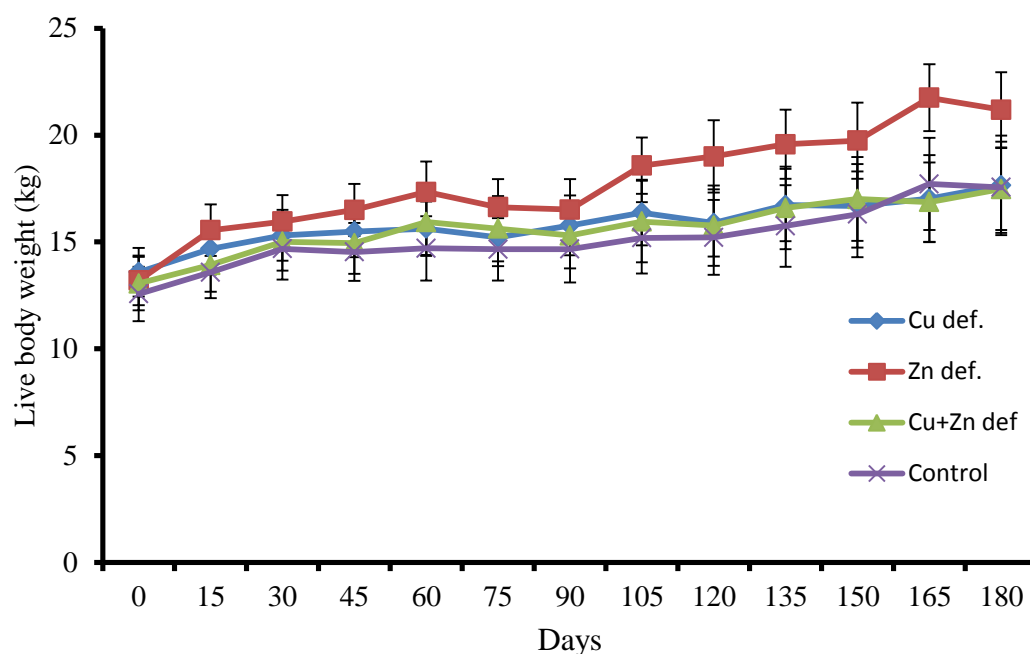
- Copper levels of >5 mg/kg (preferably 7-12 mg/kg DM) are safe unless complicating factors cause secondary Cu deficiency.
- Zinc levels of >35 mg/kg are safe in ruminants.

Experimentation:

- 6-9 month old 25 male barbari goats were procured from Barbari Unit and 11 male goats of same age group were taken from NFR&PT shed.
- Goats were kept for few weeks and fed normal feed before dividing in 4 groups with 9 animals in each.
- Experiment was started in November, 2013
 - Group-A: Copper-deficient
 - Group-B: Zinc-deficient
 - Group-C: Copper & Zinc combined-deficient
 - Group-D: Control with balanced ration

Body weight in different groups (Mean±SE):

Groups	Days Intervals												
	0	15	30	45	60	75	90	105	120	135	150	165	180
Gr-A	13.58 ±1.14	14.67 ±1.2	15.31 ±1.19	15.48 ±1.21	15.62 ±1.28	15.21 ±1.34	15.77 ±1.4	16.37 ±1.53	15.88 ±1.58	16.72 ±1.69	16.68 ±1.95	17.02 ±2.04	17.65 ±2.32
Gr-B	13.2± 1.16	15.55 ±1.21	15.95 ±1.24	16.5± 1.22	17.33 ±1.42	16.62 ±1.32	16.51 ±1.43	18.57 ±1.32	19±1. 69	19.57 ±1.62	19.74 ±1.78	21.75 ±1.57	21.18 ±1.77
Gr-C	13.05 ±1.25	13.92 ±1.25	15.01 ±1.35	14.94 ±1.44	15.93 ±1.54	15.62 ±1.52	15.3± 1.54	15.9± 1.91	15.76 ±1.88	16.58 ±1.93	17.01 ±1.96	16.86 ±1.86	17.47 ±1.81
Gr-D	12.56 ±1.28	13.57 ±1.21	14.67 ±1.45	14.53 ±1.36	14.7± 1.51	14.65 ±1.46	14.66 ±1.56	15.18 ±1.57	15.21 ±1.65	15.75 ±1.8	16.3± 1.89	17.71 ±1.9	17.55 ±1.88



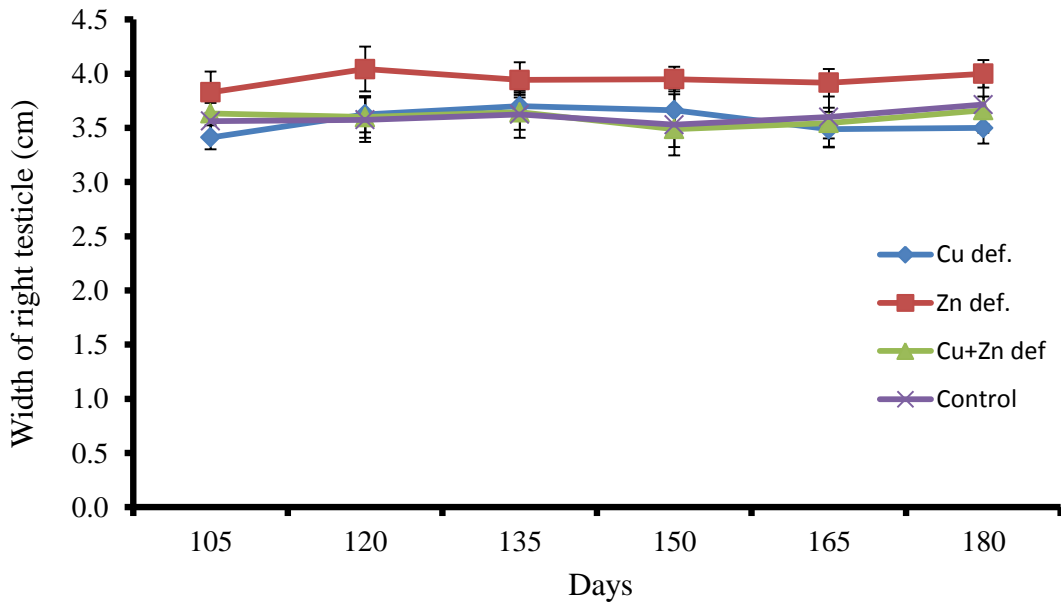
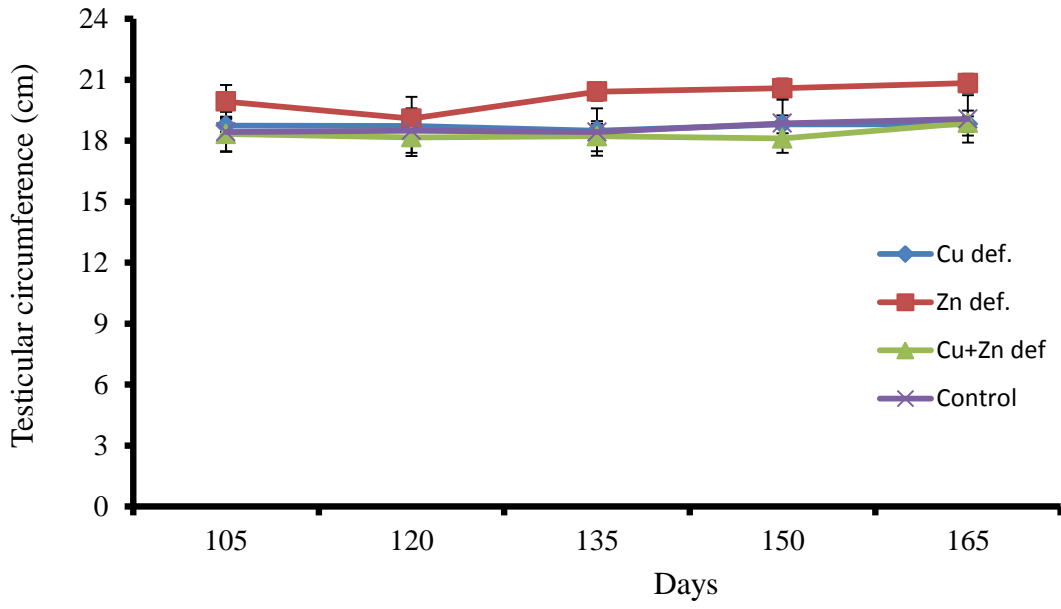
Effect of feeding Cu & Zn deficient diet on straw intake (g/d/animal) in goats:

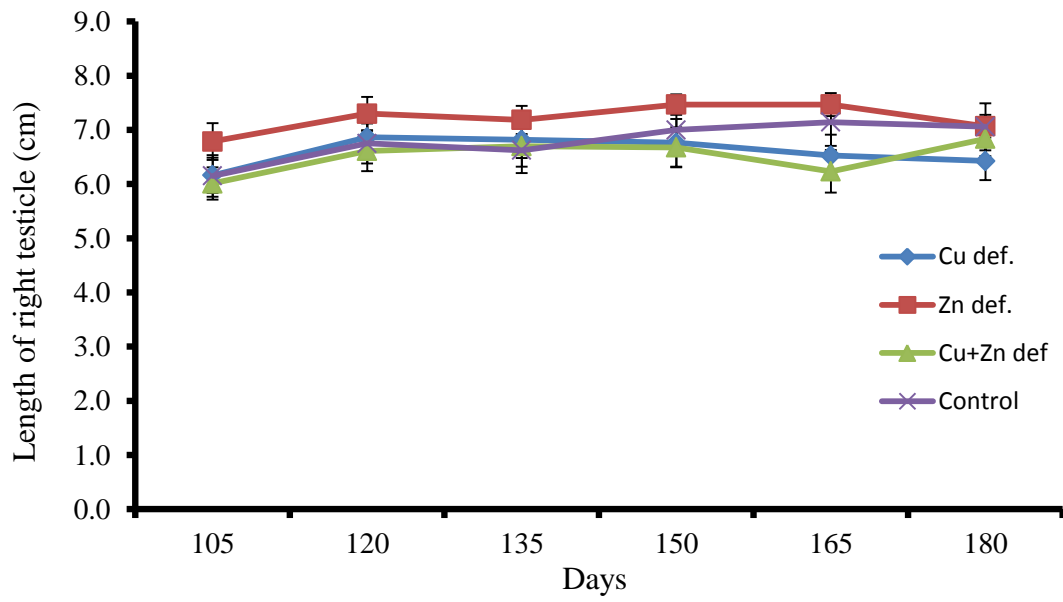
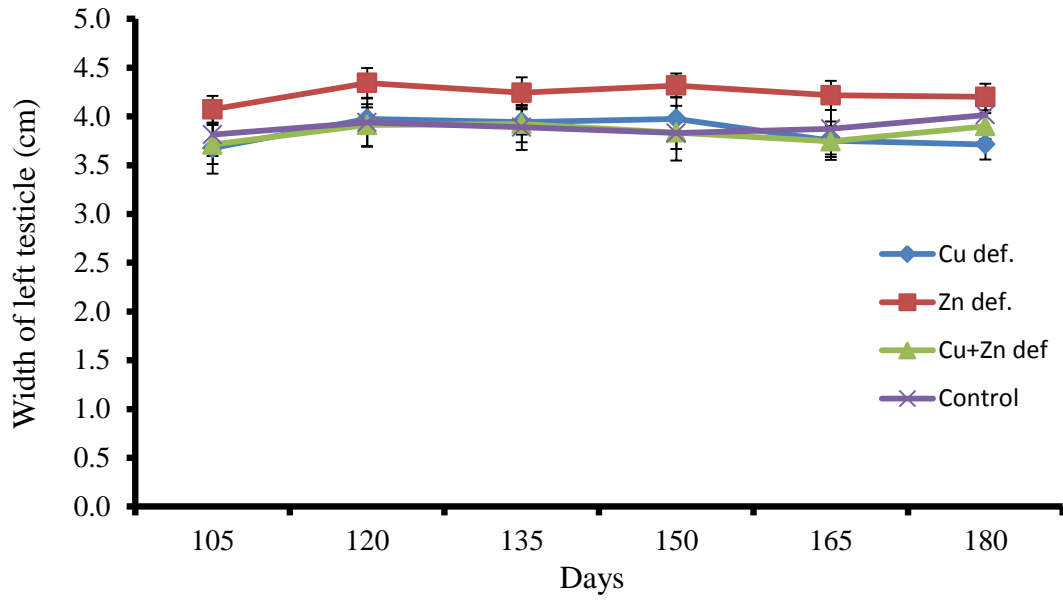
Groups	Dry matter intake (g/day/animal)
A	640.41±8.43
B	607.22±8.00
C	643.75±8.48
D	636.52±8.38

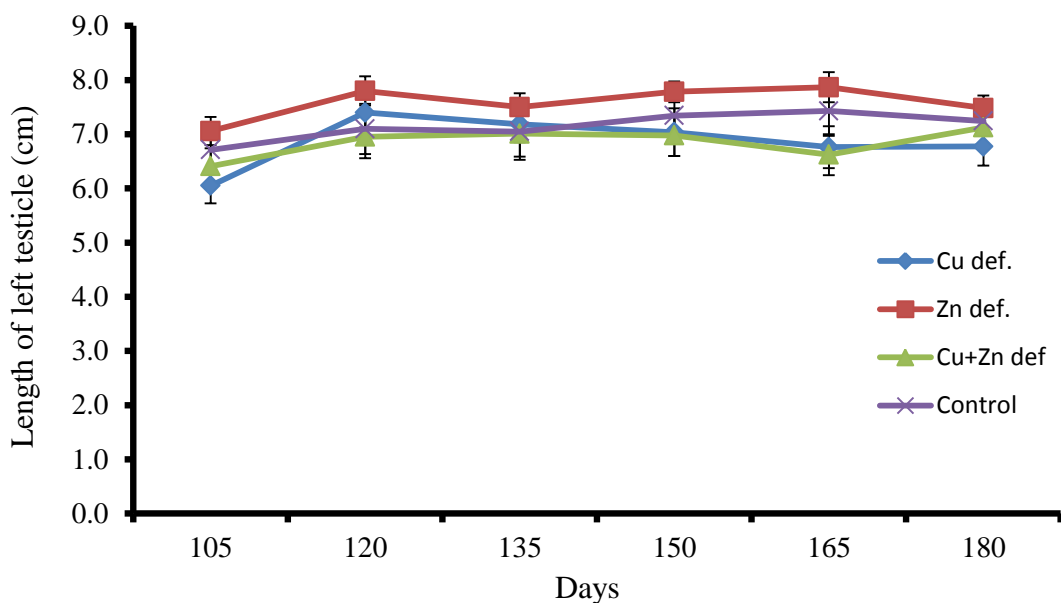
Testicular measurements of experimental animals:

Days		105	120	135	150	165	180
WIDTH (cm)							
Right							
Cu def.	Mean	3.4	3.6	3.7	3.7	3.5	3.5
	SEM	0.1	0.2	0.1	0.2	0.2	0.1
Zn def.	Mean	3.8	4.0	3.9	4.0	3.9	4.0
	SEM	0.2	0.2	0.2	0.1	0.1	0.1
Cu+Zn def	Mean	3.6	3.6	3.6	3.5	3.5	3.7
	SEM	0.2	0.2	0.2	0.2	0.1	0.1
Control	Mean	3.6	3.6	3.6	3.5	3.6	3.7
	SEM	0.2	0.2	0.2	0.3	0.3	0.3
Left							
Cu def.	Mean	3.7	4.0	3.9	4.0	3.8	3.7
	SEM	0.3	0.1	0.1	0.2	0.2	0.2

Zn def.	Mean	4.1	4.3	4.2	4.3	4.2	4.2
	SEM	0.1	0.2	0.2	0.1	0.1	0.1
Cu+Zn def	Mean	3.7	3.9	3.9	3.8	3.7	3.9
	SEM	0.2	0.2	0.2	0.2	0.2	0.1
Control	Mean	3.8	3.9	3.9	3.8	3.9	4.0
	SEM	0.2	0.2	0.2	0.3	0.3	0.3
LENGTH (cm)							
Right							
Cu def.	Mean	6.2	6.9	6.8	6.8	6.5	6.4
	SEM	0.3	0.3	0.3	0.4	0.4	0.4
Zn def.	Mean	6.8	7.3	7.2	7.5	7.5	7.1
	SEM	0.3	0.3	0.3	0.2	0.2	0.2
Cu+Zn def	Mean	6.0	6.6	6.7	6.7	6.2	6.8
	SEM	0.3	0.4	0.4	0.4	0.4	0.3
Control	Mean	6.2	6.8	6.6	7.0	7.1	7.1
	SEM	0.4	0.4	0.4	0.5	0.4	0.4
Left							
Cu def.	Mean	6.1	7.4	7.2	7.0	6.8	6.8
	SEM	0.5	0.3	0.3	0.4	0.4	0.4
Zn def.	Mean	7.1	7.8	7.5	7.8	7.9	7.5
	SEM	0.3	0.3	0.3	0.2	0.3	0.2
Cu+Zn def	Mean	6.4	7.0	7.0	7.0	6.6	7.1
	SEM	0.3	0.4	0.4	0.4	0.4	0.3
Control	Mean	6.7	7.1	7.1	7.3	7.4	7.2
	SEM	0.4	0.5	0.5	0.5	0.5	0.5
CIRCUMFERENCE (cm)							
Cu def.	Mean		18.8	18.7	18.5	18.8	18.8
	SEM		0.6	0.5	0.4	0.5	0.8
Zn def.	Mean		19.9	19.1	20.4	20.6	20.8
	SEM		0.8	1.1	0.4	0.5	0.5
Cu+Zn def	Mean		18.3	18.2	18.2	18.1	18.9
	SEM		0.9	0.9	0.7	0.7	0.6
Control	Mean		18.4	18.5	18.4	18.9	19.1
	SEM		1.0	1.1	1.2	1.2	1.2







Correlation between body weight and testes size:

Pearson's correlation co-efficient

Testes size	Body weight
Testicular circumference	0.836
Girth of right testicle	0.791
Girth of left testicle	0.815
Length of right testicle	0.707
Length of left testicle	0.732

Clinical features:

Clinically, the animals started showing signs, especially of Cu-deficiency in the A group, from 60 days onwards with progressive roughness of hair coat and increasing tendency of coarseness of hairs till 165 days as depicted in the figures below. Group-C animals fed Cu & Zn combined deficient diet also showed tendency of rough hair coat with increasing time however, the degree of changes were less intense compared to the group-A animals. Group-B (Zn-deficient) and control (group-D) animals did not show any discernible changes in their skin hair coat.

Pathology:

Gross lesions were not observed in an animal from Cu-deficient group-A which died at about 90 days of experimentation. However, the animal from Zn-deficient group-B died at about 105 days showed significantly atrophic testes in comparison to the control animal of group-D that was also died on the same day. The overall size and weight of both the testes of Zn-deficient animal was significantly decreased in comparison to the control animal as shown in the figure below. The size variables of testes in Zn-deficient (B-5) and Control (D-4) were as follow:

	B-5 (Zn-deficient)		D-4 (Control)	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)
Right testicle	9.19	5.00	26.27	6.50
Left testicle	9.39	5.50	27.74	7.20



Gross photograph showing size difference in Zn-deficient (B-5) and control (D-4) group animals.



Group-D: Control at 0 day



Group-A: Cu-deficient at 0 day



Group-B: Zn-deficient at 0 day



Group-C: Cu&Zn-deficient at 0 day



Group-D: Control at 60 days showing normal smooth hair coat.



Group-A: Cu-deficient at 90 days showing rough hair coat.



Group-A: Cu-deficient at 60 days showing relatively rough hair coat.



Group-B: Zn-deficient at 90 days showing relatively smooth, normal hair coat.



Group-D: Control at 90 days showing normal smooth hair coat.



Group-C: Cu&Zn-deficient at 90 days showing relatively normal smooth hair coat.



Group-A: Cu-deficient at 120 days showing rough hair coat.



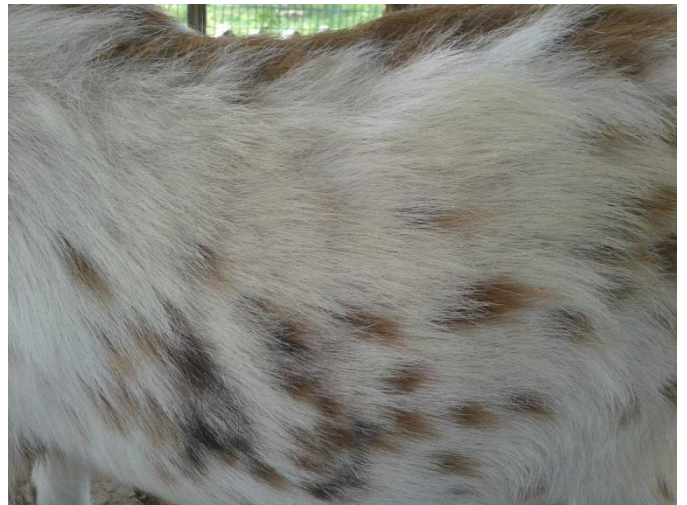
Group-B: Zn-deficient at 120 days showing relatively smooth, normal hair coat.



Group-C: Cu&Zn-deficient at 120 days showing relatively rough hair coat.



Group-D: Control animals at 150 days showing normal smooth hair coat.



Group-A: Cu-deficient at 150 days showing rough hair coat with coarse hairs.



Group-B: Zn-deficient animals at 150 days showing relatively smooth, normal hair coat.



Group-C: Cu&Zn-deficient at 150 days showing relatively rough hair coat.



Group-A: Cu-deficient at 165 days showing rough and coarse hairs.

a. List of Publications (one copy each to be submitted with RPP-II)

i. Research papers: Nil

ii. Working and Concept Papers

iii. Popular articles

iv. **Books/Book Chapters/ Lead Paper: 1**

1. RVS Pawaiya, UB Chaudhary, Nitika Sharma and N. Shivasharanappa. 2014. Nutrigenomics – exploiting the system biology for goat health. Lead paper. In: Compendium of national seminar on ‘Sheep and goat biodiversity and breeding policies: Issues and prospective’ held at Shirwal, Maharashtra during 21-22 February, 2014. LP-1-6. pp. 130-139.

b. Presentation in Workshop/Seminars/Symposia/Conferences: Nil
(relevant to the project in which scientists have participated)

c. Details of technology developed: None

(Crop-based; Animal-based, including vaccines; Biological – biofertilizer, biopesticide, etc; IT based – database, software; Any other – please specify)

d. Trainings/demonstrations organized:

e. Training received

f. Any other relevant information

8. **Constraints experienced, if any:** Scarcity of funds - Microarray analysis services could not be procured due to fund crunch. The Director is requested to kindly provide sufficient additional funds for procuring microarray analysis services.

9. Lessons Learnt

10. Evaluation

(a) Self-evaluation of the project for the period under report by the PI with rating in the scale of 1 to 10

(b) Evaluation by PI on the contribution of the team in the project including self

S. No.	Name	Status in the project (PI/CC-PI/Co-PI)	*Rating in the scale of 1 to 10
1.	RVS Pawaiya	PI	
2.	UB Chaudhary	Co-PI	
3.	Nitika Sharma	Co-PI	
4.	N Shivasharnappa	Co-PI	

11. **Signature of PI, CC-PI(s), all Co-PIs**

PI: (RVS Pawaiya)

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

12. Signature (with specific comments on progress/achievements, shortfall and constraints along with rating of the project in the scale of 1 to 10) of Head of Division/Regional Center / Section

13. Comments of IRC

14. Signature (with specific comments on progress/achievements, shortfall and constraints along with rating of the project in the scale of 1 to 10) of JD (R)/ Director

