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Review article

Full length articles

Short Communication

Review article

GENOMIC SELECTION IN SHEEP: PROSPECTS FOR INDIAN SHEEP INDUSTRY

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ABSTRACT

Small ruminants are the key component among the livestock sector that plays a significant role in sustainable livelihood of landless and small holder farmers in India. Rural human population constitutes 72.22 % out of which majority are dependent directly or indirectly on the agriculture and livestock related occupations. The total sheep in the country is 65.06 million that accounts for nearly 12.7% of total livestock population in India.Traditional breeding programs aimed at improving the productivity of sheep through selection. Nevertheless accurate, this approach always relied upon the intensity of selection and length of the generation interval (GI). Larger the GI, more time it took to improve any given trait of interest. Since advent of the genomic selection, genomic estimated breeding values (GEBV) of animals are being obtained at juvenile stage. This has resulted in significant reduction in the generation interval and faster rate of genetic improvement with more accuracy. Single Step approach has further allowed use of even non-genotyped individuals in reference for better accuracy and less bias of prediction. Genomic selection has literally replaced the pedigree selection at many places across the world barring India. In our country given the large population, sheep breeds can be easily brought under the genetic improvement programs and hence the benefits to the sheep industry can be increased many-fold. In India, given large sheep genetic resources which are yet to be included in the improvement programs due to lack of pedigree relationship can be very well brought under this umbrella. Genomic selection can be effectively used to enhance the pace and accuracy of selection programs especially for the traits which are difficult to measure, expressed in one sex and late in life or post death and have low heritability.

Keywords: Sheep Breeding, Genomic Selection, Single Step, Breeding Value, SNP

Genomic selection (GS) is built over the already existing principles of Best Linear Unbiased Prediction (BLUP) with additional information from genomic relationship matrix (**GRM**) derived from genotyping of the individuals comprised of high or low density whole genome information.

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Since time immemorial, man has been practicing the science of animal breeding for selection of the livestock for producing meat, milk and wool. Three researchers (Karl Pearson, Jay Lush, and Russell Lande) played influential role in the development of the "Breeder's equation". Traditional selection experiments have been highly successful in improvement of the traits of economic importance, a few examples being, average milk production per lactation of US Holstein cows has nearly doubled from >6000kg (year 1960) to ~12000kg (year 2000). For corn, yields have increased fourfold during the same period (Dekkers and Hospital 2002). In a typical selection experiment in Indian sheep (Malpura) there has been improvement in the live weight at six month age from 18.63 kg (year 1997) to 29.0 kg (year 2015), the genetic trend indicated 70.3 g increase per year (Figure 1) and the fit of the regression shown 94.8% coefficient of determination with the regressed value (Kumar et al. 2016).

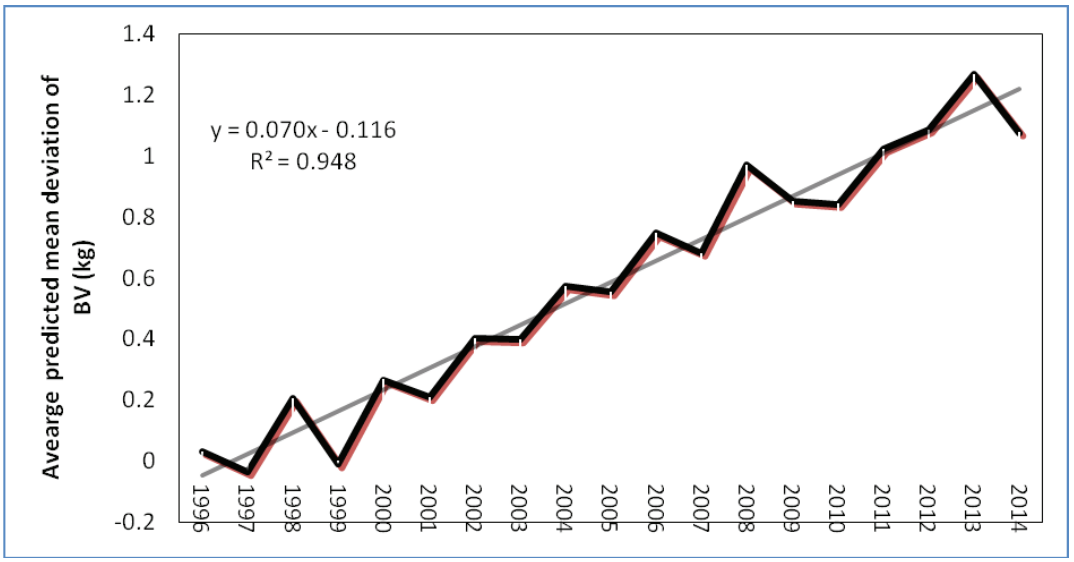

Fig 1. Genetic trend for live weight at 6 months in Malpura sheep

The breeder's equation for obtaining response to selection (R) was first conceptualised in the Animal Breeding plans by Lush (1943) as

$$\mathbf{R = h^2 S} \qquad \mathbf{(1)}$$

Where, R is response to selection; h^2 is heritability of a trait and S is the selection differential i.e. superiority of the parents as compared to the population in relation to the trait under consideration. In a typical real life animal breeding program we have overlapping generations, and hence generation interval for males and females will be different that will directly relate to the selection intensities of males and females in the selection program. This will make the response to be measured as follows (Falconer 1996)

$$R = [(i_m + i_f)/(L_m + L_f)] \times \sigma_a h \quad (2)$$

Where, intensities (i) and generation intervals (L) can be attributed to the male and females in the population. σ_a is the estimate for additive genetic standard deviation.

Limitations of Generation Interval in Genetic improvement programs

The generation interval (L) always has an inverse relationship with the response to selection (equation 2). Larger the GI, more time it took to improve any given trait of interest (Gowane and van der Werf 2017). There has not been any better way of reducing the generation interval, as most of the approaches come with a great cost to the animals with respect to reproductive efficiency. Efforts were made by adoption of MOET, however results were not encouraging. Marker Assisted Selection (MAS) has proven to be the best bet for reducing L in animal breeding programs, where potential marker for the trait under selection was utilised. However, very few examples are available where large QTL was associated with the trait and was practically used for MAS, example being Booroola fecundity gene in sheep (Sharma et al. 2004, Nimbkar et al. 2008, Arora et al. 2008, Sharma et al. 2016) and MSTN (GDF8) mutation that underpins muscle hypertrophy (Clop et al 2006). Limitation of having putative single marker for the trait does not allow use of this technology for every genetic improvement programme, as most of the traits are controlled by many number of loci. Genomic selection has resulted in significant reduction in the generation interval and faster rate of genetic improvement (Gowane and van der Werf 2017).

Sheep Breeding Programs and a need for paradigm change

In India, beef and pork have major taboos associated and hence major animal protein source for humans is mutton, chevon, chicken, egg and milk. According to the sample registration system (SRS) baseline survey (2014) released by the registrar general of India, 71 percent of Indians over the age of 15 are non-vegetarian and this proportion is bound to increase in future. This will have therefore huge pressure on sheep, goat and poultry sector for quality protein. Small ruminant breeding programmes in India were run separately for sheep and goat. Sheep breeding programme under the aegis of AICRP on Sheep started in 1971 with the major objective of increasing wool and meat. Impetus was on crossing exotic sheep (Rambouillet, Dorset and Suffolk) with low producing indigenous sheep. The success of cross-breeding with exotics for mutton was, at best, modest (Kandasamy 2009), however, for wool, in spite of producing good germplasms, they did not appear to have impact on smallholders due to rapidly declined importance of wool in the market (Naqvi et al. 2015). In 1990, AICRP took a new avatar as Network Project on Sheep Improvement (NWPSI) with major objective of intensive within breed selection. Four farm units and two field units aimed at distribution of superior germplasms (Rams) 50 and 100 each (Annual Report NWPSI 2016-17), respectively. Similarly, a new scheme called Mega Sheep Seed Project (MSSP) was initiated in 2009, where the

elite flocks of sheep (3 breeds) are given the responsibility to distribute the rams of good genetic worth to the field for improving the genetic potential of the field flocks (Annual Report MSSP 2016-17). At present the total sheep breeds covered under these two schemes are 9. Project Co-ordinator's report (2016-17) says that significant genetic gains in the set criteria for all the breeds took place over the years. However, the pace of improvement has been slow due to inherent time limit of generation interval.

Current problems with the sheep breeding programmes

1. Carcass quality evaluation: Sheep breeding programs do not incorporate the carcass quality traits, as they can only be evaluated post death of the animal. Due to this reason, in none of the sheep breeding programs in India, carcass quality has been incorporated in the selection index.

2. Non-availability of the structured pedigree: Out of 65 million sheep in India, a very few flocks maintained at Government institute at state or centre only maintain the pedigree records of the sheep. Rest of the population is therefore always not evaluated for their breeding values and thus selection of the best animals on the basis of their true genetic merit never takes place.

3. Many breeds and crossbreeds limit genetic evaluation: Usually at a given place, there is existence of the native true breed along with many crosses of the sheep. Due to different genetic groups, these populations are not considered in a single umbrella and are excluded from the genetic evaluation programme.

4. Long generation interval: Sheep usually have a generation interval of nearly three years with estimates of 3.37±0.05 years (Gowane et al. 2014a) in Malpura sheep and hence expression of the traits (reproductive traits, etc.) takes huge time that affects the selection decisions significantly.

5. The traits with low heritability estimate are very difficult to work with as predictions are weak, *viz*. selective value (h^2=0.02 in Malpura sheep, Gowane et al. 2014b), survivability (Gowane et al. 2017), etc.

6. The traits expressed only in one sex such as reproductive traits are very difficult to improve as they were essentially measured on relatives to get estimates.

Genomic selection

Best Linear Unbiased Prediction (BLUP) provides unbiased estimates of breeding values in populations under selection, however it is necessary to include all the information used in the selection decisions (Henderson 1975; Sorensen and Kennedy 1984) while analysing the

data. The relationship that follows from the recorded pedigree can be structured in the numerator relationship matrix (**NRM**) or **A**. BLUP is the most robust method of BV prediction as compared to any other method of prediction in the animal breeding programs as it accounts for the effect of selection, except for the allele frequency changes.

Genomic selection (Meuwissen et al. 2001) is a form of marker-assisted selection in which genetic markers covering the whole genome are used so that all quantitative trait loci (QTL) are in linkage disequilibrium (LD) with a dense panel of markers (Goddard and Hayes 2009). Genomic selection use single nucleotide polymorphisms (SNPs) as markers which cover the whole genome so that all genetic variance can be explained by the markers. It is assumed that the markers are dense enough that they are in LD with the QTL.

Modern day advancements in genotyping technology, along with the publication of human genome in 2001, and bovine in 2006, allowed rapid detection of SNPs and development of SNP-chips. A bovine SNP chip was initially developed for 10,000 (10k) markers and in 2007 a 56k chip was released by Illumina (van der Werf 2009). A 57k ovine chip was released in 2008, partly based on a virtual ovine genome sequence (Dalrymple et al. 2007). The OvineSNP50 chip was developed by Illumina in collaboration with the International Sheep Genomics Consortium (ISGC) and became commercially available in 2009. This microarray-based system is designed to determine the genotype of approximately 54,000 single nucleotide polymorphisms (SNPs) spaced evenly across the ovine genome (Sandenbergh et al. 2016). Farm^{IQ} in conjunction with Illumina and the International Sheep Genomics Consortium (ISGC) completed "Ovine Infinium® HD SNP BeadChip". This new chip is capable of identifying up to 600,000 SNPs points across the sheep genome and allows researchers to customize the chip or to update with new SNP discoveries. It is one of the first high-density chips developed for sheep.

Many of the animal and plant breeds have small effective population size [Holstein cattle population 150 (Zenger et al 2007), Zandi sheep 71 (Ghafouri-Kesbi 2010), Afshari sheep 50 (Ghafouri-Kesbi2012), Bharat Merino sheep 89.29 (Gowane et al. 2013) and Malpura sheep 91.74 (Gowane et al. 2014a)], that allows sharing of long chromosomal segments between the members of the population and thus strong LD. It is very essential for a marker to be in LD with a QTL across the entire population and the association must have persisted for a considerable number of generations. So, basically genomic Selection works on the principle of linkage disequilibrium.

How the Genomic Selection works?

GS has two steps, which mainly deal with estimation of marker effects and prediction of genomic estimated breeding values (GEBVs).

(I): Estimation of the effects of chromosome segments in a reference population: A sufficiently large reference population is essentially required for

estimation of the effects of chromosome segments, or SNPs which are closely related to QTLs. Reference is a group of animals which has both genotypes (SNPs ~ 50K) and phenotypes (of desired traits, such as milk yield, growth rate, etc.) which are meticulously recorded.

Genome Wide Association Study (GWAS) is performed to estimate the effects of the markers. Already known methods of assigning effects to the factors are used and found to be working good for estimation of effects. Least squares, Ridge Regression, Bayes A and Bayes B are the methods of assigning marker effects.

(II): Prediction of genomic EBVs (GEBVs) for animals not in the reference population: The selection candidates are the individuals in the generation such as progeny population, for which genomic breeding value is expected. This population does not have phenotypes recorded yet, however these individuals are genotyped using low or high density Ovine chip.

The MME are:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + \frac{m\sigma_e^2}{\sigma_G^2} I \end{bmatrix} \begin{bmatrix} \beta \\ G \end{bmatrix} = \begin{bmatrix} X'Y \\ Z'Y \end{bmatrix}$$

Fig 2: A typical genomic evaluation program using genomic data of 50K for prediction of GEBV in validation population

METHODOLOGY

The **NRM** is replaced by genomic relationship matrix (**GRM**) based on marker relationships between the individuals. **GRM** is a realised relationship matrix and hence the explained relationship is more accurate than approximations based on pedigree relationships (Figure 3). **GRM** plot the relationships exactly based on the genomic information, however **NRM** is based on expected relationships and many relationships take 0 values, which actually has a negative or near 0 values in **GRM** giving normal distribution.

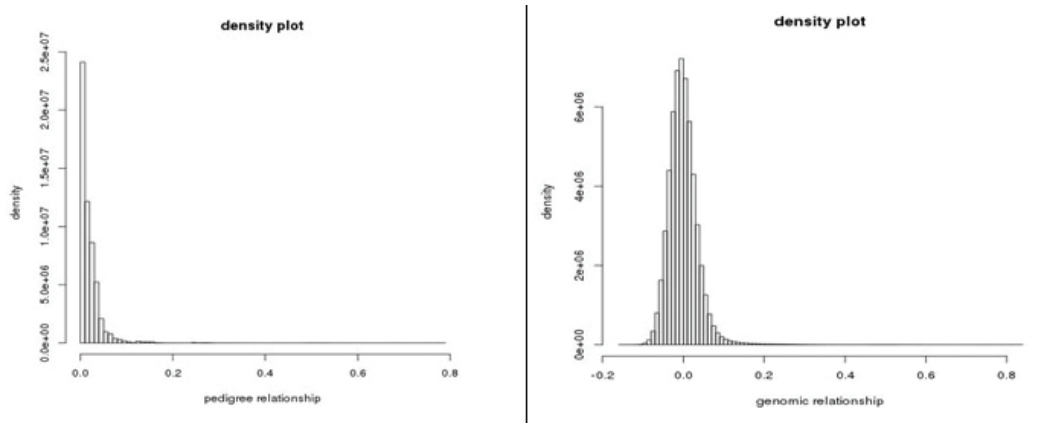

Fig 3: A relationship matrix (pedigree versus genomic) plot of 10550 animals, based on simulated data.

The mixed model equations for Genomic BLUP (GBLUP) is

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Zg} + \mathbf{e}$$

where **Y** is the vector of phenotypic values, **X** is a vector of 1s, **g** is a vector of additive genetic effects due to the ith marker allele or haplotype , **Z** is an incidence matrix and has a 0, 1, and 2 for the number of alleles for the marker effect at locus i as present in the jth animal.

The equation can also be written as

The MME are:

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} \\ \mathbf{ZX'} & \mathbf{Z'Z} + \frac{n\sigma_e^2}{\sigma_G^2}\mathbf{I} \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta} \\ \mathrm{G} \end{bmatrix} = \begin{bmatrix} \mathbf{X'Y} \\ \mathbf{Z'Y} \end{bmatrix}$$

where **X** is a vector of 1s, **G** is a vector of genetic effects due to the ith marker allele or haplotype, **Z** is an incidence matrix and has a 0, 1, and 2 for the number of alleles of type Gi present in the jth animal.

Advantages of GS for sheep breeding

The advantages of the GS might be smaller in sheep (van der Werf 2009) due to following reasons

1. Most of the traits under selection in sheep can be measured on both sexes and also before selection of animals for first mating

2. Several important traits such as growth rate, fleece yield in sheep have moderate to high estimate of additive genetic variance

Some of the traits, for which traditional BLUP selection lags behind, can be very well cashed by GS. In sheep breeding, advantages of GS can be summarised as below:

1. Some traits are difficult to measure on breeding animals, e.g. female fertility, slaughter traits, wool traits when measured on adults and parasite resistance. In a typical

parasite resistance breeding program (*Haemonchus contortus*) in sheep, it takes nearly 9 months for a sheep to express its genetic potential for susceptibility or resistance to the nematode (Prince et al. 2010). GS in such scenarios can help to estimate the breeding values even at birth of the animals, reducing the generation interval in a significant way. Reproduction traits are important but difficult to improve in sheep because they are lowly heritable and are recorded later in life, however using GS accuracy of three reproduction traits was greater when compared to pedigree methods, especially in less related animals (Daetwyler et al. 2014).

2. GS generates increased accuracy of selection as compared to the traditional pedigree based prediction by BLUP. The breeding values of young selection candidates can be predicted with reported accuracies up to 0.85 (Goddard and Hayes 2009). It uses dense marker maps for prediction of GEBV with reported higher accuracies up to 0.31 higher than those obtained by pedigree indices, without the need to phenotype the animals or their relatives (Calus 2010).

3. Apart from this, as **NRM** is replaced by GRM in the GS, even if animals are not having pedigree or if their pedigrees are not recorded, still they can be used in the validation population for prediction of the GEBVs. This is again a paradigm shift in the traditional breeding program approach, where **NRM** was essential and hence pedigree records were inseparable from the genetic evaluation methods. Recording of pedigree for long term requires a lot of time and manpower along with monetary resources. If we could compare the cost of genotyping with these, then in near future recording pedigree can easily be replaced with the GRM approach.

4. Sometimes, a crossbred of purebred is present and is not included in the recording system due to incompatibility of the pedigree. Such issues and many others can be easily sorted out given the use of **GRM** instead of **NRM** in genetic evaluation and breeding value estimation programs.

Accuracy and Bias of GS

GS is many times more accurate than PBLUP. However, accuracy of the GBLUP increases linearly with increase in the number of relatives selected for genotyping in reference (Gowane et al. 2018). Our study also revealed that traits with high h^2 had more accuracy and less bias of prediction as compared to models with low h^2.

It was seen that as we go on increasing the number of animals in the reference, the accuracy is increased linearly. Calus (2010)

reviewed several studies (Harris et al., 2008 and 2009, Berry et al., 2009, Lund and Su 2009, VanRaden et al., 2009) and reported a trend of increasing accuracy of GEBVs with increasing numbers of individuals in the reference dataset and advised to include 1000 bulls in the reference to obtain GEBVs for juvenile selection candidates with better accuracies than pedigree indices. For a respectable GS program, a reference size of 2000 animals seems to be good enough as it will assign a statistical power to the association test that is otherwise not much better with less sample size (Gowane et al. 2018). Similarly, several studies revealed that using more marker information after 50K SNP data, add little value towards accuracy of the prediction. Erbe et al (2012) revealed that for genomic predictions within a pure breed, there was no advantage of either the 800K or TRANS panel over the 50K panel when GBLUP_mod was used. When BayesR was used, there was only a very small advantage, although non-significant, given the sample size used in some cases.

Bias in genomic selection is seen when the regression coefficient of genomic breeding value on true breeding value of the animals in validation data set is not 1. Sorenson and Kennedy (1984) revealed that Henderson's MME lead to BLUP estimation of breeding values where the effects of the expected value and the variance of **a** due to selection, drift non-radom matings and inbreeding are properly accounted for, via the inverse of the relationship matrix (**A**). Thus in traditional pedigree based evaluation information about selection decision is included in phenotypes. As a result, no bias exists from selection, theoretically.

However, in GS, the case is no more the same. More efficient the pre-selection step is, further away the evaluation is from the usual assumptions in genomic selection (Patry and Ducrocq, 2011). In GS, animals in the reference do not use the allelic frequency of the base population and their value for mean is not zero but over and above the mean of the base unselected population, that tends to introduce the bias in the estimates. A few studies (VanRaden et al., 2009a, 2009b, Patry and Ducrocq 2011, Vitezica et al. 2011, Gowane et al. 2018) reported decreased accuracy and also bias in the estimates of genomic estimates of breeding value (GEBV) due to selective genotyping of sires. Now this bias will be accumulative and pass on from one generation to next very fast. The selection in GS is very stringent and hence assumptions of the traditional BLUP are almost always violated by GS.

Single Step Genomic Selection

Single Step (SS) is an improvement over the GBLUP for taking in to account the above discussed limitations of GS. Single Step combines the pedigree relationships (**NRM**) with **GRM** in a single step extending animal model to include marker genotypes. The relationship matrix that combines pedigree and markers is then called **H** matrix. Fitting $\mathbf{H^{-1}}$ in Henderson's MME gives the Single Step Genomic BLUP (SSGBLUP), which is a single estimator of breeding values that includes all available information. SSGBLUP (Legarra et al. 2009; Christensen and Lund 2010) combines genomic relationships from genotyped and pedigree relationships with non-genotyped individuals, hence this integration should

allow information on unselected animals and trace back to a conceptual unselected base population.

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix} \quad (10)$$

Where, $\mathbf{A}^{-1}$ is inverse of **NRM**, $\mathbf{G}^{-1}$ is inverse of **GRM** and $\mathbf{G}^{-1} - \mathbf{A}_{22}^{-1}$ is a correction to avoid double counting of genotyped animals.

Results of the simulation study

Genomic and phenotypic data was simulated using QMSim (Sargolzaei & Schenkel, 2009) with 25 replicates (for details of simulation refer: Gowane et al. 2018). The current generations were 10, each with 1000 population of 50:50 sex ratio. For the current study results for random mating design are shown where the genetic model of a trait has 0.5 h^2 and variance is explained by 60000 QTL. Results for accuracy (Fig 4) revealed that for pedigree BLUP (PBLUP) the accuracy was 54%. With 25% males in reference (G500) from last 4 generation (N=500) GBLUP accuracy was 46% that increased to 58% and 69% for G1000 (50% males) and G2000 (100% males), respectively. However for single step the accuracy was better. It was 66%, 70% and 75% for 25% (SS500), 50% (SS1000) and 100% (SS2000) males in reference from last 4 generations and their genomic information extended to pedigree in **H**-matrix, respectively. It was nevertheless best for GBLUP when 10500 individual's genomic data was used alone. Bias was minimum for PBLUP, SSGBLUP and GBLUP with more animals genotyped (Fig 5).

Fig 4: Accuracy obtained as correlation of TBV and GEBV for different methods of predicting EBV

Pedigree: Ony pedigree based (10500 animals), G500: is GBLUP based on 500 animals genomic data, G1000: GBLUP on 1000 animals genomic data, G2000 is GBLUP based on 2000 animals genomic data, SS500 id single step evaluation with 500 animals genomic and 10500 pedi-

gree data, SS1000: single step based on 1000 animals genomic and 10500 pedigree, SS2000: single step based on 2000 animals genomic and 10500 pedigree, G10500: genomic BLUP purely on 10500 animals genomic data.

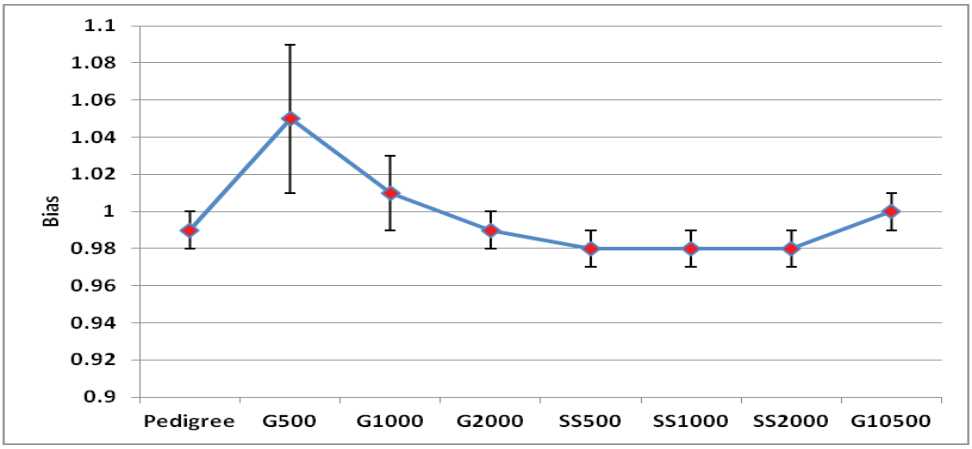

Fig 5: Bias obtained as regression of TBV and GEBV for different methods of predicting EBV

Pedigree: Ony pedigree based (10500 animals), G500: is GBLUP based on 500 animals genomic data, G1000: GBLUP on 1000 animals genomic data, G2000 is GBLUP based on 2000 animals genomic data, SS500 id single step evaluation with 500 animals genomic and 10500 pedigree data, SS1000: single step based on 1000 animals genomic and 10500 pedigree, SS2000: single step based on 2000 animals genomic and 10500 pedigree, G10500: genomic BLUP purely on 10500 animals genomic data.

Prospects of Genomic Selection in Sheep Breeding in India

BLUP has been and is being successfully used in India for livestock improvement programs. With availability of genomic selection we can definitely think and move towards adopting this approach, given a huge advantage in terms of accuracy of prediction, reduction of GI and targetting traits which are hard to measure, recorded in one sex and have low heritability. In India, we have a problem of pedigree recording in the field that makes inclusion of the animals in the field difficult in breeding programmes. GS will certainly help us to overcome this hurdle as pedigree data can be easily replaced by genomic data and **GRM** explains the relationships better. India has huge diversity of sheep genetic resources (42 breeds of sheep with 65 million population) and improvement in them is a possibility long awaited and seems to be reality in near future.

Cost of genotyping: It is one of the most important factors that need to be taken in to consideration when we think of GS. Ovine illumina 50K bead chip for sheep genome wide scanning of SNPs has three

Indian sheep breed's DNA information, namely Deccani, Garole and Changthangi. So apart from cost, it also becomes a concern, whether this cheap is better enough to scan diverse germplasm of Sheep (42 breeds and many more) with accuracy? Cost of genotyping is reducing fast as seen from the trend of decreasing cost versus high throughput sequencing over the last 2 decades. However, still in country like India, these costs are very high, as purchasing a ram of high genetic merit from the field will cost anywhere between Rupees 10000 to 20000. Average adult animal (sheep) may cost anywhere between Rs. 4000 to Rs. 7000 in India. So cost of genotyping is really high as compared to the cost of the animal. Potentially, this constraint can be overcome by genotyping selection candidates for a low density (low cost) panel of SNPs with sparse genotype coverage, imputing a much higher density of SNP genotypes using a densely genotyped reference population. These imputed genotypes would then be used with a prediction equation to produce genomic estimated breeding values (Hayes et al. 2012). The availability of pedigree also improves the accuracy of imputation, and most of the nucleus flocks in India have this information.

Can we translate the gains? Large ruminants have direct relation to industry in India, as milk is the commodity that has somehow succeeded in creating the link to industry (quantity of milk produced, fat content of milk) and thus commodity can directly be converted to money. However same is not true for other livestock commodities. Most of the shepherds in business rear sheep or goat as the way of life. They never look at this industry from business point of view, rather in true sense, we do not have livestock industries in India, we are stuck up with the livestock husbandry. Most of animals are sold as a unit and never on the basis of live weight, barring a few exceptions. Thus, it hardly makes a difference for a farmer, if his animals weigh little high or low. Thus fruits of genetic improvement for weight gain are not actually realised in real terms. He is most often compelled to sale animals during hours of need, marriage or health hazard in family, children education, etc. Middlemen or brokers who also serve as money lenders in difficult situation are essential component in this business for shepherds, however they are not really efficient for translating the gains to farmers.

A few things are essential for translating the gains of GS to farmers

(A): Transform the livestock husbandry to livestock industry

(B): Linkages between livestock producers and market

How can we work on GS?

1. There is a need to create another SNP chip for ovines which have more wider coverage of variability of the Indian sheep breeds along with exisiting variation. This itself is a new task, that needs research efforts from universities and partnership with the high end sequencing platforms.

2. We have several nucleus flocks of sheep with well recorded phenotypes (e.g. Malpura sheep,

Sirohi goat, etc.) at research institutes and universities. Today MSSP and NWPSI covers nearly 10 breeds under this umbrella. Therefore creation of reference population is not big task, given resources and time.

3. Reference needs a sizable data on phenotypes, pedigree and genotypes. Exisiting population in the nucleus can be genotyped and this can be continued for coming few more generations, till nearly 2000 individuals information is available. Training of the population with GWAS can be done on routine basis.

4. Data on alla the economically important traits is available in these nucleus flocks, however sincere efforts can be done to obtain more data on traits which were neglected e.g. Carcass traits.

5. Pedigree information must be error free and updated ad this will help in genomic evaluation of the animals using Single Step approach.

Exisiting Infrastructure can be utilised for further extending the programmes to field level. The already available infrastructure such as Government farms, Dairy co-operatives, Veterinary Health care facilities, Gram Panchayats, *etc*. can be easily involved in the program for execution (Gowane and Prince 2017). University and research institutes can be easily engaged for research and analytics, as this is the most important part of the whole story. We have high quality researchers in these institutions, however the training on this aspect is missing. Given opportunity, the same intellectual force can be capitalised for translating the gains and even generating new ideas for betterment of society. GS is a new arena opened for livestock improvement, and in particular sheep industry that will transform the genetic improvement programs will surely lead to real empowerment of farmers (Gowane and Prince 2017). Genomic selection certainly has a capacity to bring in a lot more unrelated individuals without recording system in the selection scheme and also much to offer for index selection incorporating traits like reproductive efficiency, disease resistance, carcass quality for realistic selection response.

CONCLUSION

Genomic Selection has lead to the paradigm shift in animal breeding prorammes with regards to pace and efficiency. Reduction of GI and use of GS for difficult to measure traits are a few of many gains of this technology. We have a huge sheep genetic resources that are underutilised in genetic improvemnet programs due to relationship constraints, and recording inefficiency. With GS, these resources can be easily brought in mainstream research and development. Need of the hour is to work multidimensionally. One important dimension is to bring change in existing sheep market and make it more farmer centered with better linkages. Another dimension is to incorporate the GS in breeding programs to enhance the pace of genetic improvement and translate gains

from numerical equations to monetary benefits for real stakeholders.

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Full Length Research Articles

PATHO-MORPHOLOGICAL CHANGES IN EXPERIMENTALLY INDUCED CADMIUM CHLORIDE TOXICITY IN WISTAR RATS

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ABSTRACT

The present study was undertaken to elucidate the pathomorphological changes in various visceral and immune organs of Wistar rats administered with cadmium chloride @ 100, 250 and 500 ppm, respectively in drinking water in groups II, III and IV for 12 weeks while group I rats served as control. Six randomly selected rats from each group were sacrificed at 6th and 12th week post exposure (PE). Grossly, liver and kidneys were pale and enlarged; spleen and thymus was atrophic and meningeal blood vessels were congested in toxin administered rats. Relative weights of liver, kidneys, lungs, heart and brain were significantly increased whereas relative weights of spleen and thymus declined significantly in treatment group rats. Histopathological examination revealed vacuolar degeneration, necrosis, MNC's infiltration, engorgement of blood vessel and oedema. The main target organs were found to be liver and kidneys but spleen, thymus, lungs, heart and brain were also affected. The intensity and distribution of lesions were more severe in group IV rats sacrificed at 12th week PE as compared to other groups in dose and duration dependent manner. Hence, the results of the present study indicated that cadmium chloride at dose rate of 100, 250 and 500 ppm in drinking water for 12 weeks caused severe damage to liver, kidneys, lungs, spleen, thymus and brain being hepatotoxic, nephrotoxic, immunotoxic and neurotoxic.

Key words: Wistar rats, cadmium chloride, patho-morphology.

INTRODUCTION

Cadmium (Cd) is a naturally occurring non-biodegradable environmental toxicant with a very long biological half-life (Friberg et al., 1986). It is unique among other metals because of its toxicity at a very low dosage and its low rate of excretion from the body (Jones and Cherian, 1990). It is one of the highly cumulative metal that proves to cause severe damage to a variety of organs such as liver, kidneys, lungs, brain, testes, blood system and bone (Ercal et al., 2001). Whatever the route of exposure, the liver represents an important organ for the initial accumulation of cadmium in the body and is

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potentially susceptible to cadmium induced cellular toxicities. In laboratory animals, acute cadmium poisoning produced primarily hepatic, CNS and testicular injury, whereas chronic exposure resulted in renal damage, anaemia, immunosuppression and osteotoxicity (Klaassen et al., 1999). Since no detailed systematic work particularly on cadmium induced toxicity has been undertaken, the present study was therefore, conducted with the primary aim to elucidate the pathomorphological changes in Wistar rats given cadmium toxin in different doses at various intervals.

MATERIALS AND METHODS

The study was conducted on forty eight (48) Wistar rats, approximately 21 days of age of either sex. The use of experimental animals and experimental design was duly approved by Institutional Animal Ethics Committee (IAEC). These rats were procured from Indian Institute of Integrative Medicine (RRL)**,** Jammu and maintained on commercially available feed for rats obtained from Shalimar feeds Pvt. Ltd., Bari brahmana, Jammu, India. On arrival, all the animals were examined for any abnormality and overt ill health. The animals were given a course of dewormer and all sanitary and hygienic measures were strictly observed. Rats were housed in polypropylene cages. Rice husk was provided as the bedding material. All the experimental rats were acclimatized to the experimental conditions for one week with proper identical housing, feeding and watering (*ad libitum*). After an acclimatization period of seven days, rats were randomly divided into four different groups of 12 rats in each as per the guidelines of OECD (2001). The dose selection criteria was factually based on the $1/10^{th}$ to $1/20^{th}$ of the oral lethal ($LD_{50\ i.e}$. 88mg/kg body weight) dose of cadmium chloride in rats. Following allocation, the animals were marked with picric acid solution for individual identification. Group I (Control) rats were given normal drinking water whereas group II, III and IV rats were given Cadmium chloride @ 100, 250 and 500 ppm, respectively, in drinking water for a period of 12 weeks. For observing gross changes in different organs, six randomly selected rats from each group were sacrificed at 6 and 12 weeks post exposure. All the sacrificed rats were subjected to a detailed post mortem examination. Gross lesions, if any, in different organs were recorded. Weights of different organs were taken using digital monopan balance. For histopathological examination, representative tissue samples of various visceral and immune organs were collected in 10% neutral buffer formalin and then processed for paraffin embedding employing alcohol as dehydrating agent and xylene as clearing agent. The sections were cut at 4-5µm thickness and stained by routine Harris haematoxylin and eosin method (Luna, 1968). Data generated from various parameters were presented as Mean $\pm$ SE. Statistical data analysis was done using analysis of variance (ANOVA) which was carried out in completely randomized design (CRD) and differences between sacrifice intervals were identified by Student's t test and a value of $P = 0.05$ was taken as significant employing Tukey's descriptive statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Gross pathology

Gross changes were least prominent at 6th week PE and increased in severity at 12th week of sacrifice in all treatment group rats in dose and duration dependent manner. Grossly, appreciable changes were noticed in the liver, kidneys, spleen and thymus of the toxin treated animals. As compared to control, the livers and kidneys in all the treatment group rats were moderately enlarged and pale in color at 6th week PE. Similar findings with more severity were observed at 12th week PE (Fig.1). Kidneys of group IV were more pale and enlarged than group III and II rats at 12th week PE. The findings in liver and kidney might be attributed to severe hepatocytic and renal damage, respectively, caused by cadmium as also evidenced by histopathological findings. These observations found support from earlier reports in rats (Brozska *et al*., 2001; El-Demerdash *et al*., 2004; Motup, 2012) and in chickens (Qazi *et al*., 2010; Zubair, 2012). Lungs showed mild to moderate congestion and consolidation. At 12th week PE, severe reduction in size of spleen and thymus was observed in treatment groups (Fig. 2) whereas heart did not reveal any appreciable change. Brain showed moderate to severe meningeal blood vessel congestion at 12th week PE. In the control group, rats did not show observable gross lesion in any of these organs at 6th and 12th week PE.

Relative organ weight

The data on average relative organ weights in rats is presented in Table. There was a non significant increase in the liver and kidneys body weight ratio in all treatment groups when compared to the control group at 6th week PE. However, at 12th week PE, there was significant increase in the weight of both organs than that of the control group. The increase in organ weights of these organs observed in the present study was in agreement with the findings of earlier workers in rats (Yamano *et al*., 1998; El-Demerdash *et al*., 2004) and in poultry (Zubair, 2012). At both 6th and 12th week PE, heart showed non significant increase in weight in treatment group rats as compared to that of control group rats. Lungs showed significant increase in weight amongst each other as well as control group rats at both intervals. There was non significant decrease in the spleen and thymus body weight ratio as compared to control group rats at 6th week PE. However, at 12th week PE, there was significant decrease in weight of both. This reduction in relative weights and size of spleen and thymus noticed particularly at the later stages was supported by histopathological findings of severe lymphocyte depletion and apoptosis, thereby lowering the cell mass and volume (atrophy) grossly and lowered weights of these organs in the present study. Similar observations were made by earlier workers in rats (Yamano *et al*., 1998; Rhman *et al*., 2011: Motup, 2012). Brain showed significant increase compared to control group rats at both sacrifice intervals. The significant increase in brain weights was supported by findings of Motup (2012) in rats and of Zubair (2012) in broilers as supported by oxidative stress.

Histopathological findings

Various histopathological lesions were recorded in different organs. Severity,

extent and type of lesions varied according to the affected organ, dose and period of toxin feeding.

Liver: The livers of treatment group revealed vacuolar hepatocytic degeneration and necrosis, binucleation, bile duct hyperplasia, MNC's infiltration in portal area (Fig.3) in dose and duration dependent manner. In addition, focal necrotic granulomas infiltrated by MNC's with degeneration of adjacent hepatocytes were more conspicuous at 12th week PE (Fig.4). Generally, the liver is considered as one of the critical target organs after acute and chronic exposures to cadmium (Kuester *et al*., 2002). Also, cadmium could induce lipid peroxidation in tissues including liver (Bagchi *et al*., 1996), which might lead to hepatocytic necrosis. These hepatic lesions in treatment group rats could also be possibly due to direct toxic effects of cadmium as it stimulated the intercellular signaling between Kupffer cells and hepatocytes, promoting there by proteolytic activity and liver damage (Stoll *et al*., 1976). The mechanism of liver damage in Cd exposure has been linked to a direct action of free Cd ions not bound with metallothionein (MT) and also to reactive radical production which might lead to changes in functions and structure of liver (Kowalczyk *et al*., 2002).

Kidneys: Kidneys revealed degeneration and swelling of epithelial cells of PCT's obliterating the lumen with hazzy cytoplasm. Further, presence of acellular protein cast in the lumen of PCT (Fig.5); engorged glomeruli, periglomerular MNC's infiltration with engorged interstitial blood vessel were more conspicuous in the highest toxin fed group at the end of the experiment (Fig.6). These findings corroborated well with earlier reports in rats by Brozska et al. (2001) and Motup (2012). The mechanisms of Cd-induced renal damage results from the dissolution of the Cd/metallothionein complex in the kidneys, exposing renal tissue to unbound cadmium. Cd/cell membrane binding, cellular apoptosis of renal proximal tubules, increased calcium loss in the urine, and increased protein excretion were seen in animals given long term doses of cadmium or repeated doses of Cd/metallothionein complexes (Klaassen *et al*., 1999). The results of present study showed that PCT's were more affected than DCT's. This could be due to the fact that PCT's are the primary sites of reabsorption and active transport leading to higher concentration of cadmium in the epithelial lining of these tubules (Sabolic *et al*., 2000).

Lungs: At 12 weeks PE, lungs showed presence of MNC's and macrophages infiltration in the alveolar lumen and thickening of interalveolar septa (Fig.7); degeneration and desquamation of bronchiolar epithelium with peribronchiolar lymphocytic hyperplasia and presence of cellular debris in the lumen (Fig.8). Lung sections of toxin administered rats showed marked histopathological lesions depending on dose and duration of exposure. The same findings were observed by Rao *et al*. (1998) and Motup (2012) in rats. El-Sokkary and Awadalla (2011) reported marked thickness of the alveolar septa with infiltration of inflammatory cells in rats. Effects on the lung following oral exposure to cadmium might be secondary to systemic changes. The present findings might also be due to the oxidative damage associated with

cadmium toxicity or could be possibly due to a state of anemia resulting in hypoxia and respiratory distress observed in treatment group rats (Yang *et al*., 1997).

Heart: No detectable lesions of pathological significance were observed in toxin fed group rats upto 6th week PE. However, at the end of the experiment, heart revealed engorged intermyocardial blood vessel, thinning and irregular wavy myocardial fibres, infiltration by MNC's. Severe degenerated and necrosed myocardial fibres were also observed (Fig.9). Heart lesions were dose and duration dependent and these results were in accordance with earlier reports of Qazi *et al.* (2010); Motup (2012) and Zubair (2012) who reported that the degenerative changes were due to hypoxic injury because of anemia or due to increase in free radicals and lipid peroxidation in heart muscle cells.

Spleen: Upto 6th week PE, mild depletion of lymphocytes in splenic follicle was observed in group II and III rats only. However, at the end of the experiment, sections of spleen revealed red pulp congestion, atrophy of splenic follicle with haemosiderosis and lymphocytolysis that was more conspicuous in highest toxin fed group at the end of the experiment (Fig.10).

Thymus: Microscopic changes followed the same trend as that observed in spleen upto 6 weeks. At 12 weeks PE, thymus showed engorged thymic blood vessels and multiple tiny spaces of lymphocytic depletion with presence of condensed nuclear fragments (Fig.11).

The changes in lymphoid organs (spleen and thymus) were dose and duration dependent but of almost similar nature except that changes were more severe in thymus than spleen. Depletion of lymphocytes started as single cell apoptosis in form of tiny empty spaces containing condensed nuclear fragments which coalesced to form large foci of lymphocytolysis particularly in thymus. This acute thymic atrophy may occur due to lymphocyte necrosis within thymic cortex and is secondary to anoxia of cortex resulting from capillary damage in the cortex (Morselt *et al*., 1988) or mainly due to susceptibility of primary lymphoid organs of thymus to cadmium in rats.

Brain: Lesions in brain were least prominent upto 6th week PE in all toxin fed group. Sections of brain showed engorgement of meningeal blood vessel with MNC's infiltration; increased perivascular space in white matter of cerebral cortex, focal area of gliosis with foamy appearance of neuropil (Fig.12) and shrunken deeply eosinophilic degenerated neurons more conspicuous at 12th week PE. In the present study, the dose rate was lower but the duration of feeding was much more, which might have increased in relative weights in brain in the highest dose groups and well supported by histopathological changes in brain of treated rats such as meningeal and cerebral blood vessel congestion, perineuronal oedema, neuronal degeneration and focal area of gliosis. Lesions in brain were similar to earlier reports in rats (Motup, 2012). These changes in brain might be due to increased production of ROS and free radicals and increased lipid peroxidation

(Stohs *et al*., 2001). This interpretation was confirmed by Williams (1995) who reported that oxidative stress was one of the mechanisms that contributed to structural changes and it played an important role in neurodegeneration.

CONCLUSION

The present study was carried out to elucidate the pathomorphological changes due to cadmium toxicity in Wistar rats. The appreciable gross changes in the liver, kidneys, thymus and spleen were oberved at the 12th week of sacrifice in all treatment group rats in dose and duration dependent manner. Relative weights of liver, kidneys, lungs, heart and brain were significantly increased while those of spleen and thymus were significantly decreased in treatment group rats as compared to control group rats. Histopathologically, liver, kidneys, lungs and heart showed vacuolar degeneration and necrosis, MNC's infiltration and engorged blood vessels in dose and duration dependent manner. Spleen and thymus showed depletion of lymphocytes and hemosiderosis. Brain showed engorgement of blood vessel, focal area of gliosis, shrunken deeply eosinophilic degenerated neuron and MNC's infiltration. All these changes suggesting hepatotoxic, nephrotoxic, immunotoxic and neurotoxic effects of cadmium.

Table: Organ body weight ratio (%) (Mean±SE) in rats of different groups (n=6).

Organs	Week PE	G I	G II	G III	G IV
Liver (gms)	6th	4.70 ± 0.11^{b}	4.91 ± 0.08^{abA}	5.11 ± 0.23^{abA}	5.35 ± 0.18^{aA}
	12th	4.91 ± 0.13^{d}	5.69 ± 0.12^{cB}	6.18 ± 0.08^{bB}	7.28 ± 0.14^{aB}
Kidneys (gms)	6th	0.83 ± 0.06^{b}	0.87 ± 0.06^{bA}	1.01 ± 0.01^{bA}	1.55 ± 0.03^{aA}
	12th	0.84 ± 0.07^{c}	0.92 ± 0.07^{cB}	1.38 ± 0.04^{bB}	1.59 ± 0.04^{aB}
Heart (gms)	6th	0.43 ± 0.02^{c}	0.49 ± 0.02^{bcA}	0.54 ± 0.02^{abA}	0.60 ± 0.02^{aA}
	12th	0.50 ± 0.03^{c}	0.55 ± 0.02^{bcB}	0.60 ± 0.01^{abB}	0.65 ± 0.01^{aB}
Lungs (gms)	6th	0.45 ± 0.02^{d}	0.50 ± 0.02^{cA}	0.55 ± 0.01^{bA}	0.60 ± 0.01^{aA}
	12th	0.47 ± 0.01^{d}	0.57 ± 0.03^{cB}	0.60 ± 0.00^{bB}	0.66 ± 0.01^{aB}
Spleen (gms)	6th	0.42 ± 0.02^{a}	0.36 ± 0.00^{bA}	0.32 ± 0.00^{bcA}	0.27 ± 0.01^{cA}
	12th	0.43 ± 0.03^{a}	0.31 ± 0.01^{bB}	0.26 ± 0.01^{cB}	0.21 ± 0.01^{dB}
Thymus (gms)	6th	0.26 ± 0.01^{a}	0.24 ± 0.01^{aA}	0.19 ± 0.00^{bA}	0.14 ± 0.01^{cA}
	12th	0.33 ± 0.04^{a}	0.19 ± 0.01^{bB}	0.14 ± 0.01^{bcB}	0.09 ± 0.00^{cB}
Brain (gms)	6th	0.89 ± 0.01^{b}	0.92 ± 0.01^{bA}	0.98 ± 0.01^{bA}	1.33 ± 0.10^{aA}
	12th	0.97 ± 0.04^{b}	0.98 ± 0.02^{bB}	1.02 ± 0.02^{bB}	1.99 ± 0.11^{aB}

Mean bearing at least one common superscript (a, b, c, d and A, B) do not differ significantly between groups and weeks ($P<0.05$), respectivel

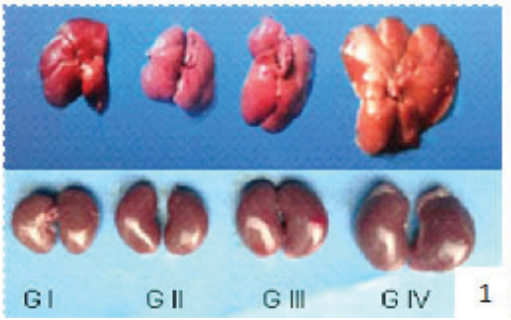

Fig.1 Dose dependent increase in size, paleness of liver and kidneys at 12 wks of intoxication

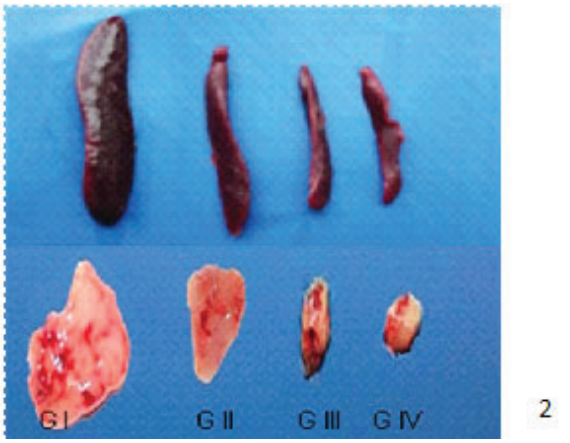

Fig.2 Dose dependent decrease in size of spleen and thymus at 12 wks

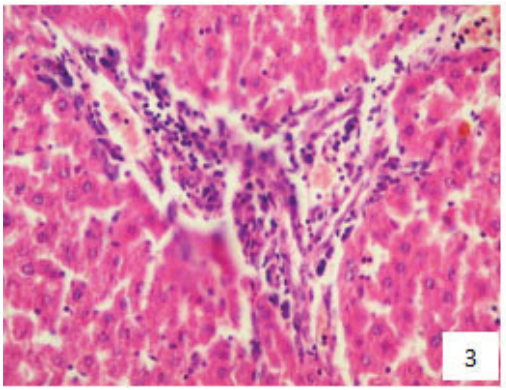

Fig.3 Liver, GIII: Portal area showing moderate MNC's infiltration, binucleation and degenerated hepatocytes with granular cytoplasm at 12th wk PE. H&E X400

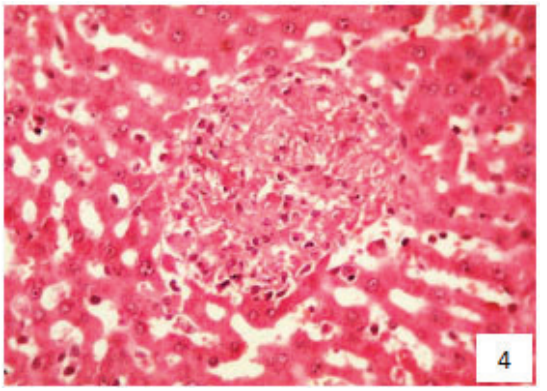

Fig.4 Liver, GIV: Vacuolar degeneration of hepatocytes and focal necrotic granuloma infiltrated by MNC's with degeneration of adjacent hepatocytes at 12th wk PE. H&EX400

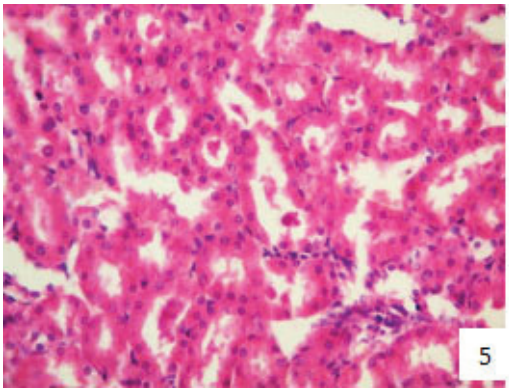

Fig.5 Kidney, GIII: Showing severe swollen and degenerated epithelial cells of PCT completely occluding the lumen. Note the presence of big protein cast in the lumen of PCT's at 12th wk PE.H&E X400

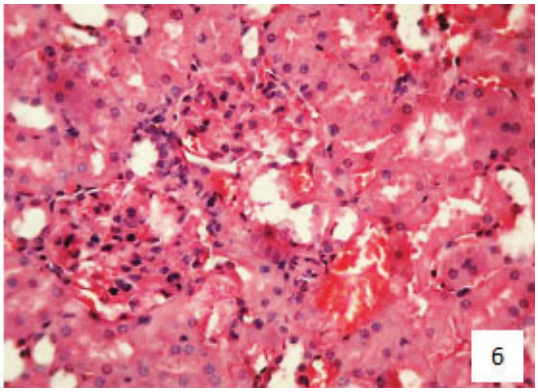

Fig.6 Kidney, GIV: Showing engorged glomeruli, mild periglomerular MNC's infiltration with engorged interstitial blood vessel and degenerated and swollen PCT epithelial cells at 12th wk PE. H&E X400

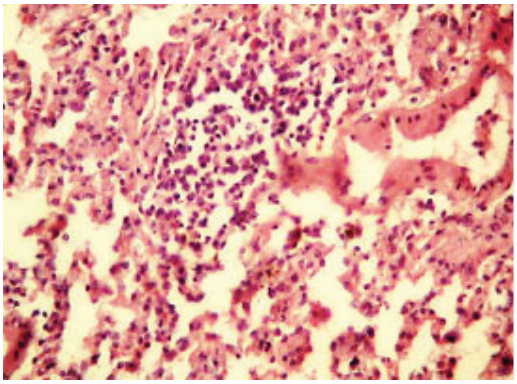

Fig.7 Lungs, GIII: Showing presence of MNC's and macrophages infiltration in the lumen of alveoli. H&E X400

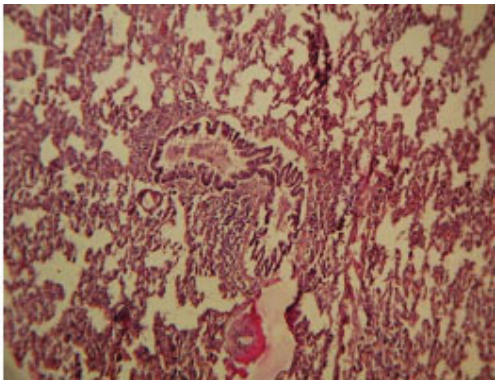

Fig.8 Lungs, GIV: Thickening of interalveolar septa with MNC's infiltration. Note degeneration, desquamation of bronchiolar epithelium, presence of cellular debris in lumen and peribronchiolar lymphocytic hyperplasia. H&E X100

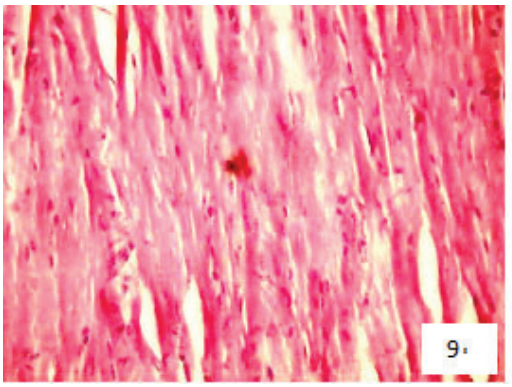

Fig.9 Heart, GIV: Severe degeneration and necrosis of myocardial fibres at 12th wk PE. H&E X400

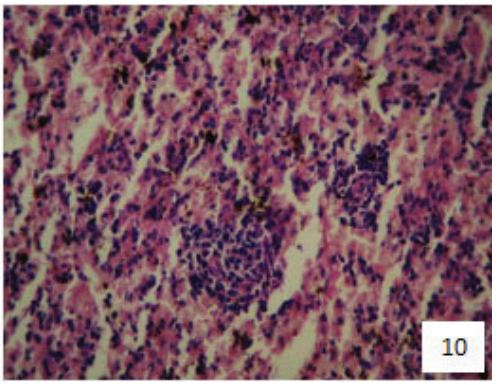

Fig.10 Spleen, GIV: Severe atrophy of splenic follicle with lymphocytolysis and haemosiderosis at 12th wk PE. H&E X400

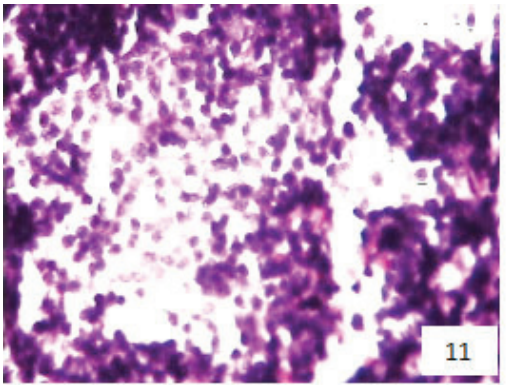

Fig.11 Thymus, GIV: Severe lymphocytic depletion with fragments of condensed nuclear material at 12th wk PE. H&E X400

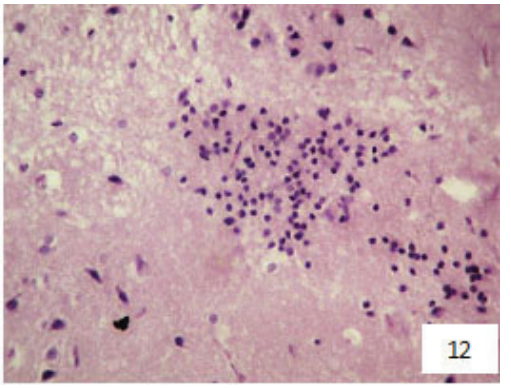

Fig.12 Brain, GIV: Focal area of gliosis with mild foamy appearance of neuropil at 12th wk PE. H&E X400

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EFFECT OFSUPPLEMENTATION OF SHRIMP WASTE ON THE IMMUNE RESPONSE, LIPID PROFILE AND CARCASS CHARACTERS OF CROSS BRED PIGS

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ABSTRACT

A study was conducted to determine the effects of supplementation of shrimp waste containing chitosonon the immune response, lipid profile and carcass characters of cross bred pigs. 40 weaned piglets (42 days) were assigned at random to 5 dietary treatments. The standard grower ration (T1) was supplemented with chlorotetracyclin (T2)while shrimp shell meal was included at 2.5% (T3), 5.0% (T4) and 7.5% (T5), as a source of chitosan.The feed and water was made adlibitum. There was a significant increase ($P < 0.01$) in serum total protein, primarily due to improvement in the globulin concentration, decrease ($P<0.01$) in serum total cholesterol, serum triglycerides, LDL Cholesterol and increase in HDL cholesterol due to shrimp waste supplementation and there was no significant difference among the pigs fed different treatments for the various carcass characters.

Key words: Shrimp waste, Chitosan, Immune response, Lipid profile, Carcass charecters

INTRODUCTION

There isa dramatic change in the food habits of the people due to the increased trend towards urbanization and improved purchasing power led them to opt for nutritionally rich foods, such as animal protein foods. Pork is comparatively cheap source of animal protein of high biological value. Maintaining the demand for pork is mainly depends on the quality of the product which is largely affected by the feed that is fed to the animals.

Modern pig production practices that are associated with regular use of antibiotics as growth promoters or to control diseases (Yang *et al.,* 2015) contributed to the spread of drug-resistant pathogens in both livestock and humans, thus posing a significant public

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health threat. Hence,many alternative feeding strategies have been studied such as acidifiers, probiotics, prebiotics etc., to reduce the use of antibiotics in animal feeds. More recently dietary inclusion of chito-oligosaccharides (COS) in swine rationsprovides a potential alternative to antibiotic supplements. Hence, the present study was carried out to study the effects of dietary inclusion of shrimp shell meal containing chitosan on the immune response, lipid profile and carcass characters in cross breed pigs**.**

MATERIALS AND METHODS

The feed ingredients like maize, soybean meal and de-oiled rice bran for preparation of standard experimental diets in ground form were procured locally and shrimp waste was procured from a shrimp processing factory in Nellore district of Andhra Pradesh. Representative samples of feed ingredients and creep feed were analyzed for proximate composition (AOAC, 2005). Fresh shrimp wastemeal contained 30 % DM. Hence, it was sundried for 3 days to about 90% DM and subjected to grinding in a hammer mill and sterilized in an autoclave for 10 min at 121˚C and 15psi pressure. The dry matter content of fresh shrimp waste was 29.0% and it contained 39.5% CP, 4.8% EE, 8.7% CF, 24.8% TA, 22.2% NFE, 7.18 % Ca, 3.45% P, and 15.5ss% chitosan. The chemical composition of shrimp waste as reported by various authors is presented in Table 1 and the chemical composition of the present study was comparable with the mean of the values reported by various authors.

Forty weaned piglets (42 days) were assigned at random to 5 dietary treatments and fed grower (15 to 35 kg body wt.) and finisher ration (35 to 70 kg body wt.) (Table 2). The standard ration (T1) was supplemented with chlortetracycline (T2) while shrimp waste was included at 2.5 (T3), 5.0 (T4) and 7.5% (T5), as a source of chitosan. Six pigs per treatment at the end of finisher phase were slaughtered to study the parameters.

Blood samples were collected at the time of slaughter into vacuum tubes containing no additive and tubes containing K_3 EDTA to obtain serum and whole blood, respectively. The red blood cells, white blood cells and lymphocyte counts of whole blood samples were determined using an automatic blood analyser (ADVIA 120, Bayer, Tarrytown, NY, USA). The serum was separated by centrifugation for 30 min at 2000 x g at 4°C and the aliquot was stored at -4°C for determination of serum profiles.

The total cholesterol, HDL and the triglycerides in the serum samples were estimated using diagnostic kits (M/s. Span Diagnostics Private Limited) by enzymatic method (Allian, 1974). The LDL cholesterol is calculated by the difference between total cholesterol and HDL cholesterol. Serum total proteins and albumin were estimated by using diagnostic kit (Monozyme India Limited) and globulin was estimated by subtracting albumin from total protein (Gornall*et al.,* 1949).

The muscle sample from each slaughtered animal was collected and preserved at – 20 ^{0} C for estimation of muscle cholesterol and triglyceride content**.**

Lipids from muscle tissue were extracted according to the procedure ofFolch*et al.* (1957). Two grams of muscle was homogenized in 7ml of methanol using Teflon homogenizer. The contents were filtered with the help of Whatman filter paper (No.40). The contents on the paper were scrapped off and homogenized with 14 ml of chloroform – methanol mixture and filtered into a flask. The residue was successively homogenized in chloroform – methanol (2:1 v/v) and each time this extract was filtered and it was repeated for two times. The pooled filtrate was evaporated to dryness.

The dried residue was dissolved in 5ml of chloroform – methanol mixture (2:1 v/v) and transferred into a centrifuge tube. To the above, 2ml of 0.1 M potassium chloride was added, shaken well and centrifuged at 3000RPM for 10 minutes. The upper aqueous layer containing gangliosides was discarded and the chloroform layer was mixed with 1 ml of chloroform – methanol – potassium chloride mixture (1:10:10 v/v) and centrifused again. The washing was repeated thrice and each time, the upper layer was discarded. The remaining mixture was evaporated to dryness and the amount of total lipids is estimated gravimetrically. The lower layer was made up to 5ml with chloroform – methanol mixture (2:1 v/v) and used for the analysis of lipid profile. The total cholesterol was estimated by enzymatic method using standard kits.

The dressing percentage was calculated from half carcass weight with intact kidneys and also with head and feet on. Loin area was traced on acetate paper by keeping it between 10^{th} and 11^{th} ribs. The traced area was measured in square centimeters. The average back fat thickness was measured at three locations *i.e*., first rib, last rib and the last lumbar vertebra. The data were subjected to one –way analysis of variance (Snedecor and Cochran, 1989) and the means were tested by least significant difference.

RESULTS AND DISCUSSION

The blood profiles of animals reflect the physiological disposition of their nutrition according to their internal and external environments. Therefore, we measured these characteristics to determine the response by which COS influenced. In the present study, serum total protein concentration was increased ($P<0.01$) in response to COS supplementation compared to control group (Table 2) which indicated that the protein status of the pigs had improved. The increased total protein concentration was primarily due to an improved globulin concentration, due to increase in serum IgG concentrationwhich are in agreement with Wang *et al*., (2009). This response indicates that COS supplementation had beneficial effects on the immune system. In addition, the lymphocyte concentration was increased ($P<0.01$) following COS supplementation which may indicate that COS also had beneficial effects on the immune system in agreement with Okomoto*et al*., (2003) Eze*et al*. (2010) and Yan and Kim (2011).

There was also significant increase ($P<0.01$) in PCV (%), Hb (g/dl) and RBC ($x10^6/\mu l$) count in pigs fed rations containing shrimp shell meal when compared to control group. Togun*et al.* (2007) observed that increase in PCV coupled with marginal

increase in RBC is indicative of more efficient erythropoiesis in the experimental animals and Nwanbe and Elechi (2009) reported lower values of PCV and Hb imply high level of blood dilution and low efficiency of cellular oxygen transportation.

There was a decrease (P<0.01) in serum total cholesterol, serum triglycerides, LDL cholesterol and increased HDL cholesterol levels in T4 compared to T3 and T5(Table 4) suggesting that COS alter the serum lipid profile by either decreasing (P<0.01) the bad cholesterol (LDL), total cholesterol or serum triglycerides or by increasing the good cholesterol which are in agreement with Tang et al., (2005).

Ma *et al.,* 2001 reported that the mechanism in reduced triglycerides (TG) and total cholesterol in serum was COS can form a gel complex with gastric acid in the gastrointestinal tract where the gel complex cannot be degraded under the high pH environment in the intestine. This gel can absorb bile acid and cholesterol and the gel, bile acid, cholesterol mixtures are discharged in faeces, thus the absorption of fat and cholesterol is decreased and the same mechanism for the decreased levels of triglycerides and cholesterol. Tang *et al.,* 2005 alsosuggested that COS formed a gel complex with gastric acid in the gastrointestinal tract and was subsequently excreted in the faeces.

The effect of supplementation of shrimp shell meal containing chitosan on muscle cholesterol and total fat can be compared with studies of Kim *et al.* (2008) where in they reported addition of COS in the diets of finishing pigs reduced the cholesterol in pork without affecting its quality. In the present study, in treatments T3 to T5 containing COS, there was decrease (P<0.01) in muscle cholesterol and total fat in finisher pigs (Table5) and the same trend was also observed in pigs fed grower rations containing COS suggesting that inclusion of COS in the rations of pigs had positive effect in lowering the muscle cholesterol and total fat in pork without affecting the carcass qualities. The effect of feeding experimental rations on carcass characteristics of pig is presented in Table6. No significant differences were found in any of the carcass characteristics among the treatments except in dressing percentage and hot carcass weight. The dressing percentage in pigs fed T4, T3 and T5 rations was significantly higher (P<0.01) than in those fed T1 and T2 rations. The hot carcass weight in pigs fed T4 rations was higher (P<0.05) than in T1, T2, T3 and T5 rations.

CONCLUSION

It was concluded that shrimp waste meal at 5% level in crossbred pig diets was beneficial as alternative to antibiotic feed additives and observed that COS alter the serum lipid profile by decreasing the bad cholesterol, total cholesterol or serum triglycerides and by increasing the good cholesterol.

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Table 1: Chemical composition and chitin (%) of shrimp shell meal (as reported by several authors)

CP	EE	TA	CF	Ca	P	Chitin	Reference
39.5	4.8	24.8	8.7	NA	NA	NA	Yugandhar kumar and Sakunthala Devi, 2015
32.6	1.7	0.3	24.9	NA	NA	14.7	Selimot*et al.,* 2013
20.1	1.3	2.3	NA	NA	NA	NA	Puga-lopez*et al.,* 2013
20.0	7.44	24.5	8.5	NA	NA	NA	Okonkwo*et al.,* 2012
24.9	6.2	46.1	NA	NA	NA	25.2	Bellaaj*et al.,* 2012
38.5	4.7	34.6	0.1	NA	NA	NA	Ehigiator*et al.,* 2011
36.6	10.2	21.7	19.5	4.9	1.2	18.9	Khempaka*et al.,* 2011
32.5	1.5	26.6	8.7	NA	NA	NA	Ravichandran*et al.,* 2009
30.0	NA	NA	NA	10.0	NA	8.0	Nguyen, 2009
40.6	**5.2**	**20.9**	**11.9**	**7.5**	**1.5**	**16.7**	**Mean**

NA: Not available

Table 2: Ingredient and chemical composition (%) of experimental diets

Ingredient	T1 (NC)	T2 (PC)	T3	T4	T5
Maize	55.0	55.0	55.0	55.0	55.0
Deioled Rice Bran	17.5	17.5	16.0	16.5	16.0
Soybean meal	25.0	25.0	24.0	21.0	19.0
Shrimp shell meal	-	-	2.5	5.0	7.5
Mineral Mixture	2.0	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5
	100	**100**	**100**	**100**	**100**
Chlorotetracycline	-	0.080	-	-	-
Lysine	0.76	0.76	0.78	0.81	0.85
Methionine	0.32	0.32	0.41	0.44	0.48
AB_2D_3	0.02	0.02	0.02	0.02	0.02
Cost per 100 kg (Rs)	22.50	22.50	20.98	20.14	19.0

Table 3: Effect of dietary treatments on haematological parameters in finishers

Parameter	T1	T2	T3	T4	T5
PCV (%)**	31.78^{b}±0.65	34.51^{a}±0.55	34.88^{a}±0.55	34.91^{a}±0.32	32.94^{b}±0.47
Hb (g/dl)**	10.73^{c}±0.20	11.94^{b}±0.42	12.27^{ab}±0.49	13.22^{a}±0.19	12.72^{ab}±0.14
RBC ($x10^{6}$/µl)**	6.10^{c}±0.44	7.60^{ab}±0.27	7.38^{b}±0.26	8.25^{a}±0.20	8.15^{ab}±0.21
WBC ($x10^{3}$/µl)^{NS}	15.54±0.66	16.25±0.54	16.25±0.54	16.62±0.69	16.26±0.48
Neutrophils (%)**	48.16^{b}±1.07	46.66^{c}±0.66	46.00^{c}±0.57	49.00^{ab}±0.36	50.33^{a}±0.49
Lymphocytes (%) **	43.16^{c}±0.94	45.50^{ab}±0.76	45.00^{bc}±0.68	47.16^{a}±0.47	45.66^{ab}±0.42
Monocytes**	4.83^{a}±0.60	5.00^{a}±0.63	5.66^{a}±0.49	2.83^{c}±0.54	3.16^{bc}±0.40
Eosinophils**	3.66^{a}±0.95	2.83^{a}±0.65	3.33^{a}±0.76	1.00^{b}±0.25	0.83^{b}±0.40
Basophils^{NS}	0	0	0	0	0
Total protein (g/dl)**	5.41^{c}±0.17	5.98^{b}±0.14	5.96^{b}±0.16	6.75^{a}±0.13	5.95^{bc}±0.28
Albumin (g/dl) ^{NS}	4.25±0.08	4.41±0.08	4.43±0.06	4.15±0.11	4.26±0.15
Globulin(g/dl)**	1.16^{c}±0.13	1.57^{bc}±0.15	1.52^{bc}±0.14	2.60^{a}±0.19	1.69^{b}±0.13
IgG (mg/dl)**	649.16^{d}±1.32	695.16^{c}±5.49		716.50^{a}±5.44	697.00^{b}±2.64

^{abc}values in a row not sharing common superscripts differ significantly **(P<0.01)

^{NS} Not significant

Table 4: Effect of treatments on serum lipid profile(mg/dl) in finishers

Parameter	T1	T2	T3	T4	T5
Total cholesterol**	77.33^{a}±1.23	75.33^{ab}±0.30	74.08^{b}±0.45	67.40^{d}±0.46	71.08^{c}±0.71
Triglycerides**	72.80^{a}±0.71	70.00^{b}±0.65	68.58^{b}±0.43	61.50^{d}±0.34	65.83^{c}±0.96
HDL**	43.08^{c}±0.56	45.83^{b}±0.35	46.16^{b}±0.72	48.75^{a}±0.47	46.66^{b}±0.55
LDL**	34.25^{a}±0.72	29.50^{b}±0.46	27.91^{b}±1.04	18.65^{d}±0.34	24.41^{c}±0.23

^{abcd}values in a row not sharing common superscripts differ significantly **(P<0.01)

Table 5: Effect of treatments on carcass fat and cholesterol content in finishers

Parameter	T1	T2	T3	T4	T5
Carcass fat (g/100g) **	12.35^{a} ±0.39	11.41^{ab}±0.23	10.60^{b}±0.45	8.58^{c}±0.22	10.91^{b}±0.35
Cholesterol (mg/100g) **	94.91^{a}±3.58	86.50^{b}±1.55	82.66^{bc}±0.47	70.41^{d}±1.86	79.25^{c}±1.79

^{abcd}values in a row not sharing common superscripts differ significantly **(P<0.01)

Table 6: Effect of dietary treatments on carcass characteristics of cross-bred finisher pigs

Treatment	Weight at slaughter (kg)^{NS}	Hot carcass weight (kg)*	Dressing percentage**	Carcass length (cm)^{NS}	Loin eye area (sq. cm)**	Average back fat thickness (cm)^{NS}	Primal cuts		
							Weight of ham (kg)^{NS}	Weight of Loin (kg)^{NS}	Weight of shoulder (kg)^{NS}
T1	70.66±0.27	55.47^{c}±0.32	78.40^{c}±0.30	70.75±0.58	29.50^{b}±0.56	2.32±0.11	14.79±0.20	12.87±0.32	9.53±0.13
T2	70.66±0.42	56.62^{b}±0.50	80.11^{b}±0.50	70.66±0.71	32.00^{b}±1.12	2.24±0.09	14.94±0.10	13.95±0.81	9.40±0.13
T3	70.83±0.38	57.94^{ab}±0.69	81.54^{ab}±0.70	70.91±0.47	37.00^{a}±0.93	2.13±0.08	15.08±0.11	13.59±0.87	9.57±0.19
T4	70.75±0.38	58.63^{a}±0.71	82.84^{a}±0.63	70.66±0.49	37.16^{a}±0.60	2.09±0.10	14.95±0.23	13.67±0.53	9.17±0.12
T5	71.58±0.27	58.18^{a}±0.27	81.27^{ab}±0.55	71.08±0.41	32.00^{b}±1.12	2.24±0.03	14.76±0.27	13.74±0.15	9.28±0.14

^{abc}values in a row not sharing common superscripts differ significantly *(P<0.05) **(P<0.01)

^{NS} Not significant

AUGMENTATION OF PLANT TOTAL NITROGEN USING FARMYARD MANURE IN ANNUAL FODDER CROPS

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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 annual fodder crops viz., Fodder maize (*Zea mays* L.) and Fodder Cowpea (*Vigna unguiculata*) 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 PTN during the harvest period (60^{th} day) varied between 1.31 to 1.45 % for T1 and 1.36 to 1.51% for T2 in Fodder Maize and between 3.04 to 3.19% for T1 and 3.08 to 3.23% for T2 in Fodder Cowpea. Significant ($P<0.05$ or $P<0.01$) difference in PTN content was evident between treatments on 60^{th} day of the trial period for both the annual fodder crops. A steady decline of PTN for both the treatments from 30^{th} day to 60^{th} day of the annual fodder crops was evident during the trial period. Integrated application of inorganic fertilizer along with farm yard manure (NPK + FYM) 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, Fodder Maize, Fodder Cowpea, Inorganic fertilizer.

INTRODUCTION

Maize is the most important multipurpose crop which is grown as food, feed and fodder crop which provides the cheapest fodder for livestock, feed for poultry and most valuable food for human beings. Its fodder is being highly relished by livestock due to its succulence and palatability (Iqbal et al., 2014). Maize has a surplus of potential for providing the energy rich forage for livestock feed and it can be safely utilized at all levels without any hazards.

Legumes are the most important forage plants that substantially improve the feed available for livestock as they can

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provide the essential protein for animals, improving soil fertility food crop production and household nutrition through a more reliable supply of milk and meat. Cowpea is an annual legume grown throughout the semiarid tropics, where it is valued as both human and livestock food. It is grown under rain-fed conditions in the tropics (Sangakkara, 1998), for its high protein content and is consumed as dry seeds, green pods or leaves. The residues of the plant are used in animal feeding. Also, cowpea can be used as a summer fodder crop fed as fresh or hay. Nutrients provided by cowpea make it extremely valuable where many people cannot afford proteins from animal sources such as meat and fish. Cowpea fixes atmospheric nitrogen up to 240 kg/ha and leaves about 60 to 70 kg nitrogen for succeeding crops. Cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation (Sanginga et al., 2003).

Integrated use of chemicals, organic fertilizers and improved management have revealed good results in terms of improved crop production, soil health which in turn decreases the chemical fertilizer requirement. Synergistic use of organic and inorganic fertilizers has many beneficial effects on crop and soil. Mostly crops produce quick response to chemical fertilizers which results in higher yield. 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, biological properties and soil health. Balanced fertilizer use along with organic manure like farm yard manure (FYM) is considered as promising agrotechnique in restoring soil fertility. Thus the integrated approach of nutrient supply by chemical fertilizers along with FYM not only reduces the use of inorganic fertilizers but is also an environment friendly approach. Combined organic and inorganic fertilization could enhance carbon storage in soils and reduce emission from N fertilizer use, which contributes to high productivity in agriculture (Pan et al., 2009). Hence, the present study was undertaken to determine the effect of inorganic fertilizer and combined effect of inorganic fertilizer with organic fertilizer (farm yard manure) on plant total nitrogen in two different annual crops viz., Fodder Maize (*Zea mays* L.) and Fodder Cowpea (*Vigna unguiculata)* in two agroclimatic zones of Tamil Nadu.

MATERIALS AND METHODS

Field experiments were carried out using the annual fodder crop, Fodder maize (*Zea mays* L.) and Fodder Cowpea (*Vigna unguiculata*) in Western and North Eastern agro climatic zone of Tamil Nadu. From each zone two districts viz., Coimbatore, Erode district (Western Zone) and Tiruvannamalai, Vellore district (North Eastern zone) were selected for the above study. Further, from each district two villages were randomly selected totaling to eight experimental sites. Soil samples were collected as per the standard agronomic practices in all the experimental villages prior to the study and analysed for the physico chemical properties and presented in Table 1**.** The study was carried out in

summer season of 2012. In Coimbatore district the experimental villages selected were Kondaiyampalayam (V1) and Idigarai (V2) and in Erode, the villages were Velankattuvalasu (V3), Velliyampalayam (V4) respectively. In the North Eastern Zone of Tiruvannamalai district, the selected experimental villages were Vannankulam (V5) and Kolathur village (V6) and in Vellore, Saduperi (V7) and Thirumani (V8) 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.

Fodder maize and Fodder cowpea were planted at 60 x 30 cm intervals on either side of the ridges as per the recommended agronomic package of practices. The experiment consisted of two treatments viz., Treatment 1 (T1) which is control with recommended dose of NPK fertilizers (60 N, 40 P_2O_5 and 20 K_2O kg/ha for Fodder Maize and 25 N, 40 P_2O_5 and 20 K_2O kg/ha for Fodder Cowpea) 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. The necessary after care operations such as hand weeding were done on 20th day after sowing. The plant protection measures have been adopted to control the pest and disease. Irrigation was carried out immediately after sowing (0th day), on 3rd day and thereafter once in 7 days.

Collection of fodder samples for estimation of Plant Total Nitrogen (PTN)

Fodder samples were collected at random just above the ground level at 30th and 60th (harvest) day for estimation of PTN. 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 Fodder Maize

The Mean values of plant total nitrogen (PTN) in Fodder Maize of the zones were

presented in Table 2. It is evident from the results that the plant total nitrogen decreased gradually in the fodder maize crop for both the treatments from 30th day to 60th day. On 60th day the PTN varied between 1.31 to 1.45 % for T1 and 1.36 to 1.51% for T2. Significant (P<0.05 or P<0.01) difference in PTN content was evident between treatments on 30th and 60th day of the trial period. The Plant total nitrogen declined for both the treatments from 30th to 60th day (harvest) of the fodder maize crop. The decrease in plant total nitrogen is due to the result of loss of leaves mass coupled with higher proportion of stems in total biomass which was generally low in total nitrogen. Moreover, the total nitrogen would be higher in immature plants than aged plants and as age progresses the crude fibre fractions in the plant enhances due to lignin deposition as the protein content gets diluted. This was in agreement with the findings of Tariq et al. (2011). On the other hand the results suggested that T2 values were significantly higher than T1 during the trial period. This increase could be due to incorporation of FYM along with NPK fertilizer which acts as a source of nitrogen for the fodder. Moreover, application of inorganic fertilizers with farm yard manure as a total basal dressing was beneficial to the 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). Also the increase in fodder total nitrogen could be due to enhanced amino acid formation with incorporation of farm yard manure (Shehzad et al., 2012).

Plant total nitrogen in Fodder Cowpea

The Mean values of plant total nitrogen (PTN) in Fodder Cowpea of the agro climatic zones were summarized in Table 3. On 60th day the PTN varied between 3.04 to 3.19% for T1 and 3.08 to 3.23% for T2. Significant (P<0.05 or P<0.01) difference in PTN content was evident between treatments on 30th and 60th day of the trial period. It could be observed from the results that the plant total nitrogen decreased gradually in fodder cowpea for both the treatments from 30th to 60th day. The decrease of plant total nitrogen from 30th to 60th day could be attributed to increased accumulation of carbohydrates and other structural materials such as lignin and silica with maturity of the crop and reduction of leaf to stem ratio which reduced the plant total nitrogen (Thavaprakaash et al., 2008). It is evident from the results that T2 values were significantly higher than T1 on 30th and 60th day. This increase in PTN values for T2 could be attributed to the effect of farm yard manure which helped in release of essential nutrients by microbes and would have contributed mainly in improvement of total nitrogen content of cowpea in fodder (Abebe et al., 2005). Also the higher plant total nitrogen content would be attributed to the ability of farm yard manure which supplied nutrients through mineralization, improvement of physical and chemical properties of soil to release nutrients gradually throughout the growing season (Ouda and Mahadeen, 2008).

The results clearly indicated that use of inorganic fertilizers alone or synergistically with organic manure/ farm yard manure resulted in augmenting significant buildup

of plant total nitrogen in Fodder Maize/ Fodder cowpea treated plots. Farmyard manure helps in significant uptake of nutrients from the soil in a steady manner which in turn possibly could increase the fodder yield as well the protein content of the fodder.

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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 Fodder Maize of Western zone and North Eastern zone of Tamil Nadu

Zone	District	Villages	30th day		t value	60th day		t value
			T1	T2		T1	T2	
			Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E	
Western	CBE	V1	1.47 ± 0.01^{cd}	1.50 ± 0.01^{cde}	2.39*	1.38 ± 0.01^{d}	1.42 ± 0.01^{d}	3.65**
		V2	1.48 ± 0.01^{de}	1.51 ± 0.02^{de}	2.27*	1.40 ± 0.01^{e}	1.47 ± 0.02^{e}	5.16**
	ERO	V3	1.53 ± 0.02^{f}	1.57 ± 0.01^{f}	5.03**	1.45 ± 0.02^{f}	1.51 ± 0.01^{f}	3.28**
		V4	1.50 ± 0.01^{e}	1.52 ± 0.01^{e}	2.25*	1.43 ± 0.01^{e}	1.45 ± 0.01^{e}	2.43*
North eastern	TVM	V5	1.44 ± 0.01^{ab}	1.46 ± 0.02^{ab}	2.43*	1.33 ± 0.01^{ab}	1.38 ± 0.01^{ab}	3.43**
		V6	1.46 ± 0.01^{bcd}	1.49 ± 0.01^{bcd}	2.30*	1.36 ± 0.02^{cd}	1.41 ± 0.02^{cd}	5.49**
	VLR	V7	1.43 ± 0.01^{a}	1.45 ± 0.01^{a}	2.42*	1.31 ± 0.01^{a}	1.36 ± 0.01^{a}	4.80**
		V8	1.45 ± 0.01^{bc}	1.48 ± 0.02^{abc}	2.26*	1.34 ± 0.2^{bc}	1.39 ± 0.01^{bc}	5.64**
		F value	21.31**	12.91**		37.64**	29.40**	

Means bearing same superscripts within columns do not differ significantly

* - Significant (P<0.05) ** - Highly Significant (P<0.01)

Table - 3. Plant Total Nitrogen (%) in Fodder Cowpea of Western zone and North Eastern zone of Tamil Nadu

Zone	District	Villages	30th day		t value	60th day		t value
			T1	T2		T1	T2	
			Mean ± S.E	Mean ± S.E		Mean ± S.E	Mean ± S.E	
Western	CBE	V1	3.16 ± 0.01^{c}	3.20 ± 0.01^{c}	2.28*	3.12 ± 0.01^{b}	3.14 ± 0.01^{c}	2.43*
		V2	3.20 ± 0.02^{d}	3.24 ± 0.01^{d}	3.19**	3.15 ± 0.01^{c}	3.20 ± 0.02^{d}	3.03*
	ERO	V3	3.25 ± 0.01^{e}	3.31 ± 0.02^{e}	3.23**	3.19 ± 0.02^{d}	3.23 ± 0.01^{e}	2.33*
		V4	3.23 ± 0.02^{de}	3.27 ± 0.01^{d}	3.20**	3.17 ± 0.02^{d}	3.21 ± 0.01^{e}	2.71*
North eastern	TVM	V5	3.12 ± 0.01^{ab}	3.16 ± 0.01^{ab}	3.41**	3.07 ± 0.02^{a}	3.11 ± 0.01^{ab}	3.07*
		V6	3.14 ± 0.01^{bc}	3.17 ± 0.02^{bc}	3.22**	3.10 ± 0.01^{b}	3.13 ± 0.02^{bc}	2.82*
	VLR	V7	3.09 ± 0.02^{a}	3.14 ± 0.01^{a}	2.96*	3.04 ± 0.01^{a}	3.08 ± 0.01^{a}	2.47*
		V8	3.11 ± 0.01^{ab}	3.15 ± 0.01^{ab}	3.14*	3.06 ± 0.02^{a}	3.10 ± 0.01^{a}	2.79*
		F value	27.08**	46.34**		40.38**	24.72**	

Means bearing same superscripts within columns do not differ significantly

* - Significant (P<0.05) ** - Highly Significant (P<0.01)

Short Communications

EVALUATION OF SERUM CYSTATIN C AS A NEW MARKER OF RENAL FAILURE IN SMALL ANIMAL MEDICINE

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In both human and veterinary medicine, diagnosing and staging renal disease can be difficult. Despite the relative clinical importance of kidney diseases, their early diagnosis can be challenging due to the absence of sensitive and specific biomarkers. Measurement of glomerular filtration rate is considered as the gold standard for assessing renal function but these techniques can be impractical for many practices (Kerl and Cook, 2005). Traditional blood markers (creatinine and blood urea nitrogen) and urinary markers (urinary casts, fractional excretion of Na^+) of kidney injury are insensitive for early diagnosis of acute kidney injury (Star, 1998). Blood Urea Nitrogen and serum creatinine concentration, which are most commonly used for measuring renal function, are relatively insensitive in detecting early renal dysfunction because about 70-75 per cent of the nephrons will be non-functional before these values rise about the normal range (Ross, 1995). When concentration of serum creatinine exceeds 2.0 mg/dl, reduction in GFR is observed (Osborne *et al*., 1972). Therefore, there is a need for better methods to diagnose and monitor patients with renal disease. Among other qualities, an ideal biomarker would identify the site and severity of injury, and correlate with renal function.

Serum cystatin C has been proposed as a simple, accurate and rapid endogenous marker of glomerular filtration rate in research and clinical practice. Cystatin C is a cysteine proteinase inhibitor produced constitutively by all nucleated cells (Abrahamson *et al*., 1986). Cystatin C has many properties that are ideal for endogenous glomerular filtration rate marker applications, such as constant production and plasma concentration in the absence of glomerular filtration rate variation, low intra individual variability, no plasma protein binding, no tubular secretion, no tubular reabsorption without catabolism, and no extra renal clearance (Seronie-Vivien *et al*., 2008). Serum cystatin C concentration correlated more strongly than serum creatinine concentration with GFR measured by creatinine clearance (Wehner *et al*., 2008). Cystatin C is an easy and

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rapid assessable marker that can be used for accurate information on renal function impairment (Gonul *et al.,* 2004).

The study was conducted on the dogs presented in the Small Animal Medicine OPD of Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu from May 2014 to June 2015. Similar to a previous study on canine Serum Cystatin C (Jensen *et al*., 2001) dogs were divided into two groups: healthy dogs (group A) and renal disease dogs (group B). Dogs were defined as healthy if their history, physical examination, CBC, biochemistry and urinalysis which included specific gravity, dipstick, sediment analysis and urine protein to creatinine ratio were unremarkable Dogs were included in group B if they were azotaemic and the anamnesis and clinical signs were consistent with intrinsic renal disease (pre-renal and post-renal diseases were excluded). The historical signs related to renal failure included polyuria and polydipsia, vomiting, anorexia, and weight loss. Azotaemia was defined as urea and creatinine concentrations above the upper limit of the reference intervals (reference intervals for urea and creatinine are 3·3 to 8 mmol/L and 36 to 120 µmol/L, respectively).

Blood samples in excess of amount required for routine diagnostic testing and with owner consent for use were obtained for this study. Samples from control and renal disease dogs were sourced from Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu. For the purpose of this study, serum creatinine, urea and cystatin were measured and recorded. The collection of urine was random and untimed. Routine complete blood count (CBC), biochemistry and urinalysis were performed within 24 hours of collection. Serum cystatin C were measured within 24 hours of collection until the stability test was carried out. Serum cystatin C (mg/L) was measured by ELISA .Glomerular filtration rate was calculated by using the following formula (Stevens and Levey, 2009)

Estimated glomerular filtration rate (eGFR) = $76.7 \times CysC^{-1.19}$

GFR is expressed as mL/min/1.73 m^2; serum CysC in nmol/L.

Conversion factors for units: serum CysC in mg/L to nmol/L × 74.9.

Dogs with Clinical characteristics of diseased dogs are reported in Table 1. Out of total cases (1580) presented in OPD, nineteen dogs (19) were found to be affected with renal failure with overall prevalence of 1.20 per cent. Out of nineteen cases, eleven cases (57.89 per cent) were of acute renal injury and eight cases (42.10 per cent) were of chronic kidney disease which is categorized on the basis of clinical signs, duration and course of disease, haemato-biochemical parameters and with the aid of radiography and ultrasonography. The clinical characteristics of control dogs are reported in Table 1.

The mean value of creatinine in control healthy dogs and diseased dogs were 0.69±0.10 mg/dl and 11.36±2.12 mg/

dl, respectively. High significantly ($p<0.01$) increase was observed in creatinine values in renal failure cases. The mean value of serum cystatin C in normal healthy dogs and diseased dogs were 0.41±0.03 mg/L and 3.05±0.45 mg/L, respectively. A highly significant ($p<0.01$) increase was observed in serum cystatin C in renal failure cases as compared to healthy dogs.

The correlation between glomerular filtration rate and reciprocal serum cystatin C (mg/L) and also with reciprocal serum creatinine (µmol/L) in dogs was estimated. The overall correlation coefficient of glomerular filtration rate with the reciprocal of serum cystatin C in control dogs was $r = 0.992$, $p< 0.01$ and in renal failure dogs was $r = 0.998$, $p< 0.01$. The overall correlation coefficient of glomerular filtration rate with the reciprocal of the serum creatinine in control dogs was $r = -0.093$ and in renal failure dogs was $r = -0.210$.

Traditional biomarkers lack sensitivity and does not allow early detection of renal injury. Serum creatinine and plasma/ serum/urine cystatin C are both functional biomarkers of renal disease. Creatinine measurement is insensitive for the diagnosis of early renal insufficiency; at least 75 per cent of nephron function is lost before creatinine elevations outside the reference interval occur (Lefebvre, 2011). Cystatin C may be superior to serum creatinine especially in early stages of renal damage (Laterza *et al*., 2002). Kavitha *et al.* (2011) also found that cystatin C is superior marker for renal dysfunction than creatinine and blood urea nitrogen. In the present study, we demonstrate that serum cystatin C can be detected in renal diseases and concentration is significantly higher in renal failure patients as compared to control.

A highly significant ($p<0.01$) increase was observed in serum cystatin C in renal failure cases as compared to healthy dogs. This was in accordance with Gonul *et al*. (2004) who reported that dogs with chronic kidney disease had significantly higher cystatin C concentrations compared with healthy dogs.

Wehner *et al.,* 2008 and Miyagawa *et al.,* 2009 found that concentrations of urinary or serum cystatin C were significantly elevated in dogs with renal disease of various causes compared to those without, and correlated strongly with glomerular filtration rate measured by creatinine or iohexol clearance in both healthy and dogs with renal disease. Serum cystatin C is primarily used as a glomerular filtration marker in assessment of chronic kidney disease in humans. Various human studies show that evaluation of cystatin C concentrations is more sensitive for detecting reduced glomerular filtration rate than evaluating creatinine concentrations and better able to detect small changes in glomerular filtration rate in the same patient (Herget-Rosenthal *et al*., 2004a). He also stated that serum Cystatin C concentration could detect development of Acute kidney injury one or two days earlier than serum Creatinine concentration in intensive care patients with ≥2 predisposing factors of acute kidney injury.

In the present study, the correlation between glomerular filtration rate and reciprocal serum cystatin C (mg/L) and also with reciprocal serum creatinine (µmol/L)

in dogs was estimated. The result was in accordance with Dharnidharka *et al*. (2002) who performed a meta-analysis of available data from various studies to compare the accuracy of Cys C and Creatinine in relation to a reference standard of glomerular filtration rate. The overall correlation coefficient for the reciprocal of serum Cys C ($r = 0.816$) was superior to that of the reciprocal of serum Cr ($r = 0.742$; $p < 0.001$).

Kumaresan and Giri (2011) carried out a study on Indian patients with chronic kidney injury with an aim to compare the diagnostic performance of serum cystatin C and creatinine with measured glomerular filtration rate and estimated glomerular filtration rate. Serum cystatin C showed significant correlation with measured glomerular filtration rate in all the three groups (r=-0.9735, r=-0.8975 and r=-0.7994 respectively) than serum creatinine (r=-0.7380, r=-0.6852 and r=-0.5127 respectively).

A highly significant increase was observed in serum cystatin C level in renal failure cases. The overall correlation coefficient of glomerular filtration rate with the reciprocal of serum cystatin C in renal failure dogs was superior to that of the reciprocal of serum creatinine. Plasma cystatin C level increases earlier than plasma creatinine as GFR decreases and may be a valuable marker in detecting early renal function impairment.

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Table 1: Baseline characteristics of dogs with renal diseases and control healthy dogs

S. No	Characteristics	Healthy control (Group A) (N=6)	Disease control (group B) (N= 6)
1	**Average Age (year)**	6.16±0.70	7.20±0.45
2	**Body weight (kg)**	22.83±3.4	20.01±1.10
3	**Rectal Temperature (^{0}F)**	101.30±0.20	102.62±0.50
4	**BUN (mg/dl)**	19.36±1.07[a]	164.06±29.57[a]
5	**Creatinine (mg/dl)**	0.69±0.10[a]	11.36±2.12[a]
6	**Serum Cystatin C (mg/l)**	0.41±0.03[a]	3.05±0.45[a]

Mean bearing similar superscript differ significantly ($p<0.05$) within group

FACTORS INFLUENCING SERUM MINERAL PROFILE OF SHEEP IN NORTH EASTERN AGRO CLIMATIC ZONE OF TAMIL NADU

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In nature all the members of animal and plant kingdom require inorganic elements: minerals, as these are needed for their survival and efficient performance. Twenty two mineral elements are believed to be "essential" for the members of the animal kingdom. These comprise seven major or macro mineral *viz.,* Calcium (Ca), Phosphorus (P), Sodium (Na), Potassium (K), Chlorine (Cl), Magnesium (Mg) and Sulphur (S) and fifteen trace or micro minerals. These mineral elements exist in the cells and tissues of the animal in specific concentrations, which is essential for the normal growth, health and productivity of the animal. Sheep and goats meet their body mineral requirements by grazing or browsing. Mineral in forages are dependent upon the interaction of number of factors including soil, plant species, stage of maturity, yield, pasture management and climate. Minerals are vital in addition to carbohydrate, fat, and protein for normal physiological functions. Various factors that influence the serum mineral concentration have not been studied well. Hence, this study was undertaken to identify the factors that influence the serum mineral profile of sheep in five districts of North Eastern Tamil Nadu.

A total of 250 sheep were randomly selected from five districts (*Kanchipuram, Thiruvallur, Thiruvannamalai, Vellore and Villupuram*) of North Eastern Tamil Nadu. All the selected animals were yearling females grazing in differentpastures and reared under similar managemental conditions (small scale farm). Blood samples were collected by jugular venipuncture, 5ml in tubes washed with acid, triple glass distilledwater and dried for harvesting serum. The separated serum was stored at -20ºC pending analysis of minerals.*The concentration of serum macro minerals viz.,*Ca, Mg and P were assessed by *Arsenazomethod, Xylidyl blue method and A*mmonium Molybdate method respectively*using*auto analyzer (A15 Biosystem). Micro minerals (Zn, Fe and Cu) were estimated using Atomic Absorption Spectrophotometer (AAS) as per the methods of AOAC (2000).*The data obtained from the above studies were analysed statistically.*

The various grazing sites like fallow land, forest and road side lands where the animals have been grazing were studied for their influence on the serum mineral concentration (Tables 1 and 2). The level

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of phosphorus was found significantly (P≤ 0.01) varied among various grazing sites, whereas, the calcium and magnesium levels remained comparable in all types of grazing sites. The sheep grazing on fallow land and roadside grazing sites were found to have significantly (P≤ 0.01) higher serum phosphorus content than those sheep grazed on forest land. Among the micro minerals status the sheep grazed on fallow land had significantly (P≤ 0.01) higher serum iron content than those sheep grazed on forest and roadside grazing sites. The serum zinc and copper levels did not vary among sheep grazed on various grazing sites. The variations in mineral profile among various grazing sites was in agreement with Rajendran and Balakrishnan (2012) who reported mineral contents of major grass species in the diet composition in mountain as well as fallow land. Their results revealed that the Ca, Fe, Cu, Zn, Mn and Co were above the critical level, but phosphorous level was below the critical level. The authors suggested that to balance the phosphorous requirement, sheep that are grazed at mountain or fallow land needs to be grazed at road side land at least on rotational basis or supplemented with area specific mineral mixture or concentrate feed.

The data collected in this study revealed only two sheep breeds were commonly raised in the North Eastern Agro Climatic Zone of Tamil Nadu *viz.,* Madras red and Ramnad White. The serum calcium and phosphorus levels of Madras Red sheep were significantly (P≤ 0.01) higher than Ramnad White. However, the magnesium level was remained comparable. The serum iron and copper levels of Madras Red were significantly (P≤ 0.01) higher than Ramnad white whereas, serum zinc level was found comparable in both the breeds. The results of mineral concentration of serum based on breed have been presented in Tables 3 and 4. The present study revealed a significantly high level of Ca, P, Cu and Fe levels in Madras Red than Ramnad White sheep. Genetic variation in the metabolism of minerals has been demonstrated in a number of species. Reetz*et al.* (1975) reported a heritability of 0.45 for plasma Cu of pigs. Differences in the metabolism of Cu among breeds of sheep have been described (Wiener and Field, 1971; Woolliams*et al.,* 1982; Wiener and Woolliams, 1983).The breed variations were also reported by Perez *et al.*(2000) in blood and wool micro mineral profile between Suffolk and Rambouillet sheep.Ranjith and Pandey (2015) reported the mineral profile in blood and milk and their interrelationship in Deccanisheep. The plasma mineral profile of West African dwarf goats was found higher than the indigenous breed (Sowande*et al.,* 2007).

The serum macro and micro mineral profile of sheep aged less than 6 month and 6 month to 1 year did not vary significantly (P ≥ 0.05) Tables 5 and 6. However, the serum levels of macro and micro minerals were found higher than the critical levels. The present study showed no significant difference in mineral profile of sheep aged less than 6 month and 6 month to one year. Dar *et al.*(2014) reported a significant variation in mineral profile in different age group of sheep. The young one had higher levels of minerals when compare to lactating, dry and pregnant animals in

Kashmir valley. Young animals were found to absorb minerals more efficiently than older animals (Sowande*et al.,* 2008).

The sheep raised under organized farms had significantly (P≤ 0.01) higher serum phosphorous, magnesium and copper levels than the sheep raised under unorganized farms (Tables 7 and8). However, the serum calcium, zinc and iron levels did not vary significantly. In the present study the sheep raised in organized farms had significantly high P, Mg and copper than the sheep raised in unorganized farms. This could be due to the mineral supplements provided in organized farms. Sharma *et al.,*(2006)also recorded lesser prevalence of deficiency in organized farms compared to unorganized farms in Western Uttar Pradesh.

The study indicates that the serum mineral profile in sheep has been affected by various factors like age, breed, farm type and different types of grazing lands. Madras Red breed was found to have higher concentration serum mineralsthan Ramnad White.Phosphorous value alone varies with different grazing lands except forest and its associated grazing sites were having higher macro mineral concentration. Sheep reared in organized farms showed higher concentration of phosphorus, magnesium and copper than unorganized farms. Minerals are important element which are having vital role in metabolism andimmunity. Hence, specific mineral supplementation can reduce the deficiency in sheep in North Eastern Agro Climatic Zone of Tamil Nadu.

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GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND MEAT QUALITY OF PEARL AND LAVENDER VARIETIES OF GUINEA FOWL (*NUMIDA MELEAGRIS*) IN TROPICAL CLIMATE OF CHHATTISGARH

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Indian poultry sector is chicken dominated and dependent on intensive system of production involving high technology with high external inputs. This is not necessarily appropriate and sustainable to the socio-economy of a densely populated country. Therefore there is a need to develop and offer a broad spectrum of poultry alternatives to meet the different local requirements. In this context, alternate poultry species which is sustainable to the landless, marginal and small farmers to improve their socio-economic profile and nutritional status becomes very important. **A**lternate poultry species like guinea fowl farming is slowly coming up in a sustainable manner through intensive farming. The guinea fowl which is still considered as semi feral has numerous advantages over village chicken. Guinea fowls are being reared in an extensive way for its gamy meat, watchfulness and ground clearance. Being native to temperate South Africa, they appear to have an inherent adaptability to both heat and cold. The bird thrives well under both intensive and semi-intensive conditions, forages well, and requires little attention. It requires little attention and can be easily raised as chickens and turkeys. The guinea fowls have better ability to protect itself against predators and better resistance to common poultry parasites and diseases e.g. Newcastle disease and Fowl pox (Micro livestock, 1991).

Guinea fowls are gaining popularity as meat birds in many developing countries including India under backyard as well as intensive system of rearing after broilers and turkeys. The delicacy and high nutritional value of meat have shown interest to many researchers to study the performance of guinea fowls under different climatic conditions in different parts of the world under both traditional and intensive management conditions. The work conducted on the growth performance of guinea fowl in the tropical climatic

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condition of Chhattisgarh is very scant. Hence, the present investigation was carried out to study the growth performance of Pearl and Lavender varieties of guinea fowl along with their growth performance, carcass characteristics and meat quality in the tropical condition of Chhattisgarh state

The present experiment was conducted in College of Veterinary Science and A.H., Anjora, Durg (Chhattisgarh),India. The district Durg of Chhattisgarh plain is located between Latitude of 20°23' - 22°02' N and the longitude of 80°46' and 81°58 E .and at a height of 317.00 M. above mean sea level. It has a dry tropical weather with an average rain fall of 1071.16 mm annually.

For the present study, 1 day old keets of Pearl and Lavender guinea fowl were procured from Central Poultry Development Organization, Bhubaneswar (Orissa) and growth performance was studied on fifty keets of each variety. The guinea fowl keets were divided into two groups depending on variety i.e. Pearl and Lavender. Further, each group was divided into five replicates of ten keets. The birds of each variety were raised separately on deep litter system under standard managemental condition for 14 th weeks of age. The birds were fed commercial Crumbro broiler starter ration available in local market on *ad libitum* basis containing 10 % moisture, 20.4 % Crude protein, 3.9 % fat, 3.8 % Crude fiber, 8.8 % total ash, 1.8 % calcium and 0.7% phosphorus as determined by NIR Spectrometer. The body weights (g) of individual birds were observed at weekly interval to find out weekly body weight gain. Weekly feed consumption (g) by each replicates was recorded by subtracting the total amount of feed left from total amount of feed offered during same 7 days. The cumulative body weight (g), body weight gain (g), feed consumption (g) and feed conversion ratio (FCR) of both varieties were calculated, separately.

At the end of experimental period six birds from each group were randomly chosen and slaughtered following standard procedure. The selected birds were kept in separate coop without access to feed 16 hours prior to slaughter but provided drinking water. The birds were weighed prior to slaughter and dressed following the procedure described by Kotula, *et al.* (1960). They were bled through section of the jugular veins, scalded in warm water (about 60^{o}C) and plucked manually. The legs and head were cut at tibio-metatarsus and atlanto-occipital joint, respectively and dressed weight and giblet weight were recorded and yields were expressed as a proportion of live weight. *C*arcasses were separated into Breast with ribs, Leg, Back with neck and wings to study cut- up parts as per the procedure described by Khanna and Panda (1983) and weighed separately. For sensory evaluation of meat quality about 250 g of meat sample of each variety of guinea fowl was cut into small pieces and 1 per cent of salt was added in it. Then cooked in pressure cooker for 20 minutes in sim flame. The cooked meat was presented to a panel of semi trained judges under identical conditions. The score sheet developed by Peryan and Pilgrim (1957) was followed for sensory evaluation of meat quality. The meat samples were judged to evaluate Colour, Flavour, Juiciness, Tenderness, Texture and Acceptance.

The data of all above parameters were recorded and expressed as mean ± standard error. Parameters of both groups were compared using independent 't' test as per method given by Snedecor and Cochran (1994). The level of significance was reported at $P < 0.05$.

The results on cumulative growth performance of Pearl and Lavender variety of guinea fowl (Table- 1) revealed that at day one the mean body weight of Lavender guinea keets were significantly higher ($P<0.05$) than Pearl guinea keets. But no significant differences were observed in mean body weight and weight gain between Pearl and Lavender variety at the end of experimental trial. The mean body weights were higher at 14 weeks of age than observed by Fajemilehin (2010) in Pearl, Ash and Black (599.24 ±0.62, 554.8 ± 0. 81 and 572.54 ±0.82 g, respectively) varieties of Greybreasted helmeted guinea fowl of Nigeria. The body weight of both varieties were lower than the values reported by Porwal *et al.* (2002) for indigenous guinea fowl which might be due to genetically slower growth rate (Ayorinde and Ayeni,1983). The significant effects of genetic groups might be the reason for differences in body weight of Pearl and Lavender guinea fowls as observed by Folasade and Obinna (2009).Non- significant differences in body weight gain at 14 th weeks of age suggested that the climatic condition of Chhattisgarh state is suitable for both Pearl and Lavender varieties. The lower body weights of guinea fowls in present investigation might be due to the diet containing low protein than recommended for guinea fowl (24-25%) in early age of growth i.e. upto 4 weeks of age (Ensminger *et al.,* 1990). Ayorinde *et al*. (1988) also suggested low live weight to be characteristics of guinea fowl. The lower body weight and body structure of guinea fowl suited for rapid flight and fast running, which are evolutionary adoptions for survival in the wild (CAB International, 1987). The total feed consumption during 14 weeks of experimental period was significantly ($P<0.01$) lower in Lavender group than Pearl group. However, overall FCR in Lavender group was significantly ($P<0.05$) better than Pearl group. The values of FCR for both varieties were better than 6.37 ± 6.71 as observed by Seabo *et al*. (2011) in guinea fowls fed commercial grower diet from 6 to 12 weeks of age under intensive system. The difference in FCR values between present and previous results could be due to age, difference of diets, management regime as well as environmental factors. Nwagu and Alawa (1995) suggested that the wildly behavior, the characteristic timid but very active flighty and noisy temperament might be attributed to the lower feed conversion efficiency of guinea fowl.

The carcass characteristics of Pearl and Lavender varieties of guinea fowl has been presented in table 2. In the present experiment no significant ($P>0.05$) difference was observed in dressing percentage and other carcass cuts as giblet weight, breast weight, leg weight, back with neck weight and wing weight between both groups. The dressing percentage of both varieties in the present experiment were lower than report of Adeyemo and Oyejola, 2004 (87.4 %) and Mareko *et al.* 2006 (92.96- 94.40%). But higher than Ayorinde (1991) who reported dressing percentage of 65- 71 percent, Nobo *et al.*

(2012) who reported 75.82 ±. 2.99 percent in female and 74.10 ± 2.99 percent in male birds. Similar findings were observed by Dahouda *et al*. (2009). The variation in dressing percentages observed in different studies might be associated with the birds strain, diets, management system and carcass dressing methods. In the present investigation the breast weight, leg weight, back with neck weight and wing weight of both groups were higher than those observed by Dahouda *et al*. (2009). Non significant differences between both varieties justifies that there was no effect of variety on carcass characteristics.

The mean values for different proximate parameters in meat of Pearl and Lavender groups have been shown in table 3. No significant (P>0.05) differences were observed in values of moisture, crude protein, total ash and cholesterol content in meat between Lavender than Pearl Variety whereas, ether extract was found significantly (P<0.05) higher in meat of Pearl variety. It was revealed that nutritive value of meat of Pearl and Lavender varieties of guinea fowl were almost similar. The moister contents in meat of both varieties were found higher than report of Mareko *et al*. 2008, who observed 56.94 ±1.5 % moister for guinea fowls raised on concrete floor. The crude protein value was higher than reported by Saina (2005) in guinea fowls managed under intensive (75.4%) and semi-extensive (72.7%) system of management, respectively. The crude protein content in present investigation was in accordance with findings of Mareko *et al*., 2008 who observed 68.18 ± 4.05 to 86.68 ± 4.05 %. The differences in protein value in present and previous findings might be due to differences in climate and rearing system. The ether extract (%) in present study was higher than that reported by Khan *et al*. (2003) in white and coloured broiler lines**.** The percentage of ash in both groups differed non-significantly and was similar to guinea fowls raised in high environmental temperature as observed by Maria *et al*. (1998) and under a typical intensive poultry system as observed by Mohamed *et al.* (2012). In the present experiment, guinea fowls reared on concrete floor were devoid of pecking of feed on ground and which might be the cause lower total ash percent in meat of both varieties. The present study revealed that cholesterol (mg/ 100 g) in meat of Pearl and Lavender guinea fowl was 68.15 ± 3.16 and 71.39 ± 2.93, respectively differed non significantly. However, it was lower than meat of red coloured broiler (80.30 ± 2.83 mg/100 g) as observed by Almeida *et a*l. 2006 in their studies. Nutritional values are indicative of nutrient contents in a food item and it is necessary to monitor intake of certain foods and nutrients and it may vary according to species, age, feed and agro-climatic condition of the place of rearing of poultry. The values of sensory attributes of Pearl control and Lavender groups has been presented in table 4. Data revealed no significant difference between different sensory characters of both the groups. However, the meat of Pearl variety was judged superior than Lavender variety in all other sensory attributes viz., flavor, juiciness, tenderness and texture.

The present study concluded that the FCR of Lavender variety was significantly better than Pearl variety whereas the mean body weight and body weight gain were not significant during 14 weeks of growth trial.

Therefore, both varieties of guinea fowl can be reared in Chhattisgarh. The higher protein content in the guinea fowl than chicken meat and other meat sources indicated that guinea fowl might be the better alternative than other poultry species.

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Table 1. Growth performance of Pearl and Lavender varieties of guinea fowl

Particulars	Pearl	Lavender	t value
Initial body weight (g)	24.80±0.36	25.18±0.46	3.22*
Final body weight(g)	1120.78±10.70	1097.88±10.99	1.49 NS
Weight gain (g)	1095.98±10.64	1072.70±10.98	1.62 NS
Feed intake (g)	5789.00±55.79	5392.98±49.84	6.03**
FCR	5.16±0.26	4.94±0.24	3.74*

*Significant at P<0.05, ** Significant at P<0.01, NS - Non-significant

Table 2. Carcass characteristics of Pearl and Lavender guinea fowl at 14 weeks of age

Carcass Traits	Pearl	Lavender	t-value
Live Weight (g)	1375 ± 88.73	1302.5 ± 54.56	0.696NS
Dressing %	78.24 ± 1.41	77.06 ± 1.17	0.643NS
Giblet Weight (g)	69.18 ± 6.90	55.33 ± 3.85	1.750NS
Breast Weight (g)	272 ± 21.07	259.33 ± 17.49	0.463NS
Leg Weight (g)	242.67 ± 16.48	239.5 ± 7.00	0.177 NS
Back with Neck Weight (g)	315.17 ± 22.25	269.67 ± 11.51	1.816 NS
Wing Weight (g)	140.5 ± 13.88	140.67 ± 4.70	0.01 NS

NS - Non-significant

Table 3. Nutrient composition of Pearl and Lavender guinea fowl meat

Nutritive traits	Pearl	Lavender	t- value
Moisture (%)	68.16 ± 0.79	69.44 ± 0.67	1.479^{NS}
Crude Protein (%)	75.99 ± 1.36	78.37 ± 1.79	1.403^{NS}
Ether Extract (%)	14.94 ± 1.49	11.31 ± 0.67	3.177 *
Total Ash (%)	4.11 ± 0.24	4.39 ± 0.23	0.912 ^{NS}
Cholesterol (mg/ 100 g meat)	68. 15 ± 3.10	71.39 ± 2.93	0.760 ^{NS}

*Significant at P<0.05 NS – Non Significant.

Table 4. Sensory attributes of Pearl and Lavender guinea fowl

Attributes	Pearl Control	Lavender	t value
Color	7.20 ± 0.18	7.20 ± 0.97	0.000
Flavour	7.20 ± 0.31	6.40 ± 0.68	0.676^{NS}
Juiciness	7.40 ± 0.13	7.20 ± 0.37	0.365^{NS}
Tenderness	7.80 ± 0.12	7.60 ± 0.40	0.365^{NS}
Texture	7.80 ± 0.16	6.60 ± 0.25	2.191^{NS}
Acceptance	8.0 0± 0.20	7.20 ± 0.58	0.930^{NS}

NS- Non Significant

NATIVE CHICKEN PRODUCTION OF TRIBAL COMMUNITY OF NICOBAR ISLANDS

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The Andaman & Nicobar Islands are a group of 572 big and small Islands and Islets in the South Eastern part of Bay of Bengal. The total geographical area is about 8249 sq. km, out of which about 86 % (7094 kms ^{2}) is covered by forest. The total area of Andaman covers 6340 sq. kms and Nicobar group covers 1841 sq. kms. Only about 53,734 ha of land are available for habitation and agriculture. The Nicobar district of A&N Islands comprises of 12 inhabited islands scattered in Bay of Bengal between 6°-10° N latitude and 92°- 94° E longitude separated from Andaman group of Islands by 10° Channel. These islands are having 63 percent ST population and the predominant feature of the demography is 'Nicobarese'. Livestock farming is the backbone to ensure nutritional security for tribal farming community of Nicobar group of Islands. Rural poultry among livestock farming is considered to be an important primary source of meeting out egg requirement and constitutes meager portion of meat consumption of tribal population of these Islands.

Major constraints associated with commercial poultry production at the level of tribal farming community are adaptability of farming system of commercial poultry and practical difficulties with establishment of commercial farms in Nicobar Islands due to transportation problems. Hence, household native chicken production in Nicobar group of Islands is the sole option for tribal farming community. Desi including Nicobari fowls form the native chicken production at backyard level contribute significantly in nutritional security of Nicobari tribes. The traditional indigenous poultry production, being integral component in balancing nutrients could be improved by studying the various bottlenecks present in the rural poultry production system of tribal farmers. Very little is known about these traditional rural poultry production system of tribal farming community. Few reports on tribal poultry production system describe them as having a very low productivity, high mortality rates and suffering from inefficient management. The study investigated the present status of indigenous native chicken production at Nicobari tribal farming community with an aim of formulating strategies to improve its production level by addressing the problems associated with the rural poultry production in tribal farming community of Nicobar Islands.

The study was conducted using the census method of complete enumeration of tribal houses in the 15 tribal tuhets located in Nicobar Islands. A pre tested well structured interview schedule was developed. The interview schedule was prepared in English since the letters of Nicobari language are similar to English and moreover, the Nicobari tribals understand well the

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English language. Data were collected on production system of rural poultry including feeding, housing and health management by Nicobari tribal farming community. The respondents were personally contacted and rapport was established to get unbiased information.

The result of the study indicated that there was only one kind of native chicken production system in the Nicobar Islands. More than eighty percent of Tribes were having poultry under the free-range low input and backyard system of rearing. It was observed that almost every family in Nicobar Islands is habituated in backyard poultry keeping and the flock size ranged from 6 to 16 chickens. Tribal family poultry comprised of mainly desi birds and Nicobari fowls. Although no definite evidence is available about the origin of this local breeds, ethnic tribal groups seem to have played a significant role for maintaining the uniqueness of the breeds and have been nurtured by Nicobari tribes for years without any introgression from outside. For ethnic Nicobari tribal groups and communities, native Nicobari fowls are of special interest because of their socio-religious use. It was recorded that the indigenous native Nicobari fowls are held in high esteem by the tribal farming community even after establishment of industrial poultry production in these Islands since native Nicobari fowl exhibit superior adaptability in their habitat and possess the ability to survive, produce and reproduce on low plane of nutrition and sub-optimal management and they possess comparatively higher immunity. Further, cock fighting is a popular sport for the Nicobari tribes and the desi birds are superior to exotic breeds in fighting. As per the 19th census (DAHVS, 2012), the total indigenous poultry population in A&N Islands has been estimated to 10,58,400 that is 98% of the total 10,80,000 poultry population of these Islands. The study indicated that the native chicken is the only source to supply 100% of the total chicken meat requirement of tribal farming community. The present study is also concurrent with the report that 80% of poultry meat come from local scavenging chicken (Paul and Islam, 2001 and Paul *et al.*, 2003). Moreover, these native Nicobari chickens are less susceptible to diseases as compared to broiler strains (Sil *et al.,* 2002 and Sunder *et al.,* 2004).

From the data obtained we observed, tribes provide the hen with bamboo basket or waste tin tubs only for laying eggs and keeping the chicks during night time. Rest of the time these birds scavenge and in night the birds rest on the trees. Tribal farmers feed poultry with rice or coconut due to abundant availability of coconuts in Nicobar Islands. These native birds get maximum nutrients by scavenging. The whole tribal farmers depend on scratch feeding for rural poultry due to non availability of commercial feed in the Nicobar Islands and unawareness on balanced feeding. It was also observed, the birds are not being vaccinated; however the percent survivability was 53.06%, which is fairly higher than the earlier reports (Panda and Nanda, 2000).

Information on egg production status in the present study revealed that the average number of eggs laid by a bird per annum is less than 60 eggs; this is much higher to the production levels of desi birds mentioned by Mandal *et al.* (2002) and meets the production levels studies by Sharma *et al.* (2001). Cockerels and Pullets

weighed about 800 to 900 g at the age of sexual maturity of 6 months old that might have attributed to low egg production since the adult body weight plays crucial role to precise more number of eggs in a biological productive life cycle of hen. This lower adult body weight again in-turn might be due to improper feeding and health management. Based on this study we could assume that each family on an average receive a total of 300 eggs from an average flock of five birds; but this is too low to meet the ICAR recommendations of 180 eggs per person per annum. Based on the data collected it is revealed that knowledge on crucial role of eggs and chicken meat in their daily nutrition and awareness on egg as a balanced and nutritious food was negligible among 95% of tribal farmers and suggest for creating awareness on nutritive value of eggs from the point of view on close association between awareness on importance of native chicken meat and egg consumption and its production enhancement.

Ninety eight percent of Nicobari tribes were not well aware of intensive chicken production and management. All the tribal homesteads consumed 80% of the eggs laid while 20% were set for hatching under broody hens as marketing of desi eggs is not practised by tribal farming community. Main interest of the tribal farmers having native indigenous poultry is not in production of eggs as source of returns. The major quantities of native and desi chickens such as cock (Meat Birds) after growing up to moderate size are consumed among tribes. Further, it is estimated that apart from exotic breed (broiler type), desi chicken production adds up to 1 ton of meat per year to meet out the consumers preference for desi bird. Indigenous birds are very popular poultry meat in these islands. Many consumers prefer desi chicken more than exotic type. Sensory evaluation study revealed that the native Nicobari fowl are harder than the exotic breeds of broiler and the taste, flavour and juiciness are almost similar to the exotic cockerel stocks. Swanson *et al.* (1984) has also advocated the suitability of desi chicks from exotic strains for preparation of chicken delicacies due to its desirable flavour, less abdominal fat and juiciness. Rural poultry at present accounts significantly in daily nutrition of tribal people. Most importantly it was observed that the rural poultry produce were of organic in nature among tribal farmers while the demand for organic egg and meat is increasing over the years in the industrially developed countries even though they cost more.

Base on the baseline survey and study, the following major constraints for the development of native chickens among tribal farming community should be given due concern:

1. No shelters for rural poultry and hence birds are vulnerable to predators and the extreme weather conditions.
2. Slow body weight gain, late sexual maturity and low egg production due to failure in supplemental feeding.
3. Risks of high mortality to Newcastle disease due to absence of vaccination
4. Lack of information to upgrade their knowledge on native chicken production

5. Lack of incubation technique for mass production of chicks
6. Lack of brooding technical know-how
7. Lack of medication and vaccination health programme

The native chicken production is an established component and has a crucial role to sustain the nutritional security of Nicobari tribal farming community. For this reason, the necessity for its development has always been recognised but insufficiently pursued. Based on the study on impact of interventions made at rural poultry farming practices, the following strategies are recommended for management of native chickens under improved conditions:

- Elevated housing : Floor made up of wooden planks and one feet raised from the ground level
- Feeding management using locally made feeder and waterer from wooden material, bamboo and used plastic cans, bottles and waste plates.
- Vaccination**:** Regularly can be done by themselves as a group to prevent the mortality.
- Low cost supplemental feeding using rice, wheat, coconut, fish, fish bone, egg shell and waste vegetables
- Brooding : Confining chicks in an area and providing warmth of broody hen with bulb protects chicks from predators
- Artificial Hatching: Hatching with mini incubator help to increase the number of table eggs and chicks from hen

It is concluded that the desi and native indigenous chicken dominates poultry production at the Nicobar group of Islands. The growing number of affluent tribal population in Nicobar Islands most likely will demand a richer desi poultry produce. Further, small holding backyard poultry production utilizing native poultry breeds therefore is expected to improve its production; if not well planned the genetic resources of native indigenous Nicobari fowl poultry shall be lost, as it has already happened in most of the Nicobar group of Islands. Conservation of native poultry germplasm should be strengthened through interventions in rural poultry production system among tribal farming community to sustain the socio-religious use of native poultry breeds and their superior adaptability in their habitat. Production improvement of native poultry breeds and their conservation for future use should be on community basis since the Nicobari tribes are living as community.

Further, production of desi meat and eggs can be enhanced by improving the present management system of rural poultry production at Nicobari tribes. Improved rural poultry farming practices including development of diets based on locally available feedstuffs, design of cheap housing units and establishment of regular vaccination program will be appropriate systems and approach to native chicken development among Nicoari tribal farming community.

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Illustrations, referred to as "figures" (Fig. 1etc.) should be on separate sheets and submitted larger than the size desired for reproduction. Information in tables must not be duplicated in figures and vice versa. Legends, should be provided for each illustration. Line drawings should be either in black ink on smooth white paper or thin board or a good quality laser printout. Photographs and photomicrographs should be printed on glossy paper with good contrast. Magnification for photomicrographs should be indicated. All illustrations should bear on the reverse side, the first author's name and the figure number, the 'top' of the figure should be indicated. While sending the manuscripts in email, and the figures should be separately sent in JPEG format but for gel pictures it should be in TIFF format with good resolution.

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