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Review article

Full length articles

Short Communication

Review article

MACRO, MICRO AND NANO – TRANSITION IN THE VET WORLD

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Nanotechnology is ubiquitous and pervasive. It is an emerging field in all areas of science, engineering and technology (Mohanraj and Chen 2006). Nanotechnology represents technology that handles nanometer sized objects, particularly those less than 100 nanometer (nm) in diameter. These extremely small particles are endowed with more surface area, more atoms to contact surface and thereby lead to creation of materials wherein the exact number of atoms can also be measured (Mohapatra *et al*., 2011). As objects get smaller they have a much greater surface area to volume ratio. For example a 10 cm cube has a surface area of 600 cm2 and a volume of 1000 cm3 (ratio = 0.6:1) while a 2 cm cube would have a surface area of 24 cm2 and a volume of 8 cm3 (ratio = 3:1). Professor Taniguchi of Tokyo Science University used the word "nanotechnology" to describe the science and technology of processing or building parts with nanometric tolerances. A nanometer is a unit of length in the metric system, equal to one billionth of a meter, one thousandth of a micrometer, 3.281×10^{-9} of a feet and 39.37×10^{-9} of an inch. At such small sizes physical properties (magnetic, electric and optical) of materials change dramatically.

Currently three different levels of products that are developed under nanotechnology are materials, devices and systems. In the last two decades, primary studies were done based on the size and characterizations of nanomaterials in research laboratories and now it has entered a commercial exploitation period. Nanoparticles formulation strategies are differing based on their applications. The detailed characterization of nanoparticles compositions and properties are very important to design smart nanomaterials. The conventional physical chemistry instruments offered well enough information about physical and chemical properties of the nanomaterials. The complete characterization of the nanomaterials requires chemical composition, molecular structure, crystal structure, physical form at room temperature, surface area, particle size distribution, average diameter, solubility in water and biological active fluids, bulk density, agglomeration state, purity, surface charge sites, particle density and dispersibility. The commercial scale production of nanomaterials involved the initial proof-of-concept development with the production range 1-100g. Second step is pilot plant studies to ensure the proof-of-process and the production range is 1-10 kg. Third step is called large scale production

and the production range is 1-10 tonnes (Charitidis *et al*., 2014).

Two different approaches such as top down and bottom down are commercially used. Metal oxide nanoparticles are produced using top down approach and the bottom down approach is used in the production of semi-conductor quantum dots, carbides, nitrides, silver, gold and metals (excluding silver and gold). In the top down approach, the dimension of bulk materials gets gradually reduced to nano dimension using physical and chemical approaches. In the bottom down approach, the nano dimension materials are produced via reaction of atoms in synthetic environment and the size of the material is controlled by growth control mechanism (Biswasb *et al*., 2012). The synthesis of nanomaterials is a complex process (Pal *et al*., 2011) and a wide range of methods are currently developed by the researchers to develop different shapes and size such as spherical, cube, prism, hexagon, octahedron, disk, wire rod, tube etc.. Some of the commonly used approaches are (i) Hot colloidal chemistry (ii) Micro emulsion chemistry (iii) Organic chemical synthesis (iv) Co-Precipitation and Re-precipitation method (v) Self-assembly (vi) Seed mediated synthesis and (vii) bio synthesis

Classification of nanomaterials

Nanomaterials are broadly classified into four major classes and they are

1. Zero dimension
2. One dimension
3. Two dimensions
4. Three dimensions

Zero dimension

Zero dimension are defined as the materials wherein all the *dimensions* are measured within the nanoscale (no *dimensions*, or 0-D, are larger than 100 nm). Example - Nanoparticles

One dimension

One dimension is defined as one dimension that is outside the nanoscale (large than 100 nm). Example - Nanotubes, nanowire and nanorods

Two dimension

Two dimensions are defined as two of the dimensions are not narrowed as nanoscale. Example - Nano films, Nano layer and nanocoating

Three dimensions

Three dimensions are defined as the materials having three arbitrary dimensions above nanoscale. Example - Bundles of nano wire, multi nanolayers

Different types of nanoparticles

1. Pure metal nanocolloids (Ag, Au, Cu, Fe, Ni, Co, etc.,)
2. Bimetallic colloids (Pt-Ru, Pt-Ni, Co-Mo, Pd-Fe/Ru)
3. Metal oxides (TiO_2, ZnO, Fe_2O_3, CrO_2 metallates, etc.)
4. Metal chalcogenides Pbs, CdS, ZnS, ZnSe, CdSe, CdTe, HgS, CuInSe,etc.

5. Ferromagnetic shape memory alloys
6. Conducting polymer-Metal nanoparticles - composites

Nanoparticles characterization techniques

Parameters	Characterization Techniques
Particle size and distribution	Dynamic Light Scattering
	Light microscope
	Scanning electron microscope (SEM)
	Transmission electron microscope
	Atomic force microscope (AFM)
Nanoparticles surface chemical characterization techniques	X-ray photoelectron spectroscopy (XPS)
	Energy dispersive X-ray detector (EDX)
	Static secondary ion mass spectrometer
	Fourier transfer spectrometer (FTIR)
Surface charge determinations	Zeta potentiometer
	Laser Doppler Anemometry
Nanoparticles–Drug /protein interactions	Differential scanning calorimetry (DSC)
	Fourier transfer spectrometer (FTIR)
	Raman spectroscopy
	NMR spectroscopy
	High performance liquid chromatography (HPLC)
Surface hydrophobicity	Rose Bengal(dye) binding
	Water contact angle measurement
	X-ray photoelectron spectroscopy
Crystallinity analysis	X-ray diffraction analyser
Magnetic Properties	Vibrating sample magnetometer (VSM)
Thermal stability analysis	Thermo Gravimetric Analysis (TGA)

Food and Drug Administration (FDA) approved nanomaterials

US Food and Drug Administration (FDA) approved 26 nanomaterial products for therapeutic applications up to 2016 and majorly based on polymeric, liposomal and nanocrystals (Bobo *et al*., 2016, Hafner *et al*., 2014). Some of the FDA approved nanoparticles drugs are listed below:

- Liposomal doxorubicin
- Liposomal vincristine
- Liposomal mifamurtide
- Liposomal irinotecan

- Iron dextran colloid
- Iron gluconate colloid
- Iron sucrose colloid
- Liposome with hepatitis A virus
- Liposome with trivalent influenza
- Liposomal amphotericin B
- Liposomal Verteporfin
- PEGylated antibody (Fab' fragment of a humanized anti-TNF-alpha antibody)
- PEGylated filgrastim (granulocyte colony-stimulating factor)
- PEGylated interferon alfa-2b
- PEGylated anti-VEGF aptamer

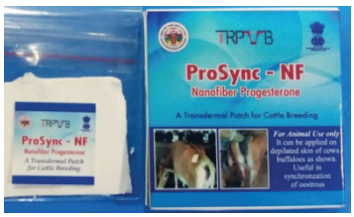
Nanoparticle applications

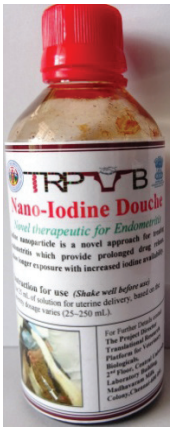
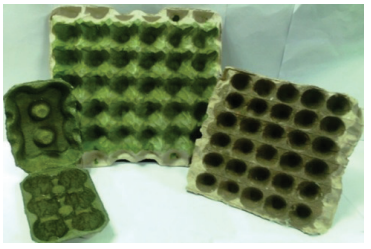
- The major medical applications of nanoparticles fall under the following categories
- Drug delivery
- Disease detection – Cancer. Virus
- Imaging

Biological applications of nanoparticles involves surface modification with different functional biomolecules by coating with DNA, drugs, peptides, carbohydrates, glycoproteins, aminoacids etc., Nanoparticles-based analytical techniques use specific antibodies, enzymes and also streptavidin against specific target sites. Surface-modified nanoparticles showed long-term stability and very small amount of samples are required for assays compared with traditional methods (Wang and Wang 2014). Currently, a lot of researchers are working on nanoparticles-based cell-targeting, delivery, imaging, and bio-labelling studies to improve traditional clinical practice. Nanoparticles-based applications currently dominate cancer studies due to their multi-functional ability and multiple therapeutic compound deliveries to specific target sites. External heating, magnetic stimulations and source-controlled activation at cellular level, improve the therapeutic efficiency of nanoparticles (Baetke *et al*., 2015, Roy Chowdhury *et al*., 2016). Fluorescent-coated nanoparticles and the semi-conductor nanoparticles exhibit strong photo stability when compared with free dyes and hence finds wide usage as imaging probes for drug /vaccine delivery studies (Li *et al*., 2011). The property of a nanoparticle to mimic a compound to inhibit/activate the specific receptors makes nanoparticles–protein interaction studies a vibrant area. Nanoparticles can also be used as a regulating tool in protein-protein and protein-nucleic acid interactions; regulation of enzyme activity and cell dynamics studies (Parak 2016, Ohta *et al*., 2016). Biophysically, nanoparticles combined with AFM cantilever is used as a technical tool to study cell adhesion, 3D imaging, development of cell microarray and so forth (Maver *et al*., 2016, Choi *et al*., 2014). Nanotechnology is also efficient in improving the bioavailability of water-insoluble drugs, protect the therapeutic agents from physiological barriers, enable the development of novel classes of bioactive macromolecules as well as carry large payloads (Yu *et al*., 2016). Liposomal drugs and polymer–drug conjugates approved by the US Food and Drug

Administration (FDA) for clinical uses are first-generation nanotechnology products that lack active targeting or controlled drug release components. Advancements in drug development with nanotechnology include synthesizing uni-lamellar and multi-lamellar liposomes with drugs and therapeutic peptides to make the therapy more effective (Panahi *et al*., 2017) Therefore, the main focus of current researchers is to design novel and multifunctional nanoparticle platforms for cell/tissue specific targeting, sustained or triggered drug delivery and co-delivery of synergistic drug combinations.

TRPVB is currently working on Nano pharmaceutical formulations and Nano therapeutic products developments related to veterinary medicine. Some of the products include the following:

S.No	Technology	Product Image	Utility
1.	**Nano Heal**		An unique combination of non-toxic and biocompatible ingredients that accelerates wound healing and exerts anti-bacterial effects. Useful for all species.
2.	**Nano Dermal Cream**		This is a unique combination of non-toxic and biocompatible ingredients that exerts multilevel antibacterial and antifungal effects
3.	**Progesterone Patch**		Controlled smart release of progesterone from impregnated nanofibers results in induction of oestrus within 6-7 days post-application

4.	**Progesterone Nano Cream**		Controlled smart release of progesterone from impregnated nanocream results in induction of oestrus within 6-7 days post-application
5.	**Teat Protect**		TEAT PROTECT is a unique germicidal teat protective spray for preventing mastitis. This gel works by preventing common mastitis causing bacteria from entering the teat canal and provides extended anti microbial protection.
6.	**Nano Iodine**		The Nano –Iodine is 5 times more potent than conventional iodine and exhibits strong anti-bacterial activity
7.	**Nanoguard**		A nano concoction coated egg trays that increases the shelf life of eggs stored especially meant for village chicken eggs

Veterinary technology should not be lagging behind in adopting this promising nano technology through its various potential applications in this naive area!!!

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Full Length Research Articles

IMPACT OF FARMYARD MANURE ON PLANT TOTAL NITROGEN IN PERENNIAL FODDER CROPS OF TAMIL NADU

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ABSTRACT

A field experiment was conducted to assess the impact of inorganic fertilizer alone (T1) and the combined effect of farmyard manure (organic) with inorganic fertilizer (T2) on plant total nitrogen (PTN) in two perennial fodder crops viz., CoCN4 Hybrid Napier (*Pennisetum purpureum* x *Pennisetum americanum*) and Desmanthus *(Desmanthus virgatus)* in North Eastern and Western Zones of Tamil Nadu, India during summer season of 2012. In Western zone two districts viz., Coimbatore and Erode districts and in North Eastern Zone Tiruvannamalai and Vellore districts were selected for the field experiments. From each district, two villages were randomly selected for field experiments totaling to eight experimental sites for the study. The plant total nitrogen (PTN) during the harvest period (90th day) varied between 1.38 to 1.64% for T1 (Recommended dose of NPK) and 1.43 to 1.68 % for T2 (Recommended dose of NPK and Farm yard manure) in CoCN4 Hybrid Napier and between 2.72 to 3.00% for T1 and 2.80 to 3.06% for T2 in Desmanthus fodder crop. Significant ($P<0.05$ or $P<0.01$) difference in plant total nitrogen content was evident between treatments on 90th day of the trial period for both the perennial fodder crops. A steady decline of plant total nitrogen for both the treatments from 30th day to 90th day (1st harvest) of the perennial fodder crops was evident during the trial period. The results recommended that integrated use of farm yard manure could be a viable option to increase the plant total nitrogen which has a definite impact on the crude protein content of the fodder crop and increased production in livestock farming systems.

Key Words: Plant total nitrogen, Farm yard manure, CoCN4 hybrid Napier, Desmanthus, Inorganic fertilizer.

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INTRODUCTION

Livestock form an integral part and vital constituent of farming systems in India. A variety of factors are responsible for lower productivity of dairy animals, but under-nourishment constitutes the biggest factor which hampers the performance of dairy animals in terms of milk production. The Napier-bajra hybrid grass is a popular fodder for rearing the livestock because of its higher biomass yield, and its suitability for feeding the dairy cattle, sheep and goats. Nitrogen is the most important plant nutrient as it is required for robust vegetative growth. Nitrogen is required by plants in greatest quantities than any other plant nutrient. Nitrogen even takes more important and vital role in forages yield and quality attributes. However, skyrocketing prices of inorganic fertilizers often result in their suboptimal use and resultantly the fodder yield suffers a serious setback. The use of organic sources of plant nutrition in combination with inorganic fertilizers holds the key to fulfill crops nutrition requirement (Iqbal *et al.,* 2015). Organic manure and inorganic fertilizer are the most common materials applied in agricultural management to improve soil quality and crop productivity (Verma and Sharma, 2007). Continuous use of inorganic fertilizers leads to deterioration in soil chemical, physical, and biological properties, and soil health. Combined organic and inorganic fertilization could enhance carbon storage in soils and reduce emission from N fertilizer use, while contributing to high productivity in agriculture (Pan *et al*., 2009). Hence the present study was undertaken to determine the effect of inorganic fertilizer and synergistic effect of inorganic fertilizer with organic fertilizer (farm yard manure) on plant total nitrogen in two different perennial crops viz., CoCN4 Hybrid Napier (*Pennisetum purpureum* x *Pennisetum americanum*) and Desmanthus *(Desmanthus virgatus)* in two agroclimatic zones of Tamil Nadu.

MATERIALS AND METHODS

A composite soil sample was collected at a depth of 0 – 15 cm in all the experimental villages prior to the study and analysed for the physico chemical properties**.** The field experiment was carried out using the Perennial fodder crop, CoCN4 Hybrid Napier (*Pennisetum purpureum* x *Pennisetum americanum*) and Desmanthus (*Desmanthus virgatus*) in two agroclimatic zones of Tamil Nadu, India viz., Western and North Eastern zone. In each zone two districts viz., Coimbatore and Erode district (Western Zone) and Tiruvannamalai and Vellore district (North Eastern zone) were selected for the field experiments. From each district, two villages were randomly selected for field experiments totaling to eight experimental sites for the study.

In Coimbatore district the 2 experimental sites were located at Kondaiyampalayam (V1) (11º32’ (N) latitude, 77º31’ (E) longitude and 679 ft above mean sea level) and Idigarai (V2) (11º07’(N) latitude, 76º53’(E) longitude and 1398 ft above mean sea level). In Erode district, the experimental sites were located at Velankattuvalasu (V3) (11º14’(N) latitude, 77º44’ (E) longitude and 685 ft above mean sea level) and Velliyampalayam (V4) (11º27’ (N) latitude, 77º28’ (E) longitude and 733 ft above

mean sea level). In the North Eastern Zone of Tiruvannamalai district, the selected experimental sites were Vannankulam (V5) (12º42' (N) latitude, 79º09' (E) longitude and 627 ft above mean sea level) and Kolathur village (V6) (12º10' (N) latitude, 79º12' (E) longitude and 467 ft above mean sea level). In Vellore district, Saduperi (V7), (12º53' (N) latitude, 79º06' (E) longitude and 714 ft above mean sea level) and Thirumani (V8) (12º36' (N) latitude, 79º21' (E) longitude and 726 ft above mean sea level) villages were selected for the study purpose.

The land was ploughed twice by a tractor with chisel ploughing followed by harrowing in all the experimental fields. The field was brought to fine tilth, leveled with a wooden plank and laid out in to the proper plot size (6 x 4 m).The experiment was laid out with six replications per treatment in all the study fields.

CoCN4 Hybrid Napier fodder slips were planted at 50 cm intervals on one side of the ridges and Desmanthus seeds were line sown on either side of the ridges as per the recommended package of practices. The experiment consisted of two treatments viz., Treatment 1 (T1) which is control with recommended dose of NPK fertilizers (100 N, 50 P_2O_5 and 40 K_2O kg/ha for CoCN4 Hybrid Napier and 10 N, 60 P_2O_5 and 30 K_2O kg/ha for Desmanthus) alone and Treatment 2 (T2) which included Farmyard Manure (Organic – Recommended dose - 12.5 t/ha) along with NPK fertilizer (inorganic – Recommended dose). The fertilizers were applied in the form of urea (N), Di-ammonium Phosphate (P_2O_5) and Muriate of Potash (K_2O). In all, 50 per cent of nitrogen and entire dose of P_2O_5 and K_2O were applied at the time of sowing and remaining 50 per cent of nitrogen was top dressed in the form of urea at 30 days after sowing (DAS). All the cultural practices were followed as per the recommended package of practices for the fodder crops usually. The necessary after care operations such as hand weeding were done as per the requirement. The plant protection measures have been adopted to control the pest and disease. Irrigation was carried out immediately after sowing (0^{th} day), on 3^{rd} day and thereafter once in 7 days.

Collection of fodder samples for estimation of Plant Total Nitrogen

Fodder samples were collected at random just above the ground level at 30^{th}, 60^{th}, 90^{th} (1^{st} harvest) and on every 45^{th} day subsequently for 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} harvests. The samples were shade dried and kept in oven at 60 - 70°C till constant weight was obtained. Finally the dried samples were ground to fine powder and subjected for chemical analysis of total nitrogen by using Analytikjena multi N/C 2100S carbon analyzer, with furnace temperature of 950°C, NDIR detector and oxygen as supportive gas.

Statistical analysis

The data collected were subjected to 't' test to find out the significant difference between treatments for all villages. In addition, One-Way ANOVA was performed using SPSS 13.0 to evaluate the significant difference between districts and zones. Also interpretation of data was done as per the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Plant total nitrogen in CoCN4 Hybrid Napier

The Physico-chemical properties of the soil in all the experimental sites are presented in Table 1. The Mean values of plant total nitrogen (PTN) in CoCN4 Hybrid Napier of western and north eastern zone of Tamil Nadu were presented in Table 2. The PTN content for CoCN4 Hybrid Napier on 30th day and 60th day varied from 2.56 to 2.82 % for T1, 2.61 to 2.88%, for T2 and 1.90 to 2.15 % for T1, 1.95 to 2.24 % for T2 respectively for all villages in the study area. During the harvest period (90th day) of the trial period the PTN content for CoCN4 Hybrid Napier varied between 1.38 to 1.64% for T1 and 1.43 to 1.68 % for T2 respectively in all villages. Significant ($P<0.05$ or $P<0.01$) difference in PTN content was evident between treatments on 90th day of the trial period. It could be observed from the results that there was a steady decline of PTN for both the treatments from 30th day to 90th day (1st harvest) of the fodder crop.

The gradual decrease of PTN in CoCN4 Hybrid Napier could be due to loss of leaf coupled with higher proportion of stems in total biomass which would be generally low in crude protein. Moreover, the PTN would be higher in immature plants than aged plants and as age progresses the crude fibre fractions in plant would have enhanced due to lignin deposition as the protein content gets diluted. This was in agreement with the findings of Tariq *et al.* (2011) who conducted a field trial to evaluate the effect of nitrogen at different levels and harvesting intervals in *Pennisetum americannuam L.* They found that the mean crude protein content decreased from 9.43 per cent on 45th day to 7.27 per cent on 75th day of harvest, indicating that the plant total nitrogen content decreased from 1.51 per cent to 1.16 per cent from 45th day to 75th day. It could be observed that the PTN was significantly higher for T2 compared with T1 from 30th day till 2nd harvest. The reason for significant increase in PTN for T2 could be due to incorporation of FYM along with NPK fertilizer which acted as a source of nitrogen for the fodder. Moreover, application of inorganic fertilizers with farm yard manure as a total basal dressing would be beneficial for balanced release of nutrients and reduction of N loss, thus increasing the N use efficiency. This was in agreement with the findings of Efthimiadou *et al.* (2010). Moreover, the enhancement in plant total nitrogen could be due to nitrogen content of farm yard manure which resulted in enhanced amino acid formation. This was in agreement with the findings of Shehzad *et al.* (2012).

In 2nd, 3rd, 4th, 5th and 6th harvest for all the villages the PTN content varied from 1.32 to 1.61 %, 1.29 to 1.59 %, 1.27 to 1.57%, 1.25 to 1.55% and 1.24 to 1.52% for T1 respectively and 1.35 to 1.64 %, 1.32 to 1.62%, 1.30 to 1.61%, 1.28 to 1.59% and 1.26 to 1.57%.01 for T2 respectively in all villages. The PTN content in CoCN4 Hybrid Napier has shown a decreasing trend in all the villages from 30th day till 6th harvest for both treatments. Moreover, it is evident from the table that PTN for CoCN4 Hybrid Napier also declined gradually from 2nd to 6th harvest for all villages. In 2nd harvest significantly higher PTN was recorded for

T2 than T1 for all villages. But no significant difference was recorded between treatments from 3rd harvest till 6th harvest, although T2 values remained higher.

In the experimental trial, recommended dose of inorganic fertilizer (T1) and farm yard manure along with inorganic fertilizer (T2) were applied at the start of the trial and no further addition of manure as well as inorganic fertilizer was carried for during the course of the experiment for CoCN4 hybrid napier in all villages. Frequent harvest affected the growth rate and chemical components due to changes in air temperature, rainfall and regrowth capability for the grass. This was in concurrence with the findings of Chengli *et al*. (2012) who studied the chemical composition of King grass at different cuttings in an experimental field of South China. They observed that the crude protein of the fodder showed a steady decline from 130.6 g kg^{-1} in first harvest to 83.6 g kg^{-1} at fourth harvest, indicating the total nitrogen fodder also tends to decrease as harvest progressed.

Inclusion of farm yard manure in T2 would have some residual effect on the plant total nitrogen as reflected by significantly higher PTN than T1 in 2nd harvest. From 3rd harvest onwards, the residual effect of farm yard manure would have decreased than the 2nd harvest and hence no significant difference was observed between the treatments. This was in agreement with the findings of Sharma *et al.* (2012) who studied the effect of different levels of nitrogen, organic manure and planting time on yield and quality of Hybrid Napier (*Pennisetum purpureum* Schum). They observed that the fodder total nitrogen reduced from 1.63 to 1.45 per cent from 1st to 3rd cut with application of farm yard manure (20 t/ha) and for inorganic fertilizer (80 kg/ha) it decreased from 1.57 to 1.31 per cent.

Plant total nitrogen in Desmanthus

The Mean values of plant total nitrogen (PTN) in Desmanthus of western and north eastern zone of Tamil Nadu were presented in Table 3. The PTN content for Desmanthus on 30th day and 60th day varied from 2.83 to 3.08% for T1, 2.90 to 3.15% for T2 and 2.73 to 3.02 % for T1, 2.82 to 3.10 % for T2 respectively for all villages in the study area. During the harvest period (90th day) of the trial period the PTN content for Desmanthus varied between 2.72 to 3.00% for T1 and 2.80 to 3.06 % for T2 respectively in all villages. Significant ($P<0.05$ or $P<0.01$) difference in PTN content was evident between treatments on 90th day of the trial period. It could be observed from the results that there was a steady decline of PTN for both the treatments from 30th day to 90th day (1st harvest) of the fodder crop.

In general the PTN would be higher in immature plants than aged plants and as age progresses the crude fibre fractions in plant would have enhanced due to lignin deposition as the protein content gets diluted. This was in agreement with the findings of Tariq *et al.* (2011). Also the decrease in plant total nitrogen was due to maturation and due to the protein factor which gets diluted. This was in concurrence with the findings of Ayub *et al.* (2009) and Ullah *et al.* (2010). It could be observed that the PTN was significantly higher for T2 compared with T1 from 30th day till 2nd harvest. The

reason for significant increase in PTN for T2 was due to incorporation of FYM along with NPK fertilizer which acted as a source of nitrogen for the fodder. The higher plant total nitrogen in T2 could be attributed to the ability of farm yard manure which supplied essential and improvement of physical and chemical properties of soil which released nutrients gradually throughout the growing season. This was in agreement with the findings of Ouda and Mahadeen (2008) who carried out an experiment to study the effect of fertilizers on growth, yield, yield components, quality and nutrient contents in Broccoli. They observed that the leaf nitrogen content significantly increased by the application of organic manure and inorganic fertilizer. Also enhancement in plant total nitrogen could be due to nitrogen content of farm yard manure which resulted in enhanced amino acid formation (Shehzad *et al.,* 2012).

In 2nd, 3rd, 4th, 5th and 6th harvest for all the villages the PTN content varied from 2.70 to 2.98 %, 2.68 to 2.95 %, 2.66 to 2.90%, 2.63 to 2.86% and 2.60 to 2.81% for T1 respectively and 2.79 to 3.03%, 2.72 to 2.98%, 2.70 to 2.93%, 2.66 to 2.90% and 2.62 to 2.85% for T2 respectively in all villages. The PTN content in Desmanthus has shown a decreasing trend in all the villages from 30th day till 6th harvest for both treatments. Moreover, it is evident from the table that PTN for the fodder crop also declined gradually from 2nd to 6th harvest for all villages. In 2nd harvest significantly higher PTN were recorded for T2 than T1 for all villages. But no significant difference was recorded between treatments from 3rd harvest to 6th harvest, although T2 values remained higher.

During the trial period, recommended dose of inorganic fertilizer (T1) and farm yard manure along with inorganic fertilizer (T2) were applied at the start of the trial and no further addition of manure as well as inorganic fertilizer was carried for during the course of the experiment in all villages. Harvesting management would affect the chemical composition of the Desmanthus. This was in accordance with the findings of Ullah *et al.* (2010) who conducted a study on the effect of nitrogen fertilization and harvesting intervals on the yield and forage quality of elephant grass. They observed that the fodder total nitrogen (% DM) decreased from 1.57 percent on 30th day to 1.02 percent on 60th day of harvest. This decrease was due to maturation and ultimately more utilization of nitrogen by the grass. Moreover, frequent harvest affected the growth rate and chemical components of due to changes in air temperature, rainfall and regrowth capability for the grass (Chengli *et al*., 2012)

Addition of farm yard manure in T2 would have some residual effect on the plant total nitrogen as indicated by significantly higher PTN than T1 in 2nd harvest. But from 3rd harvest onwards, the residual effect of farm yard manure would have been decreased than the 2nd harvest and hence no significant difference was observed between T1 and T2. This was in agreement with the findings of Sharma *et al.* (2012) who studied the effect of different levels of nitrogen, organic manure and planting time on yield and quality of Hybrid Napier (*Pennisetum purpureum* Schum). They observed that the fodder total nitrogen reduced from 1.63 to 1.45 per cent from 1st to 3rd cut with application of farm yard

manure (20 t/ha) and for inorganic fertilizer (80 kg/ha) it decreased from 1.57 to 1.31 per cent.

The results concluded that use of inorganic fertilizers alone or in synergistically with organic manure/ farm yard manure resulted in significant buildup of plant total nitrogen in CoCN4 hybrid napier and Desmanthus treated plots which in turn would increase the crude protein in the fodder crop. This will have a definite impact on production in livestock farming systems. Also the farm yard manure would help in higher uptake of plant total nitrogen from the soil in a steady manner which in turn increases the fodder yield.

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Table 1 - Physicochemical properties of the soil at experimental sites

Zone	District	Villages	Soil Properties					
			pH	Electrical conductivity (EC)	Organic Carbon (%)	Nitrogen (kg/acre)	Phosphorus (kg/acre)	Potassium (kg/acre)
Western	Coimbatore	Kondaiyampalayam (V1)	7.1	0.57	0.28	92.34	13.5	114.7
		Idigarai (V2)	7.3	0.56	0.29	91.23	13.7	116.5
	Erode	Velankattuvalasu (V3)	7.5	0.60	0.34	94.01	14.5	120.6
		Velliyampalayam (V4)	7.4	0.58	0.32	92.18	14.1	118.9
North Eastern	Tiruvannamalai	Vannankulam (V5)	7.0	0.58	0.25	91.72	12.8	112.1
		Kolathur (V6)	7.1	0.56	0.27	90.16	13.1	115.4
	Vellore	Saduperi (V7)	6.9	0.54	0.23	91.43	13.4	106.5
		Thirumani (V8)	6.8	0.53	0.24	89.22	13.2	109.8

Table – 2. Plant Total Nitrogen (in %) in CoCN4 Hybrid Napier of western zone and north eastern zone of Tamil Nadu

Zone	District	Villages	30th day			60th day			90th day(1st harvest)			2nd harvest		
			T1	T2	t value	T1	T2	t value	T1	T2	t value	T1	T2	t value
			Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E	
Western	CBE	V1	2.69 ± 0.01^{d}	2.73 ± 0.02^{de}	4.01**	1.99 ± 0.02^{cd}	2.06 ± 0.02^{c}	2.46*	1.50 ± 0.01^{d}	1.55 ± 0.02^{d}	2.58*	1.46 ± 0.01^{d}	1.51 ± 0.02^{d}	2.28*
		V2	2.72 ± 0.01^{d}	2.78 ± 0.02^{e}	2.66*	2.03 ± 0.01^{d}	2.12 ± 0.02^{d}	4.30**	1.53 ± 0.01^{d}	1.60 ± 0.02^{de}	3.61**	1.50 ± 0.01^{e}	1.55 ± 0.02^{e}	2.53*
	ERO	V3	2.82 ± 0.01^{e}	2.88 ± 0.02^{g}	2.77*	2.15 ± 0.01^{f}	2.24 ± 0.02^{e}	3.89**	1.64 ± 0.01^{f}	1.68 ± 0.01^{f}	2.37*	1.61 ± 0.01^{g}	1.64 ± 0.01^{f}	4.07**
		V4	2.78 ± 0.01^{e}	2.83 ± 0.01^{f}	2.55*	2.10 ± 0.01^{e}	2.17 ± 0.02^{d}	3.49**	1.58 ± 0.01^{e}	1.63 ± 0.02^{e}	2.39*	1.55 ± 0.01^{f}	1.59 ± 0.02^{e}	2.26*
North eastern	TVM	V5	2.59 ± 0.01^{ab}	2.64 ± 0.02^{ab}	2.35*	1.95 ± 0.02^{bc}	2.01 ± 0.02^{bc}	2.39*	1.42 ± 0.02^{b}	1.48 ± 0.02^{bc}	2.63*	1.38 ± 0.01^{bc}	1.43 ± 0.02^{bc}	2.23*
		V6	2.65 ± 0.02^{c}	2.71 ± 0.02^{cd}	2.44*	1.97 ± 0.02^{bc}	2.04 ± 0.02^{bc}	2.60*	1.46 ± 0.01^{c}	1.51 ± 0.01^{c}	3.72**	1.40 ± 0.01^{c}	1.45 ± 0.02^{c}	2.30*
	VLR	V7	2.56 ± 0.02^{a}	2.61 ± 0.01^{a}	2.71*	1.90 ± 0.01^{a}	1.95 ± 0.02^{a}	2.30*	1.38 ± 0.01^{a}	1.43 ± 0.02^{a}	2.25*	1.32 ± 0.01^{a}	1.35 ± 0.01^{a}	3.43**
		V8	2.61 ± 0.02^{bc}	2.67 ± 0.02^{bc}	2.35*	1.93 ± 0.02^{ab}	1.99 ± 0.02^{ab}	2.53*	1.40 ± 0.01^{ab}	1.46 ± 0.01^{ab}	3.83**	1.36 ± 0.01^{b}	1.39 ± 0.01^{b}	3.75**
F value			44.18**	31.22**		29.10**	23.14**		51.45**	32.50**		80.67**	48.19**	

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Villages	3rd harvest			4th harvest			5th harvest			6th harvest		
	T1	T2	t value	T1	T2	t value	T1	T2	t value	T1	T2	t value
	Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E	
V1	1.44 ± 0.02^{c}	1.49 ± 0.01^{d}	2.03^{NS}	1.43 ± 0.02^{d}	1.47 ± 0.02^{cd}	1.55^{NS}	1.40 ± 0.01^{de}	1.45 ± 0.02^{de}	1.81^{NS}	1.39 ± 0.01^{d}	1.44 ± 0.03^{de}	1.53^{NS}
V2	1.47 ± 0.02^{c}	1.50 ± 0.02^{d}	0.90^{NS}	1.44 ± 0.01^{d}	1.48 ± 0.01^{d}	2.12^{NS}	1.43 ± 0.02^{e}	1.47 ± 0.02^{e}	1.61^{NS}	1.42 ± 0.01^{d}	1.46 ± 0.02^{e}	2.19^{NS}
V3	1.59 ± 0.01^{e}	1.62 ± 0.01^{f}	1.72^{NS}	1.57 ± 0.02^{f}	1.61 ± 0.02^{f}	1.53^{NS}	1.55 ± 0.01^{g}	1.59 ± 0.02^{f}	2.04^{NS}	1.52 ± 0.01^{f}	1.57 ± 0.02^{f}	1.68^{NS}
V4	1.53 ± 0.01^{d}	1.57 ± 0.02^{e}	1.75^{NS}	1.51 ± 0.01^{e}	1.55 ± 0.01^{e}	1.90^{NS}	1.49 ± 0.01^{f}	1.54 ± 0.02^{f}	1.75^{NS}	1.47 ± 0.01^{e}	1.53 ± 0.03^{f}	2.09^{NS}
V5	1.37 ± 0.01^{b}	1.40 ± 0.01^{bc}	2.16^{NS}	1.34 ± 0.02^{bc}	1.37 ± 0.02^{b}	1.22^{NS}	1.33 ± 0.01^{bc}	1.35 ± 0.01^{bc}	1.11^{NS}	1.32 ± 0.01^{bc}	1.34 ± 0.01^{bc}	1.35^{NS}
V6	1.38 ± 0.01^{b}	1.43 ± 0.02^{c}	2.00^{NS}	1.37 ± 0.01^{c}	1.42 ± 0.03^{c}	1.72^{NS}	1.36 ± 0.01^{cd}	1.40 ± 0.02^{cd}	1.68^{NS}	1.35 ± 0.02^{c}	1.39 ± 0.02^{cd}	1.97^{NS}
V7	1.29 ± 0.02^{a}	1.32 ± 0.02^{a}	1.30^{NS}	1.27 ± 0.02^{a}	1.30 ± 0.01^{a}	1.56^{NS}	1.25 ± 0.01^{a}	1.28 ± 0.01^{a}	1.94^{NS}	1.24 ± 0.01^{a}	1.26 ± 0.01^{a}	1.53^{NS}
V8	1.34 ± 0.02^{b}	1.37 ± 0.01^{b}	1.53^{NS}	1.32 ± 0.02^{b}	1.34 ± 0.02^{ab}	0.81^{NS}	1.30 ± 0.02^{b}	1.32 ± 0.02^{ab}	0.66^{NS}	1.29 ± 0.02^{b}	1.31 ± 0.02^{ab}	1.08^{NS}
F value	41.90**	38.38**		38.27**	37.45**		49.27**	32.68**		57.98**	25.87**	

Means bearing same superscripts within columns do not differ significantly

NS – Non Significant * - Significant (P<0.05) ** - Highly Significant (P<0.01)

Table – 3. Plant Total Nitrogen (in %) in Desmanthus of western zone and north eastern zone of Tamil Nadu

Zone	District	Villages	30th day			60th day			90th day(1st harvest)			2nd harvest		
			T1	T2	t value	T1	T2	t value	T1	T2	t value	T1	T2	t value
			Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E	
Western	CBE	V1	2.94 ± 0.01b	3.02 ± 0.02de	4.06**	2.88 ± 0.01cd	2.97 ± 0.02cd	3.91**	2.86 ± 0.03c	2.93 ± 0.02cd	2.32*	2.85 ± 0.02cd	2.91 ± 0.01cd	2.89*
		V2	3.00 ± 0.01c	3.05 ± 0.01ef	3.28**	2.92 ± 0.01d	2.98 ± 0.01d	4.35**	2.90 ± 0.01d	2.96 ± 0.02d	2.77*	2.87 ± 0.02d	2.94 ± 0.02d	2.76*
	ERO	V3	3.08 ± 0.02d	3.15 ± 0.02g	2.77*	3.02 ± 0.01f	3.10 ± 0.02f	3.62**	3.00 ± 0.01f	3.06 ± 0.02f	3.26**	2.98 ± 0.02f	3.03 ± 0.01f	2.27*
		V4	3.03 ± 0.01c	3.09 ± 0.01f	2.62*	2.96 ± 0.02e	3.03 ± 0.01e	3.44**	2.94 ± 0.01e	3.01 ± 0.02e	4.13**	2.93 ± 0.01e	2.99 ± 0.01e	3.34**
North eastern	TVM	V5	2.87 ± 0.01a	2.95 ± 0.03bc	2.94*	2.80 ± 0.02b	2.87 ± 0.02b	2.76*	2.78 ± 0.02b	2.85 ± 0.02b	4.02**	2.76 ± 0.02b	2.83 ± 0.01b	3.31**
		V6	2.92 ± 0.02b	2.98 ± 0.02cd	2.65*	2.86 ± 0.02c	2.93 ± 0.01c	3.61**	2.84 ± 0.01c	2.90 ± 0.02c	4.01**	2.82 ± 0.02c	2.87 ± 0.02bc	2.24*
	VLR	V7	2.83 ± 0.02a	2.90 ± 0.01a	3.06*	2.73 ± 0.02a	2.82 ± 0.01a	3.64**	2.72 ± 0.02a	2.80 ± 0.01a	4.97**	2.70 ± 0.01a	2.79 ± 0.02a	4.08**
		V8	2.85 ± 0.02a	2.93 ± 0.02ab	2.60*	2.78 ± 0.02b	2.85 ± 0.01ab	2.81*	2.76 ± 0.01b	2.82 ± 0.02ab	3.01*	2.73 ± 0.01ab	2.76 ± 0.01a	2.56*
F value			35.70**	22.77**		36.75**	41.78**		52.06**	35.87**		36.58**	41.78**	

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Villages	3rd harvest			4th harvest			5th harvest			6th harvest		
	T1	T2	t value	T1	T2	t value	T1	T2	t value	T1	T2	t value
	Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E	
V1	2.83 ± 0.02de	2.87 ± 0.02de	1.51 ^{NS}	2.79 ± 0.01cd	2.82 ± 0.01cd	1.98^{NS}	2.76 ± 0.01de	2.78 ± 0.01bc	1.81^{NS}	2.72 ± 0.02c	2.75 ± 0.01de	1.26^{NS}
V2	2.84 ± 0.01e	2.89 ± 0.03ef	1.67 ^{NS}	2.81 ± 0.02de	2.85 ± 0.01de	2.13^{NS}	2.79 ± 0.01ef	2.82 ± 0.01cd	1.61^{NS}	2.74 ± 0.01cd	2.77 ± 0.01ef	1.65^{NS}
V3	2.95 ± 0.02g	2.98 ± 0.02g	1.30^{NS}	2.90 ± 0.02f	2.93 ± 0.01f	1.25^{NS}	2.86 ± 0.01g	2.90 ± 0.02e	1.93^{NS}	2.81 ± 0.02e	2.85 ± 0.01g	2.01^{NS}
V4	2.89 ± 0.01f	2.93 ± 0.01f	2.04^{NS}	2.85 ± 0.01e	2.88 ± 0.01e	1.88^{NS}	2.82 ± 0.01fg	2.86 ± 0.01d	2.14^{NS}	2.78 ± 0.01de	2.81 ± 0.02fg	1.47^{NS}
V5	2.74 ± 0.01bc	2.79 ± 0.01bc	2.17^{NS}	2.71 ± 0.01b	2.74 ± 0.02b	2.00^{NS}	2.67 ± 0.02bc	2.69 ± 0.02a	0.70^{NS}	2.65 ± 0.02ab	2.68 ± 0.02bc	1.17^{NS}
V6	2.79 ± 0.02cd	2.83 ± 0.02cd	1.70^{NS}	2.75 ± 0.01c	2.79 ± 0.02c	2.03^{NS}	2.72 ± 0.02cd	2.75 ± 0.01b	1.36^{NS}	2.69 ± 0.02bc	2.71 ± 0.02cd	1.12^{NS}
V7	2.68 ± 0.01a	2.72 ± 0.01a	1.87^{NS}	2.66 ± 0.02a	2.70 ± 0.02a	1.38^{NS}	2.63 ± 0.02a	2.66 ± 0.02a	1.33^{NS}	2.60 ± 0.02a	2.62 ± 0.02a	0.93^{NS}
V8	2.71 ± 0.01ab	2.74 ± 0.01ab	2.09^{NS}	2.68 ± 0.01ab	2.71 ± 0.01ab	1.69^{NS}	2.65 ± 0.02ab	2.68 ± 0.01a	1.35^{NS}	2.63 ± 0.02a	2.65 ± 0.01ab	0.84^{NS}
F value	32.59**	27.51**		35.83**	40.03**		29.78**	35.44**		17.23**	25.59**	

Means bearing same superscripts within columns do not differ significantly

NS – Non Significant * - Significant (P<0.05) ** - Highly Significant (P<0.01)

EFFECT OF EMBLICA OFFICINALIS (AMLA) ON GENERAL AND HEMATO-BIOCHEMICAL PARAMETERS IN ARSENIC INDUCED JAPANSE QUAILS

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ABSTRACT

One hundred and fifty Japanese quails (*Coturnix coturnix japonica*) were divided in 6 equal groups. Group T_1 birds were given commercial diet without any addition whereas group T_2 birds were fed with arsenic trioxide @ 300 ppm in feed. The group T_3, T_4 birds were supplemented with *Emblica officinalis* fruit powder @ 5% or 10% level, respectively. Group T_5, T_6 birds were given arsenic trioxide @ 300 ppm along with *E. officinalis* @ 5% or 10%, respectively. Average body weight, weight gain and average feed consumption was found to be highest in T_4 and T_3 group birds followed by group T_1, T_6, T_5 indicating the better performance as compare to T_2 group birds. Birds from T_2 group showed significant decrease in Hb, PCV, TEC when compared with control and other treatment groups. However in arsenic fed birds showed significant increase in plasma AST, ALT, ALP, creatinine values. The lower value of AST, ALT, and ALP were observed in arsenic and *E. officinalis* group. The creatinine values were lowered in group fed arsenic and *E. officinalis*.

Key words- *Emblica.Officinalis, hepatoptective arsenic toxicity Japanese quail*

INTRODUCTION

Arsenic occurs as an inorganic and organic compound. Inorganic form is more toxic compared organic form. It has been considered as an essential trace element for normal growth and development of animals. Aquatic shellfish contains highest concentration of arsenic. Once chicken / quail eat arsenic preparation (Roxarsone) and some of it gets evenly distributed the body tissues includes breast, thigh, leg muscles etc. The rest of arsenic is excreted and 90% of this manure is later converted into fertilizers that can contaminate crops near the lakes and eventually leaches in to drinking water. Arsenic has affinity for tissues rich in oxidative enzyme such as intestine, liver and kidney.

Emblica officinalis (Amla) plants have been proved as a defensive role against oxidative stress caused by arsenic. It is an admirable remedy to environmental toxins,

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offering protection from number of heavy metals. *E.officinalis* is recognized to have the maximum content of vitamin C of any natural plant. *E.officinalis* is good for almost each one on regular basis, as it remove the jeopardy of environmental pollutants.

MATERIAL AND METHODS

Group T_1 served as commercial control and T_2 served as positive control group for arsenic trioxide (As_2O_3, Mol. Wt. 197.84). Group T_3, T_4 were given *Emblica officinalis* fruit powder @ 5% and 10% level, respectively. Whereas group T_5, T_6, were given arsenic trioxide and *E. officinalis* fruit powder @ 5% and 10% level, respectively in the feed for a period of six weeks as under.

All experimental birds were closely observed for clinical signs weekly feed consumption, Weekly body weight (g), weekly weight gain (g) was recorded and calculated. Six birds from each group were randomly selected for collection of blood sample at the end of experimental period. The haematological parameters studied includes haemoglobin (Hb), Total erythrocyte count (TEC), Mean corpuscular volume (MCV) and differential leucocyte count (DLC) as per standard method described by (Benjamin, 2001), Packed cell volume (PCV) by (Pierson, 2000), Total erythrocyte count and Total leucocyte count was carried out with improved Neubaure's chamber using Natt and Harrick diluting fluid (Natt and Herrick, 1952) .

Plasma total protein level was estimated by Biuret method (Vatzidis, 1977). Plasma albumin levels by Bromocresol Green method, (Gustaffson, 1978), Plasma globulin levels were estimated as difference between total protein and albumin for each group. Enzyme serum glutamate oxaloactate transaminase and Serum glutamate pyruvate transaminase by (Reitman and Frankel, 1957) method, Plasma creatinine by modified Jaffes method (Bartels, 1971) and Plasma alkaline phosphatase (Young, 1997).

RESULTS AND DISCUSSION

Control and treatment groups birds did not showed any abnormal clinical signs during experiment except, birds from T_2 group. T_2 group birds showed retarded growth and were dull, depressed with drop in egg production as compared to other groups. The reduction in egg production in arsenic fed group is in agreement with Chiou *et al*. (1997) who fed arsenic@44 mg/kg to birds.

Significantly higher average total feed utilization was noted in groupT_3 and T_4 as compared to T_2, indicating tastiness of *E. officinalis* powder. The average feed utilization was significantly lower (3572±345.82 g) in group T_2 group. Similar type of reduction in feed intake has been also reported by Chiou *et al.* (1997) in layers.

The feed consumption (4250±362.17 g) in group T_5 and T_6 (4136.67±340.60 g) was recorded numerically higher as compare to group T_2. This indicates that decreased feed consumption in arsenic group birds was better, by supplementation of *E. officinalis* powder in feed which might be due to delectableness and gastro-

protective effect of *E. officinalis* as reported by Al-Rehaily *et al.* (2002).

The average weekly body weight of quails was numerically highest (236.72±3.29 g) in T_4 group followed by T_3 group (232.96±5.13 g) followed by group T_1 (229±4.54 g) although comparable significant decline statistically (209.28±8.88 g) in weekly body weight was recorded in group T_2 as compared to all other groups. The present findings agree with Chiou *et al.* (1997) and Chen *et al.* (2000). The decline in body weight by feeding arsenic in birds might be due to digestive disturbances. Arsenic may produce inflammation in proventriculus and gizzard, gelatinous exudate beneath horny lines of gizzard causing sloughing of horny lines (Roy, 2009). This may be the reason for T_5 and T_6 group showing lower body weight as compared to group T3 and T_4.

Group T_2 showed numerical lower values (25.58±6.90 g) of weight gain in quails as compared to rest of groups and was highest (28.80±7.17 g) in group T_4. The non-significant improvement in weight gain was observed in *E.officinalis* treated group. The present finding of minor progress in body weight gain in quails was observed by Rao *et al.* (2005) who reported minor perfection in weight gain in diabetic rats after oral administration of amla extracts @ 20 or 40 mg/k body weight/day.

The mean haemoglobin values in T_2 group was significantly lowest (7.83±0.25) when compared with other group birds fed either commercial diet i.e. group T_1 (10.23±0.27) or *E. officinalis* fruit powder i.e. group T_3 (10.43±0.50), group T_4 (10.5±0.59), T5 (9.20±0.27) and T6 (9.43±0.34) group. Arsenic causes inhibition of blood amino levunic acid dehydrogenase (ALAD) activity resulting in disturbed heme synthesis pathway (Padmaja *et al*., 2009). The fall of haemoglobin may possibly due to suppression of bone marrow activity as a result of toxic effect of arsenic (Halder *et al.,* 2009). The improvement of haemoglobin values in treatment groups with *E. officinalis* point out ameliorating effect of *E. officinalis* in arsenic toxicity. Result of present experiment supported with Padmaja *et al*. (2009) who found significant decline in haemoglobin values in arsenic toxicated chicks which was improved by supplementation of *E.officinalis*. Perfection in haemoglobin values might be due to stimulating effect of *E. officinalis* on bone marrow (Singh and Rana, 2007).

The mean Packed cell volume (PCV) level of T_2 group was significantly lower (25.67±1.31) than all other treatment groups. Similar results were reported by Halder *et al.* (2009) who observed decreased level of PCV in arsenic control layer chickens. Padmaja *et al.* (2009) also found significant decrease in PCV level in arsenic fed chicks @ 100 ppm. Highest value of PCV was observed in *E.officinalis* fed group. Enhancement in PCV values might be due to stimulating effect of *E. officinalis* on bone marrow Singh and Rana (2007).

Mean total erythrocyte count (TEC) values in T_2 group were significantly decreased (2.07±0.14) when compared with T_1, T_3, T_4 group. Hong *et al.* (1989) also found similar decrease in red blood cells in mice exposed to arsine gas (2.5 ppm). The

decrease level of TEC was possibly due to inhibition of bone marrow (Halder *et al*., 2009).

Statistically non significant but numerical lowest value (126.18±8.51) of MCV, (38.65±2.74) MCH, (23.35±1.31) of TLC were found in group T_2 as compared to rest of the groups and lower value (27.43±1.76) MCHC was found in group T_3.

Average mean values of plasma total protein, Albumin and globulin in group T_2 showed numerical lower values (4.12±0.22), (2.31±0.19) and (1.85±0.24) respectively as compare to other groups.

The significant increased value (347.98±37.27) of average plasma aspartate amino transferase (AST) in group T_2 as compared to rest of the groups might be due to lesion or cellular damage caused by arsenic trioxide as reported by Islam *et al*. (2009) who also reported significant increase of plasma AST value in arsenic toxicated chicks. The birds receiving either commercial diet or *E. officinalis* fed diet with or without arsenic revealed insignificant differences in the plasma AST levels. However the birds which received only *E. officinalis* powder revealed numerically lowest value (194.27±9.59) of plasma AST in group T_4 as compare to all experimental group birds. This indicates hepatoprotective and antioxidant properties of *E.officinalis* as reported by Bhattacharya *et al*. (2000) who also observed lower AST values in *E. officinalis* supplemented group.

The plasma ALT showed significantly higher values (35.95±2.88) in T_2 group than all experimental groups. Such significant increase in serum ALT value in goats administered with sodium arsenite @ 2 mg/kg body weight has been also reported by Roy *et al.* (2009).

This increase in Plasma alanine amino transferase (ALT) values might be possibly due to hepatic damage which leads to release of tissue specific enzymes in blood stream as reported by Vutukuru *et al.* (2007) in *Labeo rohita* fish fed with arsenic @ 20, 25, 30, 40 mg/l. birds showed lower value (18.88±1.07) in group T_4 as compared to arsenic fed group birds. This might be due to significantly enhanced activity of the various antioxidant enzymes and GST as well as glutathione system in the blood due to administration of *E. officinalis* (Hari Kumar *et al.,* 2004).

Significant increase value (0.98±0.07) of plasma creatinine was recorded in group T_2, as compare to other groups. The similarly concluded by Chen and Chiout (2001) in mule duck treated with arsenic (Roxarsone, AS_2O_3 and AS_2O_5). Increase in creatinine values might be due to renal damage (Brar *et al*., 2004). Whereas birds receiving arsenic along with *E. officinalis* showed reduction in creatinine value as compare to birds fed diet with arsenic. This *E. officinalis* act as nephroprotective agent Singh and Rana (2007). Reduction in creatinine value in *E. officinalis* fed broilers which where increase in arsenic @ 100 ppm fed broilers reported by Padmaja *et al.* (2009).

Group T_2 showed significant increase values (504.48±63.84) of plasma ALP as compare to other remaining groups.

Elevated level of alkaline phosphatase might be due to mitochondrial dysfunction in alcoholic rats as observed by Reddy *et al.* (2009). The similar findings were recorded by Islam *et al.* (2009) who observed significant increased ALP values in duck toxicated with arsenic trioxide @ 100 mg/l drinking water.

*G*roup T_4 showed significant lowest values (317.00±12.25) as compare to group T_2. The present finding supported to Singh and Rana. (2007) who found significant decrease values of serum ALP in *E. officinalis* group (50 mg/kg or 100 mg/ kg) whereas these values was increased in toxicated rats. The present finding reveal protective role of *E. officinalis* against arsenic toxicity.

SUMMARY AND CONCLUSIONS

E. officinalis @ 5% and 10% level with arsenic improves the feed consumption, body weight, hematological parameters and lowers the Plasma AST, Plasma ALT, Plasma ALP, Creatinine levels as compared to arsenic fed group.

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Table 1 : Average weekly feed consumption (g) in different groups

Week	Treatment						Total average feed consumption
	T_1 (Control)	T_2 (Arsenic trioxide 300ppm)	T_3 (Emblica Officinalis @5%)	T_4 (Emblica Officinalis @10%)	T_5 (Arsenic trioxide 300ppm+ Emblica Officinalis @5%)	T_6 (Arsenic trioxide 300ppm+ Emblica Officinalis @10%)	
3rd	3100	2519	3500	2900	2800	3200	**3003.17 [a] ± 152.22**
4th	4630	3000	4550	5800	5500	5000	**4746.67[b] ± 439.42**
5th	5808	3600	5100	5500	4000	4320	**4721.33[b] ± 392.72**
6th	5710	3300	5600	4900	4500	5100	**4851.67[b] ± 393.87**
7th	4589	4913	5200	5400	4100	4000	**4700.33[b] ± 256.274**
8th	4986	4100	5400	5000	4600	3200	**4547.67[b] ± 353.323**
Total average feed consumption	**4803.83[bc] ± 401.75**	**3572.00[a] ± 345.82**	**4891.67[c] ± 313.69**	**4916.67[c] ± 425.38**	**4250.00[abc] ± 362.17**	**4136.67[ab] ± 340.60**	

Table 2 : Average weekly body weight (g) per chick in different groups

Group	Age in weeks							Pooled mean
	2nd	3rd	4th	5th	6th	7th	8th	
T_1	62.56 ± 1.66	111.96 ± 2.13	153.04 ± 2.44	191.72 ± 3.92	217.72 ± 7.33	223.48 ± 6.26	229.00 ± 4.54	**169.93^b ± 4.77**
T_2	55.80 ± 2.01	101.64 ± 2.75	142.08 ± 2.17	175.08 ± 2.27	192.08 ± 2.74	207.44 ± 4.15	209.28 ± 8.88	**154.77^a ± 4.38**
T_3	62.92 ± 1.37	112.04 ± 1.52	155.36 ± 1.52	189.52 ± 3.24	222.40 ± 4.61	229.36 ± 4.79	232.96 ± 5.13	**172.08^b ± 4.77**
T_4	63.92 ± 1.92	115.44 ± 1.25	158.32 ± 1.12	191.48 ± 4.55	217.16 ± 2.25	233.00 ± 2.41	236.72 ± 3.29	**173.72^b ± 4.67**
T_5	65.48 ± 1.70	105.84 ± 2.65	158.84 ± 2.82	186.28 ± 2.07	202.28 ± 1.97	213.04 ± 1.58	221.88 ± 5.22	**164.81^b ± 4.27**
T_6	62.24 ± 1.58	111.48 ± 2.72	149.80 ± 2.32	186.80 ± 2.55	208.84 ± 2.36	218.84 ± 1.73	226.16 ± 2.21	**166.31^b ± 4.39**

Table 3 : Average weekly body weight gain (g) per chick in different groups

Group	Age in weeks						Pooled mean	
	3rd	4th	5th	6th	7th	8th		
T_1	49.4	41.08	38.68	26.00	5.76	5.52	**27.74±7.63**	**NS**
T_2	45.84	40.44	33.00	17.00	15.36	1.84	**25.58±6.90**	
T_3	49.12	43.32	34.16	32.88	6.96	3.6	**28.34±7.70**	
T_4	51.52	42.88	33.16	25.68	15.84	3.72	**28.80±7.17**	
T_5	40.36	53.00	27.44	16.00	10.76	8.84	**26.07±7.22**	
T_6	49.24	38.32	37.00	22.04	10.00	7.32	**27.32±6.89**	
Pooled mean	**47.58^e ± 1.62**	**43.17^e ± 2.10**	**33.91^d ±1.59**	**23.27^c ±2.58**	**10.78^b ±1.70**	**5.14 ^{a}±1.06**		

Mean values with common alphabet as superscript do not differ significantly

CD for week = 5.62 (significant at 1% and 5% level)

Table 4 : Haematological values in different group at the end of 6th week of experiment

Group	Hb (g/dl)	PCV (%)	TEC (10^6/ cumm)	MCV (f_l)	MCH (pg)	MCHC (%)
T_1	$10.23^{bc} \pm 0.27$	$35.00^{ab} \pm 1.00$	$2.79^{bc} \pm 0.14$	126.95 ± 5.94	37.26 ± 2.35	29.38 ± 1.23
T_2	$7.83^{a} \pm 0.25$	$25.67^{c} \pm 1.31$	$2.07^{a} \pm 0.14$	126.18 ± 8.51	38.65 ± 2.74	30.79 ± 1.27
T_3	$10.43^{c} \pm 0.50$	$38.33^{a} \pm 1.58$	$2.84^{c} \pm 0.10$	136.33 ± 8.33	37.19 ± 2.68	27.43 ± 1.76
T_4	$10.50^{c} \pm 0.59$	$38.00^{a} \pm 2.07$	$2.81^{bc} \pm 0.10$	135.14 ± 6.20	37.48 ± 2.32	27.96 ± 2.03
T_5	$9.20^{b} \pm 0.27$	$33.00^{b} \pm 1.84$	$2.35^{a} \pm 0.12$	141.26 ± 7.03	39.69 ± 2.27	28.17 ± 1.16
T_6	$9.43^{bc} \pm 0.34$	$34.33^{ab} \pm 1.74$	$2.44^{ab} \pm 0.19$	144.71 ± 12.67	39.65 ± 3.15	27.91 ± 1.87
	CD for treatment = 1.13 (significant at 1% and 5% level)	**CD for treatment = 4.71 (significant at 1% and 5% level)**	**CD for treatment = 0.39 (significant at 1% and 5% level)**	**NS**	**NS**	**NS**

Mean values with common alphabet as superscript do not differ significantly

Table 5 : Total leucocyte count (10^3/cumm) and absolute leucocyte count in different groups

Group	Total leucocyte count (TLC)	Absolute heterophil	Absolute lymphocyte	Absolute eosinophil	Absolute basophil	Absolute monocyte
T_1	28.46 ± 1.36	11617.82 ± 1255.10	15556.75 ± 1134.62	887.87 ± 192.03	0.00 ± 0.00	397.57 ± 123.40
T_2	23.35 ± 1.31	9744.33 ± 818.05	12450.95 ± 886.13	745.73 ± 96.92	39.78 ± 39.78	364.20 ± 100.93
T_3	27.29 ± 1.48	11136.27 ± 802.05	14924.43 ± 1772.08	825.83 ± 115.19	0.00 ± 0.00	406.80 ± 59.99
T_4	28.03 ± 1.49	11519.65 ± 700.60	15347.45 ± 1167.64	754.35 ± 135.80	0.00 ± 0.00	406.88 ± 103.84
T_5	26.27 ± 1.07	10779.75 ± 592.35	14287.40 ± 828.67	899.60 ± 163.59	0.00 ± 0.00	304.92 ± 86.84
T_6	26.68 ± 0.96	10977.17 ± 631.75	14608.83 ± 1143.92	687.53 ± 181.94	44.98 ± 44.98	363.15 ± 97.50
	NS	**NS**	**NS**	**NS**	**NS**	**NS**

Table 6 : Plasma total protein (g/dl), plasma albumin (g/dl), plasma globulin (g/dl), AST (IU/L), ALT (IU/L), plasma creatinine (mg/dl), ALP (IU/L)

Group	Total protein	Albumin	Globulin	AST	ALT	Creatinine	ALP
T_1	4.59 ± 0.36	2.35 ± 0.30	2.23 ± 0.22	215.13^{a} ± 9.39	22.27^{ab} ± 0.93	0.68^{ab} ± 0.08	361.27^{a} ± 7.84
T_2	4.12 ± 0.22	2.31 ± 0.19	1.85 ± 0.24	347.98^{b} ± 37.27	35.95^{c} ± 2.88	0.98^{c} ± 0.07	504.48^{b} ± 63.84
T_3	4.45 ± 0.39	2.45 ± 0.12	2.00 ± 0.29	198.05^{a} ± 15.03	19.69^{ab} ± 1.46	0.65^{ab} ± 0.04	324.85^{a} ± 8.25
T_4	4.63 ± 0.47	2.67 ± 0.32	1.97 ± 0.24	194.27^{a} ± 9.59	18.88^{a} ± 1.07	0.58^{a}± 0.03	317.00^{a} ± 12.25
T_5	4.44 ± 0.26	2.48 ± 0.23	1.96 ± 0.12	224.40^{a} ± 8.25	24.49^{b} ± 1.25	0.85^{bc} ± 0.12	397.28^{a} ± 15.14
T_6	4.30 ± 0.40	2.45 ± 0.20	1.85 ± 0.26	219.10^{a} ± 6.41	23.50^{ab} ± 1.63	0.70^{ab} ± 0.09	387.70^{a} ± 34.99
	NS	**NS**	**NS**	**CD for treatment =51.45 (significant at 1% and 5%level)**	**CD for treatment = 4.81 (significant at 1% and 5% level)**	**CD for treatment = 0.23 (significant at 5% level)**	**CD for treatment = 89.85 (significant at 5% level)**

IDENTIFICATION OF POLYMORPHISM IN KAPPA CASEIN GENE AND THEIR ASSOCIATION WITH DIFFERENT MILK COMPOSITION TRAITS

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ABSTRACT

Jersey, Red Sindhi and crossbred cattle were genotyped for Kappa casein locus through RFLP from blood samples and using PAGE in milk samples. Results from blood and milk gave identical results. The different genotypes were associated to milk composition traits. Among the genetic groups Jersey cattle was superior in fat per cent, protein per cent, casein per cent and total solids. B allele of Kappa casein was superior in terms of all the milk composition traits. Variation in the Kappa casein locus and results of association were indicative of potential scope for improvement in milk composition traits through marker selection.

Key words: RFLP, Kappa casein, polymorphism, genotype, milk composition

INTRODUCTION

Biochemical polymorphism of milk proteins was used as markers to identify superior animals through their association with better performance with respect to economic traits. Casein is the major constituent of protein in bovine milk and is present as ά, β and k-Casein (k-CN), each known to occur in the form of two or more variants (AA, AB, BB). These variations could be identified only after the animal was in lactation and therefore, selection was delayed until first calving. However, it is possible to identity variation in the genes responsible for the particular change in milk protein using DNA isolated from blood immediately after birth. The present study was undertaken to identify the variation in the k-Casein gene through PCR - RFLP. The different genotypes were associated with milk composition traits of Jersey, Red Sindhi and Jersey crossbred animals.

MATERIALS AND METHODS

Genomic DNA was isolated from the blood of 95 Red Sindhi, 50 Jersey and 60 Jersey crossbred cows as per the method of Montgomery and Sise (1990) with slight modifications.. The blood samples were collected from (i) District Livestock Farm, Hosur, (ii) Nucleus Jersey and Stud Farm, Udhagamandalam, (iii) Exotic Cattle Breeding Farm, Eachenkottai, and (iv) Livestock Research Station, Kattupakkam. The yield and purity of the samples were estimated through spectrophotometer. Samples having an OD ratio between 1.7 and 2.0 were used for the study.

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PCR was used to amplify a 872 bp region between exon IV and intron V of the bovine k-CN gene. Primers described by Pinder *et al.* (1991) were used for the amplification of k-CN gene. The sequences of forward and reverse primers are given below. PCR reactions were carried out in 100 µl as per the method described by Chung *et al.* (1995).

Forward primer-----
5'-GTGCTGAGTAGGTATCCTAG-3'

Reverse primer-----
5'-GTAGAGTGCAACAACACTGG-3'

The amplified DNA was digested using 20 units of *Pst I* at 37°C for 4 hours. Digested PCR products were run on 1.4 % agarose gel and stained with ethidium bromide to identify variations in the fragments produced. The alleles of k-CN gene were identified based on the number and size of fragments produced in comparison with DNA ladder.

k-CN variants in milk from the Red Sindhi, Jersey and Jersey crossbred cows were also typed by using 8 % PAGE. The fat per cent, protein per cent and casein per cent in milk were estimated using standard protocols (IS-1479 Part II, 1961)

RESULTS AND DISCUSSION

The results of genotyping of k-Cn proteins in milk and DNA are presented in Tables 1 and 2 respectively. The gene and genotypic frequencies of the k-Cn variants were the same for milk and blood in Red Sindhi and Crossbred Jersey. The breed-wise and variant-wise performance with respect to different milk composition traits are as follows: The gene frequency of k-Cn variants found in the milk of Red Sindhi cattle in the present study concur with the values of 0.74 and 0.26 found for A and B alleles respectively by Aschaffenburg *et al.* (1968) in the same breed.

Milk fat

Jersey had significantly (P<0.01) higher fat percentage pooled over variants followed by Jersey crossbred and Red Sindhi animals. Similar effect was noticed with respect to different variants (Table 3). This is in agreement with findings of McLean *et al*. (1984) who had stated that fat concentrations were significantly higher in Jersey milk when compared to others. The BB genotype had significantly (P<0.01) higher fat percentage as seen in Table 3, followed by AB and AA genotypes in all the breeds under study , as observed by several authors in different breeds. Ng-Kwai-Hang *et al*. (1986) recorded higher fat content in k-casein BB than in AA genotypes. Similar observations were made in earlier studies (Lin *et al.,* 1986; Horne *et al.,* 1997; Winkelman and Wickham, 1997;).

Milk protein

Jersey had significantly (P<0.01) higher protein percentage followed by Jersey crossbred and Red Sindhi animals. (Table 4). The results are in agreement with McLean *et al*. (1984) who stated that the crude total protein concentrations in Jersey was 31.6 grams per litre compared to other breeds under study.

The BB and AB genotypes had significantly (P<0.01) highest protein percentage followed by AA milk. Several authors have noticed similar results.

Winkelman and Wickham (1997) found that k-Cn B- allele was associated with a significantly higher protein production, with BB animals producing 3.5 percentage more protein than AA animals.

Similar trend was noticed by Kroeker *et al.*(1985) and Ng-Kwai-Hang *et al*. (1987) who had consistently shown that the B variant of k-casein was associated with high total casein and protein. Bovenhuis *et al.* (1992) had observed similar significant effect on protein content and reported that the k-casein BB cows produced milk with a 0.08 percentage higher protein content than that of the AA cows.

Milk casein

Jersey had significantly (P<0.01) higher casein percentage (Table 5) followed by Jersey crossbred and Red Sindhi animals as observed by McLean*, et al*. (1984). The BB and AB genotypes had significantly (P<0.01) higher casein percentage than AA genotypes in different breeds taken for this study. The values observed in this study are in concordance with the values obtained by Schaar (1984), Kroeker *et al*. (1985), Ng-Kwai-Hang *et al*. (1986) and Ng-Kwai-Hang *et al*. (1987) in different breeds of cattle.

Milk total solids

Results shown in Table6 revealed significantly (P<0.01) higher values in Jersey followed by Jersey crossbred and Red Sindhi animals. Similar effect was seen with respect to K-Cn variants. McLean *et al*. (1984) had reported a similar observation that the concentrations of total solids were significantly higher in Jersey milk than in Friesian milk. The BB and AB genotypes had significantly (P<0.01) highest total solids percentage followed by AA milk in different breeds taken for this study.

CONCLUSION

Jersey animals were superior in terms of all the milk composition traits. The BB and AB variants for k-casein was found to be superior in terms of fat, protein, casein , and total solids percentage. Further association studies with larger sample size could be helpful in ascertaining the possibility of selection using these genotypic variants.

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Table 1. Genotype and gene frequencies of k-CN variants in milk of different breeds of cattle

BREED	k-Cn GENOTYPE FREQUENCIES			k – Cn GENE FREQUENCIES	
	AA	AB	BB	A	B
RED SINDHI	0.59 (56)	0.32 (30)	0.09 (9)	0.75	0.25
JERSEY CROSSBRED	0.48 (20)	0.34 (29)	0.18 (11)	0.58	0.42
JERSEY	-----	0.47 (9)	0.52 (10)	0.23	0.76

Numbers in the parentheses denote no. of animals

Table 2. Genotype and gene frequencies of k-CN variants in DNA of different breeds of cattle

BREED	k-Cn GENOTYPE FREQUENCIES			k – Cn GENE FREQUENCIES	
	AA	AB	BB	A	B
RED SINDHI	0.59 (56)	0.32 (30)	0.09 (9)	0.75	0.25
JERSEY CROSSBRED	0.48 (20)	0.34 (29)	0.18 (11)	0.58	0.42
JERSEY	0.16 (8)	0.36 (18)	0.48 (24)	0.34	0.66

Numbers in the parentheses denote no. of animals

Table 3. Least-squares means for fat percentage in different breeds and k-Cn variants

VARIANTS	BREED			
	JERSEY CROSSBRED	RED SINDHI	JERSEY	MEAN OF k-Cn VARIANTS
AA	4.53±0.15 (20)	3.81±0.01 (56)	-----	4.17 ± 0.08^{x} (76)
AB	4.95±0.11 (29)	3.91±0.05 (30)	5.25±0.28 (9)	4.70 ± 0.15^{y} (68)
BB	5.39±0.17 (11)	3.92±0.05 (9)	6.03±0.42 (10)	5.11 ± 0.22^{y} (30)
MEAN OF BREEDS	4.96 ± 0.15^{x} (60)	3.88 ± 0.04^{x} (95)	5.64 ± 0.35^{y} (19)	4.72±0.16 (174)

Numbers in the parentheses denote no. of animals. Means bearing different superscript differ significantly ($P<0.01$)

Table 4. Least-squares means for protein percentage in different breeds and k-Cn variants

VARIANTS	BREED			
	JERSEY CROSSBRED	RED SINDHI	JERSEY	MEAN OF k-Cn VARIANTS
AA	3.45±0.03 (20)	2.99±0.05 (56)	-----	3.22±0.05^{x} (76)
AB	3.56±0.03 (29)	3.04±0.02 (30)	3.86±0.08 (9)	3.48±0.04^{y} (68)
BB	3.64±0.18 (11)	3.27±0.01 (9)	4.01±0.09 (10)	3.64±0.04^{y} (30)
MEAN OF BREEDS	3.55±0.03^{x} (60)	3.10±0.03^{x} (95)	3.93±0.08^{y} (19)	3.48±0.06 (174)

Numbers in the parentheses denote no. of animals

Means bearing different superscript differ significantly (P<0.01)

Table 5. Least-squares means for casein percentage in different breeds and k-Cn variants

VARIANTS	BREED			
	JERSEY CROSSBRED	RED SINDHI	JERSEY	MEAN OF k-Cn VARIANTS
AA	2.50±0.03 (20)	2.38±0.02 (56)	-----	2.44±0.02^{x} (76)
AB	2.69±0.05 (29)	2.44±0.02 (30)	3.08±0.06 (9)	2.73±0.05^{y} (68)
BB	2.74±0.04 (11)	2.66±0.01 (9)	3.29±0.09 (10)	2.90±0.05^{y} (30)
MEAN OF BREEDS	2.64±0.04^{x} (60)	2.49±0.02^{x} (95)	3.19±0.08^{y} (19)	2.72±0.04 (174)

Numbers in the parentheses denote no. of animals

Means bearing different superscript differ significantly (P<0.01)

Table 6. Least-squares means for total solids percentage in different breeds and k-Cn variants

VARIANTS	BREED			
	JERSEY CROSSBRED	**RED SINDHI**	**JERSEY**	**MEAN OF k-Cn VARIANTS**
AA	12.70±0.10 (20)	12.46±0.02 (56)	-----	12.58±0.07^{x} (76)
AB	13.03±0.05 (29)	12.79±0.12 (30)	13.92±0.28 (9)	13.25±0.15^{y} (68)
BB	13.35±0.16 (11)	13.09±0.09 (9)	14.07±0.09 (10)	13.50±0.12^{y} (30)
MEAN OF BREEDS	13.03±0.10^{x} (60)	12.78±0.11^{x} (95)	14.01±0.28^{y} (19)	13.18±0.11 (174)

Numbers in the parentheses denote no. of animals

Means bearing different superscript differ significantly (P<0.01)

Short Communications

EFFECT OF HYGIENIC PRACTICES ON FISH LANDING PRICE OF COMMERCIALY IMPORTANT MARINE FISHES OF THOOTHUKUDI DISTRICT, TAMILNADU

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Post harvest handling of catch is the most important step in the production of a high quality finished product (Devadasan, 2004). To achieve safe fish, the primary fish handlers and fish retailers must be educated on good hygiene and sanitation practices. Most of them are unaware that they are potential carriers of pathogenic microorganisms, and that poor personal hygiene makes the fish unsafe for consumption (Rao*et al*., 2005). Several studies indicate that better knowledge leads to better adoption of hygienic practices (Sanoria and Sharma, 1983; Pathak and Sasmal, 1992).Hygienic measures involve not only the activities that deal with handling operations from onboard fishing vessels to fish landing centres but also focus on infrastructure facilities at fish landing centre. The adoption of the same will be further extended to the subsequent channels of marketing intermediaries until it reaches the consumer. Hence, dissemination of proper technical information onhygienic fish handling and maintenance practices for fishermen and marketing intermediaries would be essentially important. In this line, the study aims at presenting a comparative economic assessment of marine fishing practices between two fishing villages with special reference to adoption of hygienic practices.

The specific objectives of the study are:

- To assess the extent of adoption of hygienic practices by fishermen and the availability of infrastructure facilities in fish landing centres
- To estimate and compare the fish landing price of some of the commercially important fishes landed and economics of marine capture fisheries

Thoothukudi with a coastal length of 163.5 km is a maritime district of Southern Tamil Nadu covering 24 fishing villages. Two fishing villages namely Therespuram and Kombuthurai were exclusively selected for the study based on the type of the fishing craft operated (motorized crafts with OBM),

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adoption of fishing practices (Gill netting and line fishing), adoption of hygienic practices from onboard to fish landing centre and availability of infrastructure facilities at the landing centres.In the proposed study, the total sample size was assigned as 100 for fishermen and 100 for buyers. From each of the fish landing centres, 50 fishermen and 50 buyers were randomly selected thus constituting a total of 200 respondents as sample size. The primary data was collected with a structured survey schedule based on the objectives of the study.

The extent of adoption of hygienic practices by fishermen at on board and on beach was measured on a three-point scale rating viz., adopted, partially adopted and not adopted, with the scoring pattern of 3,2 and 1, respectively. By simple observation method, the availability of infrastructure facilities was recorded. The economics of marine capture fisheries of the selected fishing villages was estimated using different costing techniques and price variation of selected commercially important fishes like Emperors, barracudas, groupers, trevallies and seer fisheswhich were commonly landed in both fish landing centres was also documented.

Garrett ranking technique was employed to rank the order of preference of infrastructure facilities by fishermenand deciding attributes in choosing the fish landing centres by the buyers. The order of merit given by the respondents was transmitted into scores. For converting the scores assigned by the respondents towards the particular factor, percent position for each rank was worked out using the following formula.

$$\text{Percent Position} = \frac{100\,(Rij - 0.05)}{Nj}$$

where,

Rij = Rank given for the i^{th} factor by j^{th} individual

Nj = Number of factors ranked by j^{th}individual

Fishing harbours plays an important role in determining the quality of sea food produced as it is the main area where fish is handled after their landing at shore (Thomas et al., 2015). The extent of adoption of hygienic practices at on board and on beach at Therespuram and Kombuthurai fish landing centres are analysed and presented (Table 1). A total of eight hygienic practices were measured and the table revealed that the overall adoption index of hygienic practices was 2.46±0.56 with Coefficient of variation of 26.22%. Hygienic practices like use of ice for preserving fish (3.00±0), cleaning of fish hold and insulated ice box (2.85±0.36) and use of clean seawater for washing and cleaning (2.48±0.83) were adopted on board by majority of the respondents, whereas the lower adoption was observed towards use of hygienic materials for fish handling like gloves (1.95±0.98). With respect to the adoption of hygienic practices at fish landing centre, the highest score was observed for cleaning of fishing vessels and nets periodically (3.00±0). Balasubramanium et al. (2009) reported thatlow level of scores was obtained for use of adequate clean water for washing fish (39.49%) and prompt disposal of waste (40%) in fish landing centres of

Vishakapattinam. At Therespuram, use of hygienic materials like gloves, gumboots, apron, mouth guard and head gear at fish landing was adopted at lower rate (1.00±0) as same as on board. Hence, training and awareness programmes on adoption of hygienic practices have to be provided to the fishermen for gaining better knowledge for producing quality fishes. The results revealed that Kombuthurai fishermen were found to adopt better hygienic practices at on board and on beach (2.82±0.13) when compared to Therespuram fishermen (2.1±0.3).

Besides the hygienic practices, unhygienic practices like spitting, urination, walking with slippers / barefoot, throwing liquor bottles and plastic wastes at fish landing centres were also documented(Table 2). The table clearly revealed that all the fishermen (100%) were walking either with slippers or bare foot at fish landing centres. Urination and the prevalence of liquor bottles (100%) were not noticed in Kombuthurai fish landing centre. Spitting (62%) and plastic wastes (50%) were observed on higher side in Therespuram fish landing centre.

The extent of availability of infrastructure facilities of the study area is given in Table 3. It was found that auction hall, street lights, notice boards, and refreshment shops were present in both fish landing centres. The infrastructure facilities like first aid box, drainage system, drinking water and waste disposal facility were noticed only in Kombuthurai fish landing centre. Infrastructures like cold storage, ice plant, drying yard, rest room, vehicle parking shed, marine engine repair and service shop and net mending/repair hall were not observed in the selected fish landing centres. Das et al (2013) reported that improved fishing techniques and infrastructure resulted in increased fish catch, better marketing, processing and curing facilities. Hygienically dried and well packaged dried fish are sold at attractive prices in the supermarkets in India as well as in gulf countries fetching a good income for the fisher folk of Visakhapatnam.

The cost and returns part of the two fishing villages clearly indicated that the total investment was higher for Kombuthurai fishermen (Rs. 96.10 lakhs) with a mean of Rs. 1.92 lakhs per fisherman (Table 4). Similarly the profit on investment (Rs. 85.48 lakhs) was also on a higher side with a mean value of Rs. 1.71 lakhs. The mean total investment and profit on investment in marine capture fisheries for Therespuram fishermen depicted a value of Rs. 1.59 lakhs and Rs. 1.25 lakhs, respectively. The Benefit Cost Ratio (BCR) indicated that the return on investment was higher for Kombuthurai fishermen (1.89) when compared to Therespuram fishermen (1.79).

The price behaviour for selected marine fishes was represented in Fig 1. The annual mean fish landing price under different grades for the selected fishes was collected and tabulated. For Emperors, Groupers and Barracudas the size varied with< 0.5 kg, 0.5 -1.5 kg, 1.6 – 3 kg and 3.1-5 kg. Additionally, 5.1-10 kg sized fish category was assigned for Trevallies and for Seer fishes it was up to 20 kg. The results showed that annual mean selling price was higher at Kombuthurai fish landing

centre when compared to Therespuram fish landing centre for 1.6 – 3.0 kg (Rs. 282.50 and 225.83) and 3.1-5 kg (Rs.338.33 and 275.00) sized Emperors fish, respectively. Similar situation was observed for 3.1–5.0 kg sized Groupers (Rs. 318.33 and 260.83). In Kombuthurai, 1.6-3.0 kg sized Groupers were landed only during January to March of every year.For Barracudas,it was Rs. 301.67at Therespuram fish landing centre and Rs. 380.83at Kombuthurai fish landing centre for 3.1-5.0 kg sized fishes. While the highest price was observed as Rs. 260.83 for 3.1 -5.0 kg and Rs. 213.33 for 5.1 – 10 kg for Trevallies at Kombudurai, it was Rs. 215.83 and Rs. 191.67 at Therespuram, respectively. It has been noticed that as size increases, the selling price of fish tends to decrease. The price range was observed very higher for Kombuthurai seer fishes. The size grading ranged between < 0.5 kg and 20 kg. Comparatively, Kombuthurai fishermen realised a higher annual mean selling price in selling 15.1-20 kg sized seer fishes (Rs. 1150.00) than Therespuram fishermen (Rs. 916.67). There was no evidence in harvesting seer fishes up to 3 kg sized fishes in both centres. European commission (2011) in a report on adding value to local fishery and aquaculture products stated that the product differentiation had triggered an increase in themarket value of line caught bass, doubling its sale price and repositioning it as a "high end" product. Thisimprovement in the sale price enabled fishermen not only to recover revenue levels equivalent to the pre-crisissituation, but also to improve their margin by approximately 20%.Singh et al. (2012) reported that adoption of hygienic fish handling practices leads to increase in the market values of fishes among the various stakeholders and consumers.Hence, it is clearly evidenced from the study that Kombuthurai fishermen going for Hook and line fishing areharvesting only large sized fishes which ensures the sustainable management of fishery resources and high landing price due to size specificity and quality of fishes.

The factors as perceived by the buyers in choosing the fish landing centre was given in Table 5.The order of merit varied with fish landing centres. Among the eleven reasons, only eight reasons were ranked by the buyers of Therespuram landing centre. Low price (mean score 77.92) was the major determinant followed by accessibility (71.10), high landings (69.44), regular supply (66.82) etc. Buyers of Kombuthurai fish landing centre ranked five determinants which decide their purchasing centre. All the buyers felt that good quality fishes (77.80) due to cold chain maintenance (74.64) were observed to be the primary reason for opting Kombuthurai fish landing centre. Third and fourth place was occupied by size specificity and cleanliness, respectively.

It is concluded that the availability of infrastructure facilities and extent of adoption of hygienic practices was predominant in Kombuthurai fish landing centre ultimately resulting in higher price realisation and profit margin when compared to Therespuram fish landing centre. There finds a gap in disseminating technical information on adoption of hygienic practices to Therespuram fishermen stating the economic loss in different stages of post harvesting and health hazards of human consumption when not practiced. Hence, it is recommended that awareness

on Community based management system and hygienic living standards could be done through trainings, awareness programmes, short term courses and audio-visualsto the fishermen and buyers so as to ensure food quality and safety.

General cleanliness in Fish landing centres could be addressed jointly through local district administration and Fisheries department. Necessary infrastructures in Fish landing centres could be established. Practice of Co-Management in Kombuthurai fishing village could be replicated for sustainability in other fishing villages also.

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Table 1. Adoption of hygienic practices by fishermen

S.No	Hygienic practices	Therespuram *(n=50)*	Kombuthurai *(n=50)*	Overall *(n=100)*
Onboard				
1	Use of clean seawater for washing and cleaning	1.96±0.92	3.00±0	2.48±0.83
		1-3	3	1-3
		46.94	0	33.47
2	Use of ice for preserving fish	3.00±0	3.00±0	3.00±0
		3	3	3
		0	0	0
3	Cleaning of fish hold	2.80±0.40	2.90±0.30	2.85±0.36
		2-3	2-3	2-3
		14.29	10.34	12.63
4	Cleaning of insulated ice box	2.70±0.46	3.00±0	2.85±0.36
		2-3	3	2-3
		17.04	0	12.63
5	Use of hygienic materials for fish handling like gloves	1±0	2.90±0.30	1.95±0.98
		1	2-3	1-3
		0	10.34	50.26
On beach				
1	Use of clean water for cleaning fish	1.30±0.61	3.00±0	2.15±0.96
		1-3	3	1-3
		46.92	0	44.65
2	Use of hygienic materials like gloves, gumboots, apron, mouth guard and head gear	1±0	1.78±0.42	1.39±0.49
		1	1-2	1-2
		0	23.60	35.25
3	Cleaning of fishing vessels and nets periodically	3.00±0	3.00±0	3.00±0
		3	3	3
		0	0	0
	Overall	2.1±0.3	2.82±0.13	2.46±0.56
		--	--	--
		15.65	5.54	26.22

(The values in first, second and third rows indicate mean and S.D, range and C.V, respectively)

Table 2. Adoption of unhygienic practices at fish landing centre

S. No.	Unhygienic practices	Therespuram*(n=50)*		Kombuthurai*(n=50)*	
		Practiced	Not Practiced	Practiced	Not Practiced
1	Spitting	62	38	12	88
2	Urination	20	80	--	100
3	Use of slippers / bare foot	100	--	100	--
4	Throwing liquor bottles	10	90	--	100
5	Throwing plastic wastes	50	50	12	88

(The figures indicate percentage)

Table 3. Availability of infrastructure facilities in fish landing centres

S.No.	Infrastructural facilities	Availability index	
		Therespuram*(n=50)*	Kombuthurai*(n=50)*
1	Cold storage	--	--
2	Auction hall	+	+
3	Ice plant	--	--
4	Street lights	+	+
5	Drying yard	--	--
6	Approachable road during night	+	-
7	Drainage system	--	+
8	Drinking water	--	+
9	Rest room	--	--
10	First Aid box	--	+
11	Notice boards	+	+
12	Vehicle parking shed	--	--
13	Waste disposal	--	+
14	Engine repair and service shop	--	--
15	Refreshment shop	+	+
16	Net mending/repair hall	--	--

(+ : Available, -- : Not Available)

Table 4. Economics of marine capture fisheries

(Rounded off to the nearest figure) (Rs. In lakhs)

Particulars		TCC	TFC	TVC	TC	TR	NR	BCR
Therespuram	Total	143.62	76.74	2.85	79.59	142.23	62.64	1.79
	Mean	2.87	1.53	0.06	1.59	2.84	1.25	--
Kombuthurai	Total	184.17	91.90	4.20	96.10	181.58	85.48	1.89
	Mean	3.68	1.84	0.08	1.92	3.63	1.71	--

(TCC – Total Capital Cost, TFC – Total Fixed Cost, TVC – Total Variable Cost,
TR – Total Returns, NR – Net Returns)

Table 5. Reasons as attributed by buyers in choosing fish landing centre

S. No	Reasons	Therespuram *(n=50)*			Kombuthurai *(n=50)*		
		Score	Mean score	Order of merit	Score	Mean score	Order of merit
1	Cleanliness of fish landing centre	2993	59.86	VII	3663	73.26	IV
2	Size specificity	3042	60.84	V	3275	65.50	III
3	Accessibility	3555	71.10	II	--	--	--
4	Cold chain maintenance	--	--	--	3732	74.64	II
5	Good quality fishes	--	--	--	3890	77.80	I
6	Low price	3896	77.92	I	--	--	--
7	High landings	3472	69.44	III	--	--	--
8	Value added services	2771	55.42	VII	--	--	--
9	Regular supply	3341	66.82	IV	--	--	--
10	Credit possibility	3005	60.10	VI	--	--	--
11	Marketing practices followed	--	--	--	3413	68.26	V

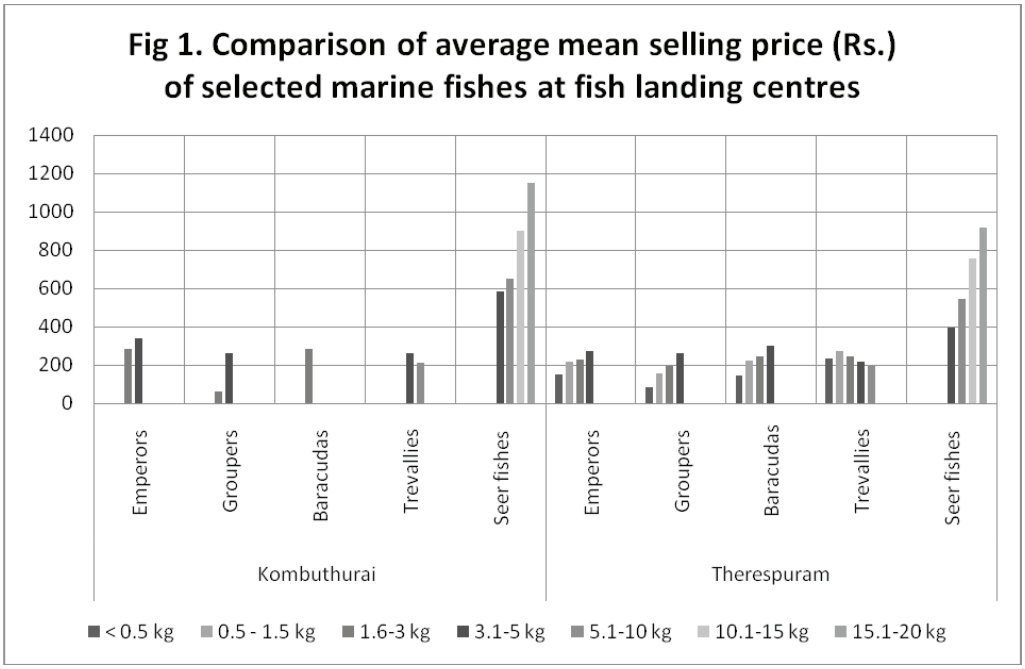
Fig 1. Comparison of average mean selling price (Rs.) of selected marine fishes at fish landing centres
1400
1200
1000
800
600
400
200
0
Emperors
Groupers
Baracudas
Trevallies
Seer fishes
Emperors
Groupers
Baracudas
Trevallies
Seer fishes
Kombuthurai
Therespuram
< 0.5 kg
0.5 - 1.5 kg
1.6-3 kg
3.1-5 kg
5.1-10 kg
10.1-15 kg
15.1-20 kg

PERFORMANCE OF TRADITIONAL MUD CRAB FISHING GEARS USED IN PULICAT LAKE, TAMIL NADU

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Pulicat lake has a rich diversity of nearly 29 species of crabs(Joel *et.al.,* 1986) representing brackishwater, freshwater and terrestrial water. Of these, the mud crab (*Scylla serrata* and *S.tranquebarica*) and the euryhalineportunid(*Portunuspelagicus*) crab were the common species occurring all over the lake and all through the year. Over the last two decades, exploitation of mud crabs from the known natural habitats, particularly from the estuarine areas, has been intensified in many south-east Asian countries, mainly for live export of mud crabs. The mud crabs, *Scylla spp*., represent a valuable component of small-scale coastal fisheries in many countries of tropical and sub-tropical Asia and African coast including India. Mud crab is one of the important Indian estuarine species that played major role not only for domestic consumption but also for export earnings.Different types of gears are being employed for the capture of mud crabs throughout the world. In India, Pulicatlake is an important mud crab fishing ground.Among all the brackishwater bodies in India, Pulicatlakeproduces the largest amount of mud crabs(Sivasubramanianand Angel, 1992). It has been estimated that the potential resources of crabs particularly from the estuaries were recorded as 13,209 tonnes in Indian coastal waters and apparently the southern part of the coasts are potentially richer than the northern part(Rao*et.al.*, 1973).The annual production of crab is estimated to be more than 10,000 tonnes(Zafarand Siddiqui, 2000).Several types of gears (nonselective and selective) are being employed by the fishermen in and around Pulicatlake. However, the detailed studies on the fishing gears and their efficiency to capture the mud crabs have not been reported from this lake. The study was carried out to analyze the mud crab fishery and their catch composition by using four different types of fishing gears inPulicatlakefor the period of one year.

The study was carried out at Pulicatfish landing center (Lat. 9^0 14' N, Long. 78^0 47' E) (Fig.1) for the period of one year (October, 2015 to September, 2016). The Pulicat Lake, located on the southeast coastline of Tamil Nadu is an estuarine lagoon and it is well known for mud crab (*Scyllaserrate* sp.and *Scylla tranquebarica*) and shrimp fisheries(Sanjeeva Raj, 2000).

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Different crab fishing gears and methods practiced by crab fishers in Pulicatlake were studied through field visits and interactions with the fisherfolk. Specifications of each gear were recorded during the survey by measuring different parameters like dimensions, mesh size etc. The mud crab fishing gears usedwere(i)Baited loop line known as '*Surukku'* (Fig.2-a) (ii)Ring crab trap known as '*Kaccha'* (Fig.2-b) (iii) Scoop net known as '*Chikkan*' (Fig.2-c) (iv) Wooden cover crab pot known as '*Vutha*' (Fig.2-d) and (v)Monofilament gill net locally known as '*Settuvalai*' (Fig.2-e). Once in a month four numbers of each gear were operated for 2to 5 hrs.at each fishing ground. All gears were operated during high tide except the scoop net. Among the four crab fishing gears, gill net and scoop net were operated during night hours.These gears were taken out for observation after a soaking period of 2-5 h. Catch rate was expressed as number of crab caught from each gear and the carapace length were measured by using verniercaliber. The catch among the gears and months were analyzed using Two-way ANOVA ($P<0.05$).

The design of traditional fishing gears like ring crab trap, baited loop line, scoop net, monofilament gill net and crab cover pot were operated for mud crab fishery along the Pulicatlake. Thesecan be classified into two categories viz., selective gears and non-selective gears. In the non-selective gears, it was observed all size of mud crabs (early juveniles to adult crabs) were caught, whereas in selective gears particular size group of crabs (>100g) only caught. Some of the gears were used throughout the year and some were operated during a particular season. Among the five types of fishing gear, four were of selective type and one was non-selective (Table 1).

This is the most efficient crab fishing gear operated to target big size of mud crab from the lake. This gear is operated in and around the Pulicat lake during the North-east monsoon (October 2015to January 2016). The gear locally known as 'Surukku', generally circle in shape. The gear is made up of iron frame attached with 3-4 monofilament lines.Synthetic rope of 4mm dia. is attached in upper side of the iron frame and knotted at a single point from the center of the net. A float made up of broken thermocoleis attached to the other end of the rope. The float was attached for the easy identification of the trap.The length of the rope normally depends upon the depth of operation. The diameter of the iron frame is 5-7cm. Catfish is most commonly used as bait. A person can operate more than 20 numbers of net at a time. The crabs come to the gear being attracted by the bait. During hauling the gear after 30 to 45mins, claw of the crabs were trapped in the loop line. Finally the crabs were collected using scoop nets. The distance between setting of each baited loop line was about 5 to 7m to cover a wide area. The gear is operated by two people. While one person is engaged in punting and manoeuvring of the boat, the other person is engaged in laying the loop lines, hauling and collection of crabs. This gear is operated only during day time. The catch rate varies with the season and from place to place depending on the density of crab population. The gear is selective to catch medium and large size crabs only (>100mm CW). Baited long line is

a common gear used in mud crab fishing along the east coast of India and Kerala coast (Mahesh Raj, 1992).

*Krishanthan*et. al. (2015) reported the ring crab traps are used mainly in Kokilai and Legombo lagoons in Sri Lanka.*In Pulicatlake, it* is the most efficient crab fishing gear operated to catch all size of mud crabs. This gear is operated in and around the Pulicatlakethroughout the year. But the intensity of operationdecreases from monsoon towards summer. The gear is very widely used by the fisherfolk of the villageThonirevu, Jamila bath, Naduoormadhakuppam and Annamalacherry. The gear locally known as 'Kaccha', it is generally circular in shape. The entire gear is made up of iron frame (6mm dia.)and the bottom part of the gear is covered by Polyethylene (PE) netting. In centre of the net, a single rope is tied which is used for attaching the bait. Threesynthetic ropes (4mm dia.)were attachedat three points in the iron frame in equal distanceof the net which are knotted at a single point keeping a distance of about 0.5 - 0.75m from thecenterof the net and a single rope (6mm dia.) was attached at this point.The float was attached for the easy identification of the trap.The size of the ring crab trap is 40 – 50cm diameter. The gear is normally operated during day time. The operation of ring crab trap was similar to that of baited loop line. By using this gear fisherman used to catch 2-3 numbers of crabs at a time, which corresponds to a catch of 0.7-1.0kg/ day.

This gear is operated mainly in summer season especially during high salinity and low turbidity condition, when the water depth becomes low. The gear is made up of a conical Polyethylene bag (20mm stretched mesh) which is attached to the circular iron frame (6mm dia.) with short handle. The crab was attracted towards the light, suddenly fisherman scoop out the crab. The diameter of the metal ring is 60cm. The fishers used to target sub-adult and adult crabs with this net. The use of scoop net in mud crab fishery also very common in other Asian countries such as Indonesia(Cholik and Hanafi, 1992), Thailand (Rattanachote and Dangwatanakul, 1992) and Sri Lanka (How-Cheong and Amandakoon, 1992).

This gearis made up of nylon monofilament webbing of 4mm dia. rope and is rigged with both head rope and foot rope. The mesh size varies from 70 – 85mm (stretched). It is about 85-100m long and about 1.5-2m in depth. The gill nets are operated with anchor-sinker at one end of the foot rope and the other end is fastened to a bamboo or casuarina pole. Baits are attached to the foot rope at several points to attract the crabs. Fishermen use cork as floats in the gill net. The numbers of floats used should be depending upon water depth and length of the net in operation. The gill net is mostly operated during night. The crabs are caught by entanglement and are collected by hauling the net in the morning. The gill nets are also operated during day time and entangled crabs are collected by lifting the net at regular intervals. Crabs of all sizes are caught in the gill net. These gill nets are mostly operated in Tamil Nadu region only. In India, Monofilament crab gill nets are used mainly inChilka lake and Killai estuary (Mahesh Raj, 1992)and also used in Kokilai lagoon, Sri Lanka(*Krishanthanet. al., 2015*)

Earlier, this gear was deployed in Pulicatlake for crab fishing, locally called as 'Vutha'. It is a falling gear which is operated by a single fisherman in shallow waters mainly during dry seasons. It is a conical cover pot made of bamboo sticks woven together by coir rope and open at both the ends. Circumference of the upper opening is about 15cm and the diameter of the bottom opening is around 60cm. Height of the gear is generally 60cm and there is gap of 0.5cm between adjacent sticks. During the presence of crab fisherman plunges the gear into the water with broad opening faces downward at the area and caught by hand picking.

The mean annual catch rate of different crab fishing gears operated in Pulicat lake are given in Table 2. The mean catch rate was highest for ring crab trap (45±6.82), followed by baited loop line (16.9±4.47), monofilament gill net (17.0±3.65), and scoop net (8.25±3.04). Mohapatra*et. al.*,(2011) reported the mean catch rate was highest for crab lift net (0.78±0.04), followed by crab pot (0.61±0.06), baited long line (0.53±0.03), monofilament gill net (0.44±0.02), single hook hand line (0.37±0.016), monofilament screen barrier (0.34±0.02), triangular push net (0.29±0.02), split bamboo traps (0.19±0.01) and scoop net (0.10±0.006) inChilka lake.Significant differences could be observed between the catch rates of mud crab belonging to different gears ($P<0.05$) (Table 3).The catch composition of the mud crab was almost same for baited loop line and monofilament gill net. Among the fishing gears, big size crab was caught in loop line, followed by scoop net, ring crab trap and monofilament gill net. The study revealed better catch efficiency of ring crab trap followed by baited loop line; monofilament gill net and scoop net (Table 2). Ring crab trap is used in and around the Pulicatlake whereas loop line is operated only in Pazhaverkadu region during the North-east monsoon. Scoop net, which is used throughout the year to catch mud crabs, is also deployed for collecting crabs which are caught by baited loop line operation.The use of monofilament gill net in which the catch of juvenile crabs predominate need to be regulated, in order to support the conservation of crab fishery resources in Pulicat lake.

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Table1. Design description of the mud crab fishing gears operated in Pulicatlake

S. No	Particulars	Ring crab trap (Kacha)	Baited loop line (Surukku)	Scoop net (Chikkan)	Monofilament crab gill net (Settuvalai)	Wooden cover crab pot (Vutha)
1	Webbing material	Polyethylene Mesh size – 40 mm Twine diameter – 1.5 mm dia.	Nylon (Monofilament) Twine diameter – 0.75 mm dia.	Nylon (Multifilament) Mesh size – 20 mm Twine diameter – 0.5 mm dia.	Nylon (Monofilament) Mesh size – 85 mm Twine diameter - 0.7 mm dia.	Bamboo stick Stick thickness – 2 cm
2	Frame	Mild steel (50 cm dia.) Thickness- 6 mm dia.	Mild steel (7 cm dia.) Thickness – 8 mm dia.	Mild steel (60 cm dia.) Thickness – 6 mm dia.	Nil	Bamboo stick
3	Horizontal hanging co-efficient	Nil	Nil	Nil	0.68	Nil
4	Vertical hanging co-efficient	Nil	Nil	Nil	0.72	Nil
5	Floats	Thermocole	Thermocole	Nil	Cork	Nil
6	Sinkers	Nil	Nil	Nil	5 g lead	Nil
7	Hauling rope	Polypropylene – 4 mm dia.	Polypropylene – 4 mm dia.	Nil	Polypropylene	Nil
8	Marker float	Thermocole	Thermocole	Nil	Thermocole	Nil

Table 2. Month wise catch details of mud crab caught in the different gears (N.h^{-1} ± SE)

S. No.	Month	Ring crab trap			Loop line			Scoop net			Monofilament gill net		
		Numbers caught	Carapace length range (cm)	Percentage contribution (%)	Numbers caught	Carapace length range (cm)	Percentage contribution (%)	Numbers caught	Carapace length range (cm)	Percentage contribution (%)	Numbers caught	Carapace length range	Percentage contribution (%)
1	October	50± 4.16	4.5-19.0	47	22± 2.16	12.5-19.7	20	13± 1.82	7.5-17.4	12	23± 1.82	3.2-15.5	21
2	November	55± 1.71	5.2-18.7	49	24± 1.15	13.2-19.4	21	14± 2.45	6.5-15.4	12	20± 2.94	3.9-14.7	18
3	December	53± 4.42	5.0-19.5	49	22± 2.16	13.9-18.6	21	11± 1.18	6.9-18.8	10	21± 3.55	4.1-13.6	20
4	January	54± 3.91	4.7-17.8	52	20± 2.94	14.5-18.0	20	10± 2.44	7.9-17.9	10	18± 3.16	3.7-13.7	18
5	February	50± 5.35	4.4-17.4	53	18± 2.44	13.4-18.5	19	7± 1.15	6.5-14.6	8	19± 3.74	4.7-14.4	20
6	March	45± 3.16	4.0-16.9	51	20± 2.94	13.9-17.8	23	5± 1.71	7.8-15.9	6	18± 1.15	4.5-14.2	20
7	April	42± 2.94	4.3-18.3	52	15± 3.91	12.8-17.6	18	7±1.0	8.8-16.7	9	17± 3.91	3.3-15.7	21
8	May	37± 2.94	4.9-16.5	50	13± 2.16	12.2-17.9	18	7± 1.82	7.3-17.3	9	17± 4.24	4.1-15.3	23
9	June	39± 3.16	5.0-17.2	55	14± 3.36	12.9-16.8	20	6± 1.15	6.3-15.2	7	12± 2.45	4.3-16.4	17
10	July	36± 3.55	4.8-17.4	58	11± 1.82	13.0-17.6	18	5± 1.32	7.1-16.5	8	10± 2.94	3.8-12.6	16
11	August	40± 3.16	4.0-16.3	56	13± 2.45	13.2-18.3	18	6±1.15	6.6-15.7	9	12± 4.03	3.9-13.6	17
12	September	43± 4.69	4.6-18.5	52	17± 3.59	12.7-17.7	20	8±1.0	5.5-17.1	10	15± 2.16	4.0-14.4	18
	Total	544± 6.82			209± 4.47			99± 3.04			202± 3.65		
	Mean catch rate	45 ± 6.82	Na1n		16.9± 4.47			8.25± 3.04			17.0± 3.65		

Table.3. ANOVA for theimpact of designs and seasons in the catch rate of mud crab

Source	df	Sum of square	Mean square	Sig.
Season	11	370.5	33.68	<0.05
Design	3	9374.5	3124.83	<0.05

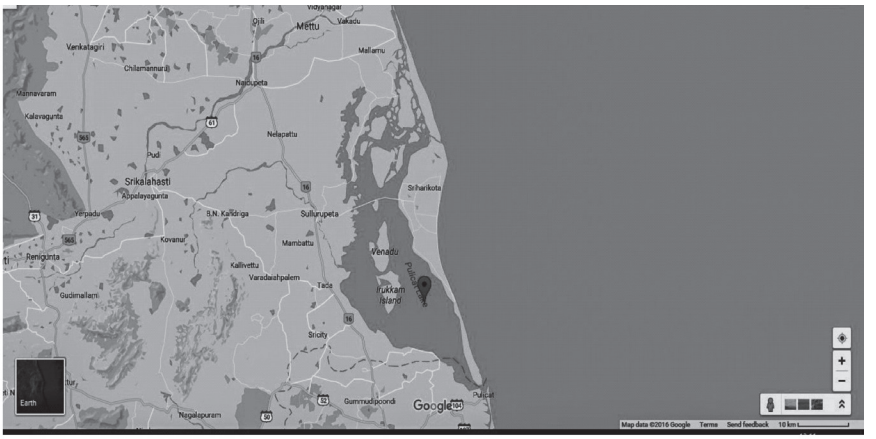

Fig.1. Description of the study area

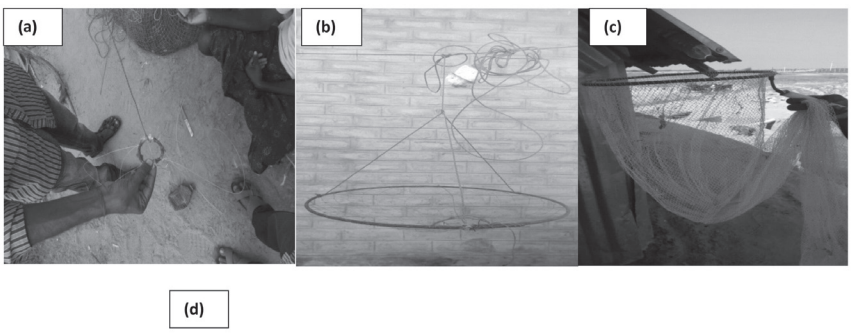

Fig.2. a-e–(a) A baited loop line; (b) A ring crab trap; (c) A scoop net; (d) A Wooden cover crab pot; e) A monofilament gill net

THE USEFULNESS OF ROSE BENGAL TEST AND MILK RING TEST IN THE DIAGNOSIS OF BOVINE BRUCELLOSIS

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Brucellosis is one of the highly contagious, abortifacient reproductive diseases of dairy animals with worldwide distribution and the disease is commonly present among the bovine population of India (Patel *et al.,* 2014). In India, brucellosis was first recognized in 1942 and is now endemic throughout the country. *Brucella abortus* is the etiological agent for bovine brucellosis which causes abortion, infertility, retention of placenta, stillbirth and calf loss in animals, with huge economic losses to dairy farmers (Radostits *et al.,* 2010).

Brucellosis is the second most important zoonotic disease commonly encountered throughout world (FAO, 2005). In India, more than three fourth of the general population residing in rural setup are in close contact bovines which increase the risk of getting this zoonotic pathogen. In order to prevent this transmission, various intervention strategies were developed to detect the pathogen and to develop control strategies. In this view, various diagnostic test *viz.,* Rose Bengal Plate Agglutination Test (RBT), Standard Tube Agglutination Test (STAT), Enzyme Linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT), Fluorescent Polarization Assay (FPA), Milk Ring Test (MRT) and milk ELISA have been utilised to ascertain the true status of bovine brucellosis under field conditions (Bronner *et al.,* 2014). Based on the above facts, the present study was focused to screen the lactating animals against brucellosis by using screening tests *viz.,* RBT and MRT.

The present study was conducted in certain districts of Tamil Nadu, *viz.,* Erode, Salem, Kancheepuram, Tiruvallur, Tiruvannamalai, Viluppuram, Thiruvarur, Pudukkottai, Virudhunagar and Tirunelveli to prove the utility of MRT and RBT as a screening test for bovine brucellosis (Fig 1).

Sexually matured cattle comprising of lactating animals calved once or more than once were selected randomly from the study area. Blood samples (3 ml) were collected from 483 cattle by jugular vein puncture in sterile test tubes (5 ml) and they were allowed to clot and then centrifuged at 2000 rpm for 15 minutes. Serum was separated and stored at - 20°C until further

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use. Milk samples were collected from 483 cattle (excluding dry cows, mastitis milk animals and colostrum milk cows). The udder was thoroughly washed and cleaned with potassium permanganate solution (1:1000) and dried using sterile gauze. Teat openings were disinfected with 70 per cent ethyl alcohol. After discarding the first few drops of milk, approximately 10 ml of milk from each quarter was collected in two sets of sterile screw capped plastic vials (50 ml) and transported on ice to the laboratory.

Rose Bengal test and Milk Ring test antigen were obtained from Institute of Veterinary Preventive Medicine, Ranipet, Vellore. The antigen was stored at 4°C until use. The RBT and MRT were performed as per Alton *et al.,* 1988.

The statistical analysis with various risk factors and prevalence rate were assessed statistically as per the procedure of Snedecor and Cochran, 1994.

In the present study, the prevalence of bovine brucellosis was found to be 3.10 per cent (15/483) by RBT and 4.35 per cent (21/483) by MRT (Table 1). Among these, 11 samples showed positivity by both RBT and MRT. RBT results of our study are in agreement with Nizeyimana *et al.,* 2013 who have documented 4.7 per cent sensitivity of RBT in bovines. Other workers have found higher positivity by RBT such as, 7.74 per cent (Reddy *et al.,* 2014), 9.8 per cent (Ganesan, 2013) and 26 per cent (Akhtar *et al.,* 2010). The variation in the positive percentage by RBT by different workers could be due to false positive and negative reactions, variations in sample, interpretation variation by individuals, pH influence on RBT antigen, demography and different infective stages of animals.

Prevalence of *Brucella* infection in milk samples by MRT was 4.35 per cent (Table 1). These findings varied with the studies of Mahato *et al.,* 2004 (35.82 %) and Junaidu *et al.,* 2011 (25.25 %) who found higher prevalence than the present study. The difference in prevalence between our study and the previous studies may be partly explained by the methodology used in the study protocol and false positive reactions with abnormal milk (mastitis and colostrum), individual variation during interpretation and collection at end stage of lactation or hormonal disorders (Bercovinch and Moerman, 1979).

A total of 11 samples were positive by both RBT and MRT with 2.27 per cent positivity for bovine brucellosis. These results were significantly contradicted with reports of NIVEDI, 2014 which recorded more correlation between RBT and MRT. The variation with the test results might be due to sampling size, lack of specificity, interpretation variation among individuals and variation of antibody level in serum and milk.

Districts wise prevalence of brucellosis by RBT and MRT

In district-wise prevalence of bovine brucellosis, the highest seroprevalence was found in Virudhunagar and Tiruvannamalai followed by other districts (Table 1).

In Tamil Nadu, few studies were conducted to find out the district-wise prevalence of bovine brucellosis. According

to Ganesan, 2013, 7.37 per cent of cattle were detected positive for brucellosis by RBT which includes 9.90 per cent in Erode, 3.47 per cent in Tirunelveli, 9.03 per cent in Tiruvannamalai and Vellore, 6.85 per cent in Salem and Namakkal area.

The results of our study showed a lesser prevalence rate from Erode, Tirunelveli, Tiruvannamalai and Salem district as compared to the study done by Ganesan, 2013. This might be due to implementation of intervention strategies like appropriate diagnosis and treatment, veterinary services and awareness among farmers about the disease with the adaptation of recent control strategies against brucellosis.

In this study, the highest prevalence in milk were encountered in Virudhunagar and Tiruvannamalai (9.76%), followed by other districts (Table 1). In these areas, only limited research work was carried out to assess the prevalence of brucellosis in milk samples. In our study, the prevalence of *Brucella* antibody in serum and milk was almost same in all the selected districts except Tiruvallur.

Prevalence of brucellosis from the study area may be attributed to the clustering of animals, free animal movement, mixed species farming and lack of awareness of farmers against brucellosis screening and prevention.

Age-wise prevalence of *Brucella* infection in serum and milk was assessed. The highest prevalence was noticed in group of animals above 7 years age by RBT – 5.07 % and MRT – 8.12 % followed by other age groups (Table 2). This present study was in agreement with (Silva *et al.,* 2000, Amin *et al.,* 2005 and Islam *et al.,* 2013).

The prevalence of *Brucella* infection in dairy cows was found to be higher as age advances (Aulakh *et al.,* 2008). This is in accordance with the present study. The reason for higher positivity in advanced ages might be due to resistance of young cattle to *Brucella* infection (Aulakh *et al.,* 2008) or post natal immunity derived from dam to calves through colostrum (Silva *et al.,* 2000). This study concludes that the animals might have been exposed to *Brucella* before reaching maturity, but did not seroconvert at the time of testing. Sex hormones and erythritol concentrations are found to be higher with old age and sexually matured animals which facilitate the growth and multiplication of *Brucella* organisms (Radostits *et al.,* 2010).

Jersey crossbred cattle were more susceptible (RBT – 3.54% and MRT – 4.60%) than Holstein Friesian crossbred and non-descript cattle (Table 2). Our results were in agreement with Salman *et al.,* 1984 and Akbarmehr and Ghiyamirad, 2011. The low prevalence in ND breeds might be due to natural resistance pattern adoption in the local environment and innate immunity (Salman *et al.,* 1984). This study concludes that most of the ND breeds were reared in single cow herd whereas, crossbreds were mostly maintained in intensive or semi intensive systems and also crossbreds cattle are found to be highly susceptible to various stress conditions than other breeds.

This study showed bovine brucellosis from the study area was 4.35 per cent by MRT and 3.11 per cent by RBT. Under field

conditions, RBT and MRT can be used as screening test and further confirmation can be done by primary binding assays to rule out the exact prevalence of bovine brucellosis. Presence of *Brucella* antibodies from the study area indicates that the pathogen is circulating among bovine population which warrants the need for developing effective control strategies.

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Table 1:- Prevalence and comparison of various diagnostic tests in milking animals

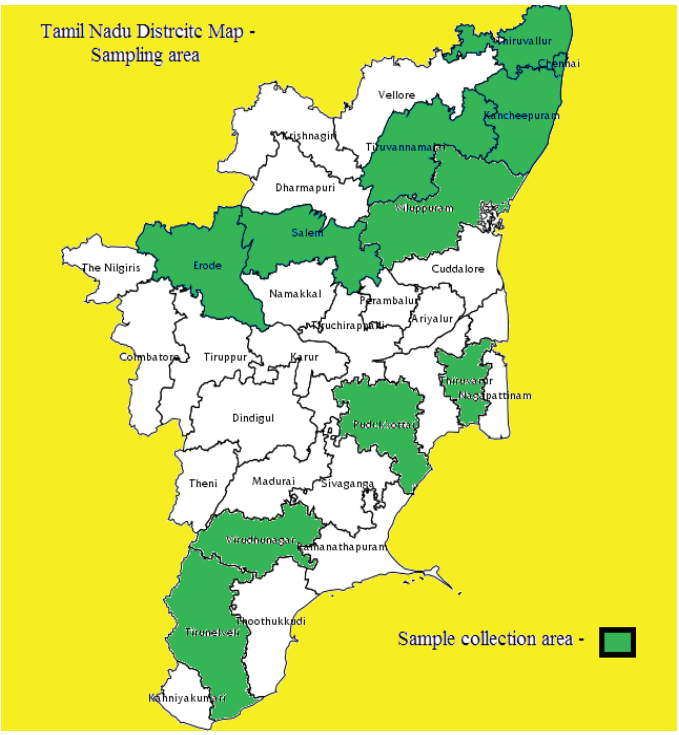
Districts	Samples analysed	Positive samples	
		MRT No (%)	RBT No (%)
Erode	82	3 (3.66%)	2 (2.44%)
Salem	56	2 (3.57%)	2 (3.57%)
Kancheepuram	68	4 (5.88%)	1 (1.47%)
Villupuram	45	2 (4.44%)	2 (4.44%)
Tiruvannamalai	47	3 (6.38%)	3 (6.38%)
Tiruvallur	33	1 (3.03%)	-
Tirunelveli	37	1 (2.70%)	1 (2.70%)
Pudhukkottai	45	2 (4.44 %)	1 (2.22%)
Tiruvarur	39	1 (2.56%)	1 (2.56%)
Virudhunagar	31	2 (6.45%)	2 (6.45%)
	483	**21 (4.34 %)**	**15 (3.10%)**

Table 2:- Age and breed wise prevalence brucellosis in milking animals by RBT and MRT

Risk factors	Age wise prevalence				Breed wise prevalence			
	2 – 4 years	4 – 7 years	>7 years	Total	Jersey cross	Holstein Friesian cross	Non-Descript breed	Total
No. of samples	**120**	**166**	**197**	**483**	**282**	**170**	**31**	**483**
RBT	**2 (1.67%)**	**3 (1.81%)**	**10 (5.07%)**	**15 (3.10%)**	**10 (3.54%)**	**5 (2.94%)**	**0**	**15 (3.10%)**
Chi square	**$(4.01)^{NS}$**				**(10.34)****			
MRT	**2 (1.67%)**	**3 (1.81%)**	**16 (8.12%)**	**21 (4.35%)**	**13 (4.60%)**	**7 (4.11%)**	**1 (3.23%)**	**21 (4.35%)**
Chi square	**$(1.14)^{NS}$**				**$(0.14)^{NS}$**			

(* NS – Non significant, ** - highly significant, P<0.01, *- significant, P<0.05)

Fig. 1 Tamil Nadu – District wise sampling area



HELMINTH PARASITIC SURVEY OF CAPTIVE SPOTTED DEER (*Axis axis*) IN TAMIL NADU

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The Spotted Deer (also known as Chital) is the most common deer species found in Indian forests distributed from the base of Himalayas, practically throughout the Peninsular India and Ceylon. Chital is listed as "Least Concern" by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (2015) because it occurs over a very wide range within which there are many large populations (Duckworth *et al*., 2015). Although, it is still declining in some sites (particularly outside of protected areas), at the species level any such declines are at nowhere near the rate required to qualify for listing even as Near Threatened.

Gastrointestinal parasites are the major health issues in wild and captive cervids mainly kept in relatively small enclosures (Goossens *et al.*, 2005). Although outbreaks of parasitic diseases in Spotted Deer are not so deadly but it is out most important to keep the Spotted Deer free from parasites. Mohan and Coumarane (2007) reported that the occurrence of endoparasite infection in Spotted Deer (*Axis axis*) at Puducherry with eggs of *Trichostrongylus* sp., *Cooperia punctata* and *Capillaria bovis* by direct smear and centrifugal flotation technique. Due to paucity of systematic investigation, information on parasitic infections of wild animals is meager. In addition, some parasites are zoonotic and pose a risk to human health. Hence, the study was carried out in Zoo animals.

During the study period, about 25g of faecal samples (20 pooled samples/ month) of Spotted Deer from Arignar Anna Zoological Park (AAZP) and V.O. Chidambaranar (VOC) Park and Zoo were collected in 10 per cent formalin during rainy, winter and summer seasons and the samples were properly sealed and labeled. The samples were processed by both

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sedimentation and floatation technique for endoparasites examination as per the methods described by Soulsby, 1982. Identification of helminthic eggs was done by observing their characters (Soulsby, 1982; Zajac and Conboy, 2013 and Bowman, 2014). The findings of this study were statistically analyzed using chi-square test.

The pooled samples from AAZP were examined for endoparasites and 21 samples were found positive for helminthic eggs, out of 140 samples.

In AAZP, season-wise helminthic prevalence revealed that the helminthic infection with regard to rainy season was 22.50% (n=40); winter season 16.67% (n=60) and summer season 5.00% (n=40) (Table 1). Strongyle infection was present in all the seasons. The highest rate of Strongyle infection was noticed during rainy season (15.00%) followed by winter season (8.33%) and with least infection found during summer season (5.00%) at AAZP. But, *Strongyloides papillosus* found in its peak during winter season (8.33%) followed by rainy season (2.50%) and no infection was found during summer season. The helminthic prevalence in the mixed infections that composed of Strongyle with *Strongyloides papillosus* were 5.00% during rainy season and no mixed infection was noticed during winter and summer season. Statistical analysis revealed that there was no significant difference in prevalence of helminths noticed between seasons (Table 1 and Figure 1).

Spotted Deer reared at V.O.C. Park and Zoo revealed that the season-wise helminths with regard to rainy season was 11.25% (n=80), winter season was 6.67% (n=60) and summer season was 0.00% (n=40) (Table 1 and Figure 2).

Among the various seasons, a higher Strongyle infection was noticed during rainy season (6.25%) followed by winter season (5.00%) and no helminth was found during summer season. Similarly, the highest infection of *Strongyloides papillosus* was found during rainy season (3.75%) followed by winter season (1.67%) and no infection was found during summer season. Mixed infection (Strongyle and *Strongyloides papillosus*) (1.25%) was only found during rainy season. During this study period, the prevalence of helminths in V.O.C. Park and Zoo in Spotted Deer did not differ significantly between seasons (Table 1 and Figure 2).

In Spotted Deer of Arignar Anna Zoological Park, the most detected helminth was of Strongyle (9.29%) followed by *Strongyloides papillosus* (4.29%) and mixed infection of Strongyle and *Strongyloides papillosus* (1.43%) (Table 2). Similarly, Spotted Deer of V.O.C. Park and Zoo also revealed more prevalence of Strongyle (4.44%) followed by *Strongyloides papillosus* (2.22%) and mixed infection of both Strongyle and *Strongyloides papillosus* (0.56%) (Table 2).

Strongyle was the predominant helminth noticed in both the zoos followed by *Strongyloides* spp. and mixed infection of both. There was a highly significant difference noticed between helminthic species of Spotted Deer of AAZP, Chennai. But helminthic species of Spotted Deer,

V.O.C. Park and Zoo failed to show significant differences among them. The findings are in accordance with Singh *et al*. (2009) who recorded higher incidence of helminthic infection during rainy season in Spotted Deer and the higher rate of incidence due to the availability of lush herbage pasture on account of favourable micro-climate for the survival and propagation of free-living larval stages.

Overall helminthic prevalence in Spotted Deer of AAZP, Chennai was found to be 15.00% whereas in V.O.C. Park and Zoo, it was 7.22% (Figure 3). The statatistical analysis revealed that there was significant variation at 5% level noticed between Spotted Deer of two study regions while comparing the overall helminthic prevalence. Desired anthelmintics coupled with better sanitary measures will be able to reduce the parasitic incidence in captive wild animals.

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TABLE 1 SEASON-WISE PREVALENCE OF HELMINTHS IN SPOTTED DEER

Study area	Arignar Anna Zoological Park			V.O.C. Park and Zoo		
Season	Rainy (n=40)	Winter (n=60)	Summer (n=40)	Rainy (n=80)	Winter (n=60)	Summer (n=40)
Positive Samples	9 (22.50%)	10 (16.67%)	2 (5.00%)	9 (11.25%)	4 (6.67%)	0 (0.00%)
Strongyloides papillosus	1^{a} (2.50%)	5^{a} (8.33%)	0^{a} (0.00%)	3^{b} (3.75%)	1^{b} (1.67%)	-
Strongyle	6^{c} (15.00%)	5^{c} (8.33%)	2^{c} (5.00%)	5^{d} (6.25%)	3^{d} (5.00%)	-
Strongyloides papillosus + Strongyle	2^{e} (5.00%)	0^{e} (0.00%)	0^{e} (0.00%)	1^{f} (1.25%)	-	-
χ^2	5.08^{NS}			5.03^{NS}		

n – Nmber of samples collected

NS - Not Significant (P>0.05)

Figures bearing same superscript do not differ significantly

TABLE 2 SPECIES-WISE HELMINTHIC PREVALENCE IN SPOTTED DEER

Study area	V.O.Chidambaranar Park and Zoo	Arignar Anna Zoological Park
Species	**Spotted Deer (n=180)**	**Spotted Deer (n=140)**
Strongyloides spp.	4 (2.22%)	6 (4.29%)
Strongyle	8 (4.44%)	13 (9.29%)
Strongyloides spp. + Strongyle	1 (0.56%)	2 (1.43%)
χ^2	5.83^{NS}	9.32**

n – Number of samples collected

NS - Not Significant (P>0.05)

**Significant (P<0.01)

FIGURE 1- SEASON-WISE PREVALENCE OF HELMINTHS IN SPOTTED DEER AT AAZP

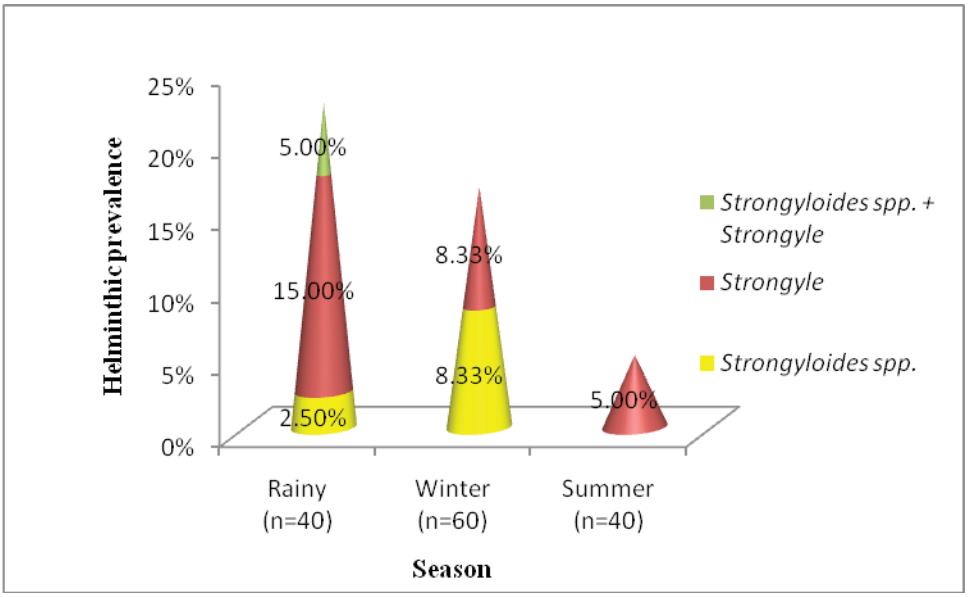


FIGURE 2. SEASON-WISE PREVALENCE OF HELMINTHS IN SPOTTED DEER AT V.O.C. PARK AND ZOO

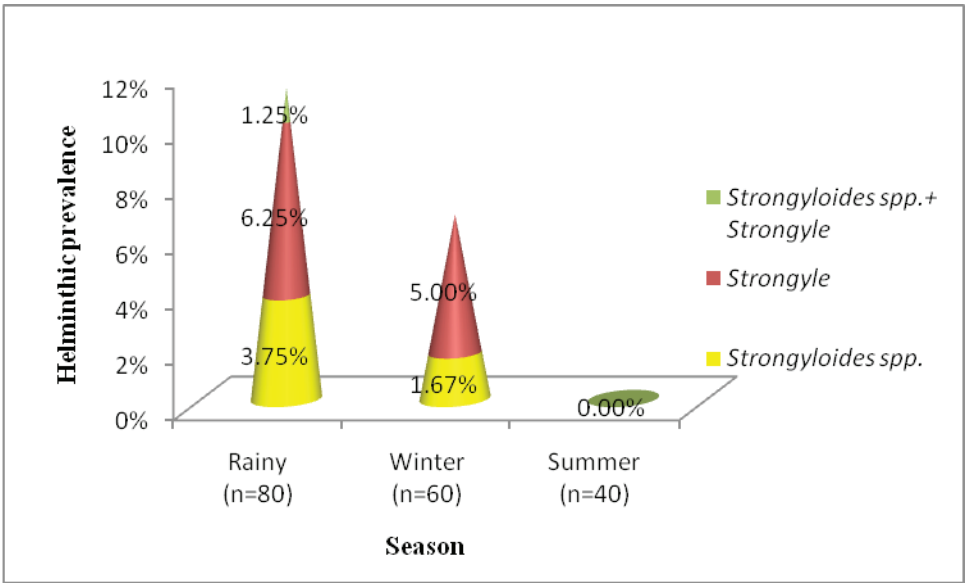
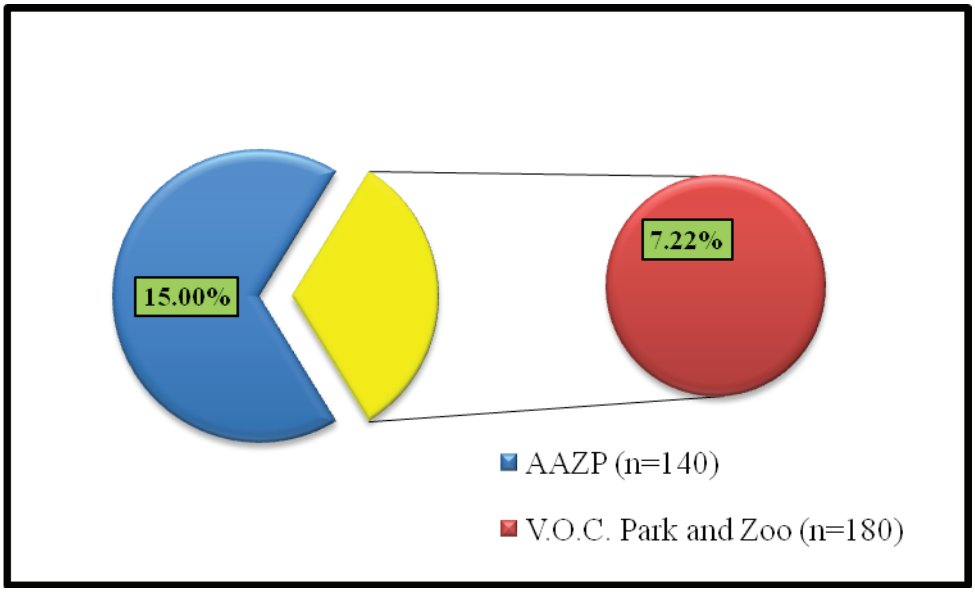


FIGURE 3. OVERALL PREVALENCE OF HELMINTHS IN SPOTTED DEER



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Books and articles within edited books

Rundall, C.J. (1991). A colour Atlas of Diseases of the Domestic Fowl and Turkey. 2nd ed. London. Wolf Publishing Ltd. 175 p.

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