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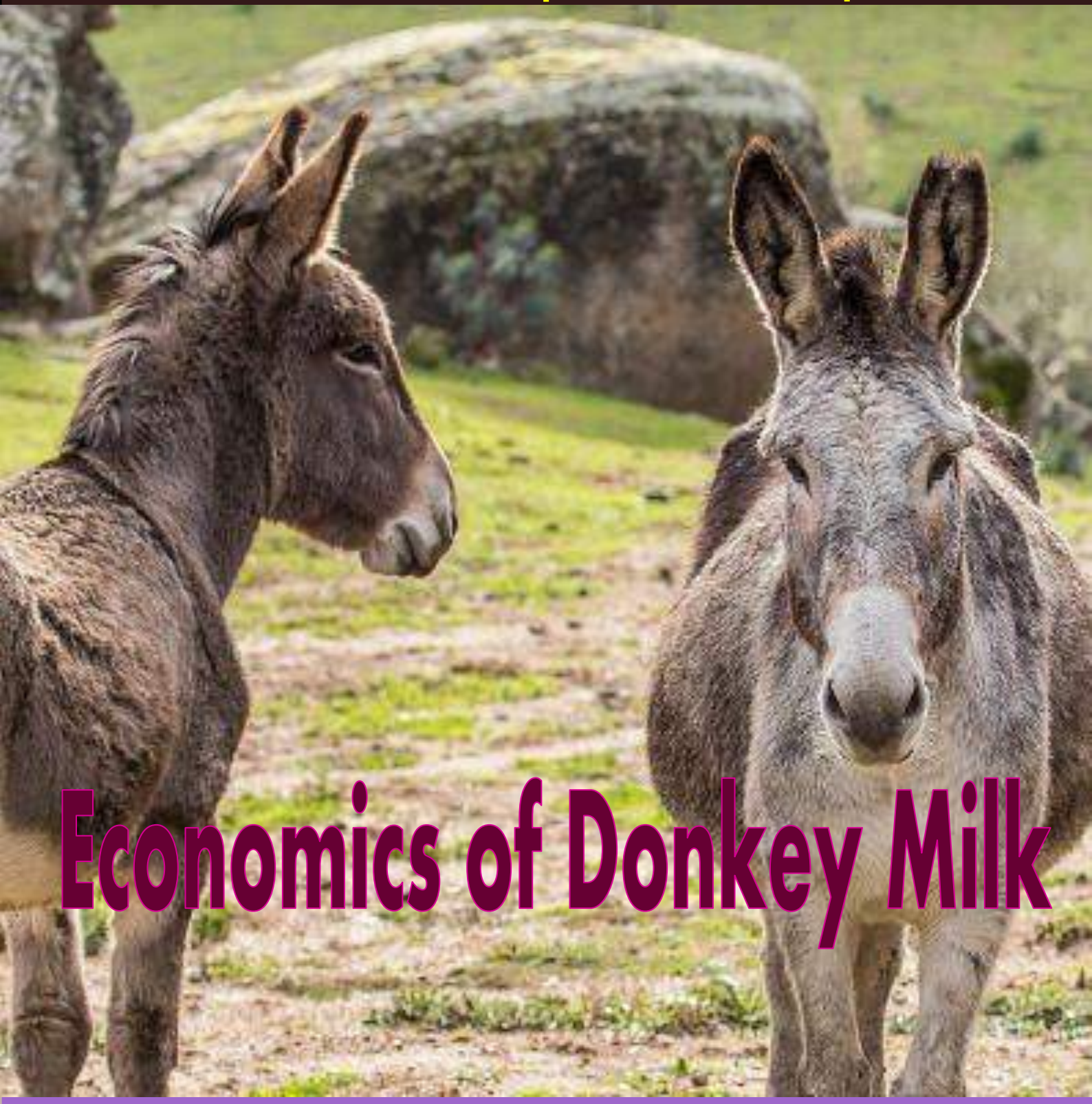

Economics of Donkey Milk

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Metagenomics and their Applications in Veterinary Science

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ABSTRACT

Metagenomics is analogous approach to the genomics with the difference that it does not deal with the single genome from a clone or microbe cultured or characterized in laboratory. It deals with the entire microbial community present in an environmental sample hence it is also called as community genome. The challenge of microbiologists for decades is how to access the microorganisms that cannot be cultured in the laboratory. Almost all of our knowledge of microbial life is based on organisms raised in pure culture but metagenomics provides an additional set of tools to study uncultured species. This new field offers an approach to study microbial communities as entire units, without cultivating individual members. Metagenomics entails extraction of DNA from a community and these genomes are usually fragmented and cloned into an organism that can be cultured to create 'metagenomic libraries', and these libraries are then subjected to analysis based on DNA sequence or on functions conferred on the surrogate host by the metagenomic DNA. The metagenomic information allows in-depth understanding of the ecological role, metabolism, and evolutionary history of microbes in a ecosystem. One of the most outstanding discoveries of metagenomics is the first description of proteorhodopsin in marine bacteria. Metagenomics will help in continuous detection and description of novel viruses in the future that leads to a better understanding of both emerging and existing diseases as well as to the increased knowledge of the role of different viruses in humans and animals.

Keywords: Metagenomics; veterinary science; rRNA sequencing

INTRODUCTION

Metagenomics has become one of the indispensable tools in microbial ecology for the last few decades especially in massive data production and substantial cost reduction in next-generation sequencing have led to the rapid growth of metagenomic research both quantitatively and qualitatively (Teeling and Glockner, 2012). Metagenomics is the study of the collective genomes of the members of a microbial community. It involves cloning and analyzing the genomes without culturing the organisms in the community, thereby offering the opportunity to describe the planet's diverse microbial inhabitants, many of which cannot yet be

cultured. The term "metagenomics" was first used by Jo Handelsman in 1998. One of the most outstanding discoveries of metagenomics is the first description of proteorhodopsin in marine bacteria (Beja *et al.,* 2000). Metagenomics bypasses the need for isolation or cultivation of microorganisms. Metagenomic approaches based on direct isolation of nucleic acids from environmental samples have proven to be powerful tools for comparing and for exploring the ecology (Biddle, 2008) and metabolic profiling of complex environmental microbial communities, as well as for identifying novel biomolecules by use of libraries constructed from isolated nucleic acids (Ferrer *et al.,* 2009).Metagenomics is often based on a general strategy of producing large amount of environmental DNA to achieve two goals:
(1) Discovery of new gene sequences coding for enzymes and drugs
(2) Random sampling and archiving of the genomes from a small subset of organisms present in an environment for subsequent in silico analysis.

Specific aims of Metagenomics

1. Examining phylogenetic diversity using 16S rRNA sequence analysis
2. Diversity patterns of microorganisms can be used for monitoring and predicting environmental conditions and change.
3. Examining genes/operons for desirable enzyme candidates (e.g., cellulases, chitinases, lipases, antibiotics, other natural products) these may be exploited for industrial or medical applications.
4. Examining secretory, regulatory, and signal transduction mechanisms associated with samples or genes of interest.
5. Examining bacteriophage or plasmid sequences. They are potentially influence diversity and structure of microbial communities.
6. Examining potential lateral gene transfer events. Knowledge of genome plasticity may give an idea of selective pressures for gene capture and evolution within a habitat.
7. Examining metabolic pathways.
8. Directed approach towards designing culture media for the growth of previously-uncultured microbes.
9. Examining genes that predominate in a given environment compared to others.
10. Finally, metagenomic data and metadata can be leveraged towards designing low- and high-throughput experiments focused on defining the roles of genes and microorganisms in the establishment of a dynamic microbial community.

rRNA Analysis

One of the basic approach for identification of microbes in a complex community by exploiting universal and conserved targets such as rRNA genes. By amplifying selected target regions within 16S rRNA genes, microbes especially bacteria and Archaea, can be identified by the effective combination of conserved primer binding sites and intervening variable sequences that facilitate genus and species identification. Usually 5S and 16S rRNA

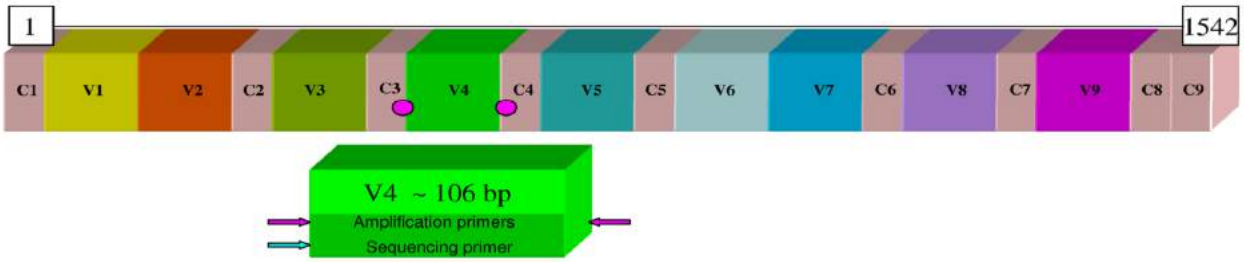

Figure 1: Conserved and hypervariable regions in the 16S rRNA gene

sequences are used as "Evolutionary Chronometer" due to their very slow mutation rate. The 16S rRNA gene in bacteria is comprised of interspersed conserved and variable sequences including a total of 9 hypervariable regions (V1–V9) as shown in fig 1. These hypervariable regions range in size from approximately 50–100 bases in length, and sequences differ with respect to variation and corresponding utility for universal microbial identification. The interspersed conserved regions (C1–C9) are shown in gray, and the hypervariable regions (V1–V9) are depicted in different colors. An example of primer selection for DNA amplification and sequencing-based microbial identification is provided in the figure (V4 sub region with pink circles and arrows representing primer binding sites).

Approaches in the metagenomics

Sample preparation

One of the most important, difficult tasks of viral metagenomics is to prepare the sample in such a way that the contaminating host nucleic acids are depleted while the viral nucleic acids are preserved (Delwart, 2007). For viral genome sample preparations, the viral nucleic acid only constitutes a small proportion of all nucleic acids. Most viruses are smaller than bacteria and filtration using a 0.22-mm filter is a common procedure for removing bacteria when searching for viruses (Thurber *et al.,* 2009). However, a number of viruses have been discovered that are as large as bacteria (Van Etten *et al.,* 2010) and these would be lost using this procedure. The viral particles can also be separated from other components by density centrifugation using sucrose or cesium chloride gradients (Thurber et al. 2009). By treating the samples with nucleases prior to nucleic acid extraction, the host nucleic acid is degraded while the virus nucleic acid is preserved (Allander *et al.,* 2001).

Approximately 90% of all RNA in a cell is estimated to be ribosomal RNA (rRNA). The combination of the abundance of rRNA and its stability through its association with ribosomes makes the detection of RNA viruses difficult. As RNases are not very effective in the degradation of rRNA, other strategies have been employed. One strategy is based on subtractive hybridization where biotin-labeled probes that target ribosomal sequences are used to extract rRNA from the samples (He *et al.,* 2010). Another rRNA depletion strategy is to use random primers in the cDNA synthesis that do not target rRNA sequences (Endoh *et al.,* 2005). rRNA-

blocking oligos were used to further decrease rRNA transcription.

Metagenomic library construction and analysis

There are two distinct strategies used in metagenomics, according to the primary goal:
(1) Large-insert libraries (cosmid, fosmid, or bacterial artificial chromosomes) are constructed for archiving and sequence homology screening purposes: to capture the largest amount of the available genetic resources available in the sample and archive it for further studies/interrogation.
(2) Small-insert expression libraries, especially those made in lambda phage vectors, are constructed for activity screening. The small size of the cloned fragments will be under the influence of the extremely strong vector expression signals, and thus have a good chance of being expressed and detected by activity screens.

Sequence-based metagenomic analysis

Sequence-based metagenomics is used to collect genomic information from microbes without culturing them. In contrast to functional screening, this approach relies on sequence analysis to provide the basis for predictions about function. Massive datasets are now catalogued in the 'Environmental Genomic Sequence' database, and each sequencing project is more informative than the last because of the accumulated data from diverse environments. Some studies use a gene of interest or 'anchor' to identify metagenomic clones of interest. A metagenomic library is constructed and screened using PCR to amplify the anchor. Anchors are often a ribosomal RNA gene, but can also be a metabolic gene (e.g., a polyketide synthase). The clones that contain the anchor are then sequenced or further analyzed to provide information about the genomic context of the anchor that facilitate the quick focus on a clone of interest.

Function-based metagenomic analysis

Functional metagenomics involves identification of clones that express activities conferred by the metagenomic DNA. Sequence-based metagenomics has revealing physiological and ecological capacity for very low culturable microbes. Function-based metagenomics, unlike sequence-driven approaches, does not require that genes have homology to genes of known function, and it offers the opportunity to add functional information to the nucleic acid and protein databases. A powerful approach to metagenomic analysis is to identify clones that express a function. For expression of particular gene function requires faithful transcription and translation of that gene or genes of interest and secretion of the gene product. Functional analysis has identified novel antibiotics (Courtois *et al.,* 2003), antibiotic resistance genes (Diaz-Torres *et al.*, 2003) Na(Li)/H transporters and degradative enzymes (Healy *et al.,* 1995). Metagenomics has the potential to identify entirely new classes of genes for new or known functions. Because it does not require that the genes of interest to be recognizable by sequence analysis. The significant limitation is that many genes will not be

expressed in any particular host bacterium selected for cloning. The frequency of expression of metagenomic clone activity is low. Henne *et al.* (2000) screened the lipolytic activity of clones derived from soil in that they documented only 1 in 730,000 clones expressed the lipolytic activity.

Viral metagenomics

New diseases continue to emerge in both human and animal populations, and the importance of animals, as reservoirs for viruses that can cause zoonoses are evident. Thus, an increased knowledge of the viral flora in animals, both in healthy and diseased individuals and important for animal and human health. Only a small portion of all existing viruses has been characterized. Furthermore, new emerging infectious diseases (EIDs) continue to emerge in the human and animal populations. The increased knowledge and discovery of viruses circulating in domestic animals and in wildlife can help to control diseases. Therefore, to be prepared for new diseases in humans and animals, it is important to have tools that can rapidly identify agents behind new disease outbreaks. The investigation of the viral flora, as well as new viral discoveries, has been hindered by the fact that many viruses are difficult to grow in cell culture, and a number of viruses, even if grown in culture, do not yield any cytopathic effect. In addition, there is no common viral gene that can be targeted with PCR assays, and there is high genetic diversity between viruses within the same family. These reasons make it difficult to develop pan-viral PCR assays for the detection of all viruses within a given group.

The viral metagenomics does not require prior knowledge of the genetic viral composition in the samples. This procedure aims to provide the genetic composition of the complete viral populations of a sample in an unbiased and culture-independent manner (Delwart, 2007). This approach has been successfully used to investigate viral populations in different environments such as seawater (Breitbart *et al.,* 2002), gastrointestinal tracts (Victoria *et al.,* 2009), and respiratory samples (Allander *et al.,* 2007) and have demonstrated that there is a high diversity among viruses, and that there is a vast number of viruses that are yet to be discovered. The table 1 shows the newly discovered viruses using the metogenomics approaches.

Conclusions and future aspects

The use of viral metagenomics in veterinary science has substantially increased during the past decade through the advancement of new molecular tools and through an increased understanding of the importance of animal viruses to both animal and human welfare this technique has proven to be a powerful tool for the investigation of diseases of unknown etiology. These studies will increase our understanding of common and often poorly understood disorders, such as encephalitis, different gastrointestinal disorders, and chronic diseases as well as acute emerging diseases. It can also show the complexity of many diseases where the clinical picture and disease emergence is dependent on the presence of a combination of different

viruses that interact with each other as well as with the host. Wildlife often act as a reservoir for viruses in nature, and as the interface between wildlife, humans and domestic animals increases due to encroachment, these viruses have the potential to spill-over and cause diseases and/or death in the new hosts (Daszak *et al.,* 2000). Approximately, 72% of all emerging zoonotic diseases are estimated to have a wildlife reservoir, including SARS and Hantavirus pulmonary disease (Jones *et al.,* 2008). Viral metagenomic studies have demonstrated the abundance and divergence of viruses present in bats, and these studies have identified bats as important viral carriers of known and currently unknown viruses. In addition, many animal and human diseases are dependent on arthropod vectors for their transmission, and approximately 29% of EID events have been vector related in the past decade (Jones *et al.,* 2008). In animals, this has been observed with the emergence of bluetongue and African swine fever. With a better understanding of the viruses that are maintained and transmitted through wildlife and arthropod vectors, we can be better prepared for new EIDs and set up strategies to better prevent and control current and novel diseases. Viral metagenomics and the tools for the analysis of the data produced will continue to be developed and refined, which will enable the continued detection and description of novel viruses in the future. This will lead to a better understanding of both emerging and existing diseases as well as to the increased knowledge of the roles of different viruses in humans and animals.

Table 1: Newly discovered viruses using the metogenomics approaches.

Disease	Sample type	Virus identified	Method
PMC	Sera	Bungowannah virus	SISPA, CLONING & sanger sequecing
Proventricular dilatation disease in parrots	fibropapillomas	Sea turtile tornovirus I	Phi 29 and shotgun sequencing
Shaking mink syndrome	Brain	Astrovirus	rPCR, 454 sequecig
Post weaning multi systemic wasting syndrome	Lymph nodes	PCV-2, torque teno virus, porcine bocavirus	Phi 29 and shotgun sequencing
Viral flora- 1. Honey bee	bees	Chronic bee paralysis virus, sacbrood virus, Acute bee paralysis virus	rPCR, 454 sequencing
2. Temporal analysis of honey bee	bees	Lake sinai virus	rPCR, illumina and microarray
Viral flora-bats	1. Fecal	Parvovirus, Coronovirus	rPCR, 454 sequencing
	2. Fecal and oral	Coronovirus, flavivirus, herpes virus	SISPA, 454 sequencing
Viral flora-monkeys	1.Feacal 2. Sera	1.Chimpanzee stool-associated circular virus 2.Simian hemorrhagic fever virus	1.rPCR cloning and sangar sequencing 2.rPCR, 454 sequencing

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Phytase and its importance in Poultry Nutrition

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Feed ingredients of plant origin contain a number of components that cannot be digested by monogastric species. Examples of such antinutritive components include phytic acid (PA). Phytic acid is present in grains and seeds as a mixed salt, phytate which refers to the phytic acid molecule chelated to minerals, proteins, starch, lipids, or both starch and lipids. Phytate is the major form of phosphorus found in cereal grains, beans and oilseed meals fed to poultry birds (Ravindran *et al.,* 1995). Approximately 61–70% phosphorus found in poultry diet ingredients is in the form of phytate phosphorus. The monogastric animals like poultry birds are unable to utilize this phytate phosphorus, as they lack endogenous phytase, which necessitates in the addition of inorganic feed containing phosphates to poultry diets in order to meet the phosphorus requirements of poultry (Yu *et al.*, 2004). The dietary addition of feed phosphatase not only increases the feed and production cost, but may also lead to an increase of soluble P in the litter resulting in the potential for water contamination from excess P in soil. It results in relatively large amounts of phosphorus in the manure that contribute to environmental pollution (Guo *et al.,* 2009). Exogenous phytase of microbial origin can be used as an alternative that can help to reduce phosphorus excretion in poultry (Yu *et al.,* 2004). The beneficial effect of exogenous phytases in poultry ration has been supposed to be due to the direct hydrolytic effects on phytate and the subsequent improvement in the availability of minerals, amino acids and energy (Selle and Ravindran, 2007). It has also been suggested that phytase in poultry diets improves gut health as indicated by reduced secretions from the gastrointestinal tract (GIT) which consequently improves the efficiency of utilization of energy (Oduguwa *et al.,* 2007; Pirgozliev *et al.,* 2008).

PHYTASE

Phytase are phosphatases capable of hydrolyzing one or more phosphate groups and yielding lower myo-inositol phosphates, inositol and inorganic P. Depending on the position of the phosphate group on the myo-inositol ring which they hydrolyse first, they belong to one of two sub-classes: 3-phytase and 6-

phytase. The 3-phytase initiates phytate hydrolysis by removing phosphate residue from position three of the myo-inositol ring, and 6-phytase from position six or four of the phytic acid molecule (Żyła *et al.,* 2004).

Several distinct microbial phytase products are now commercially available. The three commonly used phytase feed enzymes are derived from *A. niger*, which is a 3-phytase and *Peniophora lycii* and *Escherichia coli*, which are 6-phytases. Phytase feed enzymes may be included in poultry rations as granulates or as liquids, via post-pelleting application systems, to avoid thermostability problems at high pelleting temperatures (>80°C). Phytate hydrolysis mainly takes place in the fore-stomach (crop, proventriculus, gizzard) where the pH is more conducive to phytase activity. The crop is probably the primary site of phytate degradation by exogenous phytase (Kerr *et al.,* 2000). However, there is evidence that *E. Coli* derived phytase is more active in the small intestine than phytase derived from *P. lycii* (Onyango *et al.,* 2005), which may be attributable to the greater resistance of *E. Coli* derived phytase to endogenous, proteolytic enzymes (Igbasan *et al.,* 2000). The defined measurement unit of phytase activity depends on assay conditions including concentration of substrate (sodium phytate) used, assay temperature and pH. Phytase activity is defined as *fytase* units (FTU), where one FTU is the amount of enzyme that liberates one mole inorganic orthophosphate/min from 0.0051 mol L^{-1} of sodium phytate at pH 5.5 and a temperature of 37°C (Engelen *et al*., 1994).

EFFECT OF PHYTASE ON GROWTH PERFORMANCE

Juanpere *et al.* (2004) reported that phytase addition (500 FTU kg^{-1}) to diets containing 2.7 g kg^{-1} total P increased weight gain and feed efficiency of broiler chicks from 7 to 21 days of age. Subsequently, Pirgozliev *et al.* (2010) reported that phytase addition (250 FTU kg^{-1}) to diets increased weight gain (32.2 g/bird/day versus 29.6 g/bird/day) and feed efficiency (1.47 versus 1.52) of broilers. Ptak *et al.* (2013) reported that the effect of addition of 6-phytase products, and particularly the phytase II on body weight gains, was more pronounced than that obtained by 3-phytase (phytase I) supplementation. This effect was not observed in relation to the FCR.

EFFECT OF PHYTASE ON FEED CONVERSION RATIO

Jamal *et al*.(2009) reported that Phytase supplementation at different levels improved ($P<0.05$) feed con**v**ersion ratio of broilers at weight of marketing compared to with low P diets. Similarly Mondal *et al.* (2007) who reported that phytase supplementation to broiler diets caused numerical improvement in feed efficiency of broil**e**rs fed a P-deficient diets fed without phytase.

EFFECT ON BONE GROWTH AND MINERAL RETENTION

Phytase supplementation to low available phosphorus diets significantly affected tibia weight, tibia ash and calcium content in tibia ash of broiler chicks. Brenes *et al.* (2003) reported that phytase supplementation increased tibia ash (up to 4%), and Ca (up to 2%), P (up to 1%) *and* Zn (up to 4%) contents in tibia ash,

while Mg concentration was not affected by phytase supplementation. Ptak *et al.* (2013) reported that phytases improved the tibial bone breaking force. Phytase supplementation to diets in**c**reased the content of Ca and P in the tibia compared to diets containing low P. This is a good indication of increased availability of P from phytate min**e**ral complex by the action of phytase (Mondal, *et al.,* 2007). Jamal *et al*.(2009) reported that bone Zn level was increased (P<0.05) when phytase was added in the diet.

EFFECTS ON SERUM BIOCHEMICAL PARAMETERS

Wang *et al.*(2013) reported that diets supplemented with phytase increased (P < 0.05) the serum phosphorus, which was similar to results by Dendow *et al*. (1995). They also reported non significant difference (P >0.05) in serum alkaline phosphatase, albumin, total protein, urea nitrogen and growth hormone of broilers receiving phytase than control birds

EFFECT OF PHYTASE ON LAYER DIET

Generally Ca and P levels in diet seem to be the important factors affecting egg production, egg mass and egg weight as well as eggshell quality in laying hens (Wu *et al.,* 2006). Lim *et al*. (2003) concluded that phytase supplementation improved egg production and reduced percentages of broken and soft eggs and P excretion. They also found significant increased in egg phosphorus content with phytase supplementation in layers fed low Ca (30 g kg^{-1}) compared to those fed high Ca (40 g Ca kg^{-1}) diet. The reduced performance in layers fed higher Ca levels in phytase supplemented groups could be due to the possible inhibitory effect of higher Ca on phytin digestibility and activity of phytase. Supplemental phytase is reported to increase the release of Ca from phytate molecule (Um and Paik, 1999), which further increased the available Ca concentration in gut of layers fed adequate or higher Ca concentration. Punna and Roland (1999) reported significant improvement in shell quality in layers fed low phosphorus diets supplemented with phytase. Hughes *et al*.(2008), who observed improved eggshell quality in hens fed diets supplemented with phytase.

CONCLUSION

It may be concluded that supplementation of phytase in poultry ration increases body weight gain, feed conversion efficiency, egg shell quality and minerals concentration in birds

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Allele Minning: An Approach for Development of Sustainable Agriculture

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ABSTRACT

Allele mining study aimed at identifying allelic variation of relevant traits within germplasm collections.It is important approach to use genomic information for the identification and isolation of novel and superior alleles of agronomically important genes from crop gene pools to suitably deploy for the development of improved cultivars. Allele mining is a promising technique to dissect naturally occurring allelic difference at target genes controlling agronomic traits which has potential applications in crop improvement.

1. INTRODUCTION

Superior and high yielding varieties of agriculture crops were develop through plant breeding technique. High yielding varieties of agriculture crops was made possible by accumulation of beneficial alleles from huge plant genetic resources existing worldwide. Superior alleles were not utilized as these were left behind during evolution and domestication. Superior genetic variation was existing in wild and land races of crop plants that variation could be exploited for development of high yielding varieties. Introgressions of novel alleles from wild relatives of crop plants into cultivated varieties. Available germplasm resources need to be relooked for novel alleles to further enhance the genetic enhancement of crop varieties for different agronomic traits. With rapid accumulation of sequence and expression data in various genomic databases, accelerated discovery and annotation of new genes can be expected which would enable the development of allele-specific markers. Based on gene and genome sequences, polymerase chain reaction (PCR) strategies are devised to isolate useful alleles of genes from a wide range of species. Based on gene and genome sequences, polymerase chain reaction (PCR) strategies are devised to isolate useful alleles of genes from a wide range of species. This capability enables direct access to key alleles conferring resistance to biotic and abiotic stresses, greater nutrient use efficiency, enhanced yield and improved quality. Using novel genomic tools, similar alleles responsible for a given trait and their variants in other genotypes can be identified. This is often referred to as 'dissection of naturally occurring variation at candidate

genes/loci' or simply 'allele mining'. Identification of allelic variants from germplasm collections not only provides new germplasm for delivering novel alleles to targeted trait improvement but also categorizes the germplasm entries for their conservation.

Mutation is considered as an evolutionary driving force which underlies existing allelic diversity in any crop species. For creation of new alleles or causing variations in the existing allele and allelic combinations, mutations in the genic regions of the genome either as single nucleotide polymorphism (SNP) or as insertion and deletion (InDel) are important. The mutations in coding regions and/or regulatory regions may have tremendous effect on the phenotype by altering the encoded protein structure and/or function while those that occur in noncoding regions of a gene could often be silent without any effect on the phenotype.

2. ALLELE MINING

Initial research of allele mining have focused only on the identification of SNPs/InDels at coding sequences or exons of the gene, since these variations were expected to affect the encoded protein structure and/or function. Ample examples are available to demonstrate the effect of such sequence variations in genic regions in altering the phenotypes. However, recent reports indicate that the nucleotide changes in non-coding regions also have significant effect on transcript synthesis and accumulation which in turn alter the trait expression. Role of intronic mutations in gene regulation was evident in the expression of some genes like tubulin and rubi3 (polyubiquitin gene) in rice as well VRN-1 (which affect vernalization response) in barley and wheat. Thus, 'true' allele mining should also include analysis of non-coding and regulatory regions of the candidate genes in addition to analysing sequence variations in the coding regions of the agronomically important genes so as to cover most of the functional variations of relevance in the genes. Realizing the importance of 'mining' for variation in regulatory regions, promoters of genes have been chosen as the potential target for allele mining which is termed as 'promoter mining'. Promoter mining coupled with in silico analysis aimed to identify nucleotide variation in transcription factor binding motif, number/frequency, and location of regulatory elements binding sites in promoter regions and their corresponding transcription factors which are associated with specific expression pattern in response to constitutive, developmental, tissue-specific, hormonal and environmental regulation. Two major approaches are available for the identification of sequence polymorphisms for a given gene in the naturally occurring populations, (i) modified TILLING (Targeting Induced Local Lesions in Genomes) procedure called EcoTilling and (ii) Sequencing based allele mining.

2.1 ECOTILLING

TILLING can identify polymorphisms (more specifically point mutations) resulting from induced mutations in a target gene by heteroduplex analysis. EcoTilling, represents a means to determine the extent of natural variation in selected genes in crops. The method is

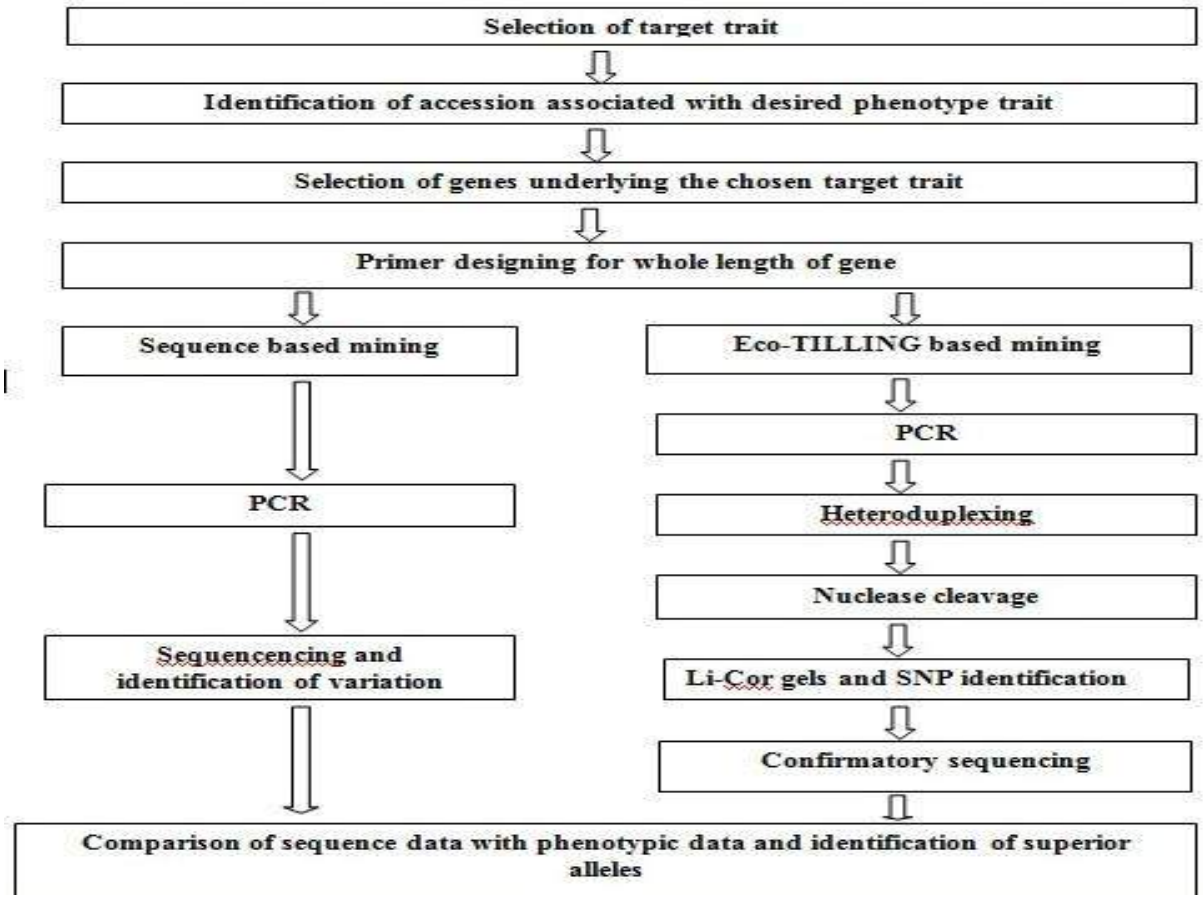

Figure 1: Steps involved in allele mining

essentially same as TILLING except that the mutations are not induced artificially and are detected from naturally occurring alleles in the primary and secondary crop gene pools. Like TILLING, EcoTilling also relies on the enzymatic cleavage of heteroduplexed DNA with a single strand specific nuclease under specific conditions. At point mutations, there will be a cleavage by the nuclease to produce two cleaved products whose sizes will be equal to the size of full length product.

The presence, type and location of point mutation or SNP will be confirmed by sequencing the amplicon from the test genotype that carry the mutation. TILLING and EcoTilling were proposed as cost effective approaches for haplotyping and SNP discovery. Recently, a rapid and cost-effective method for detecting novel allelic variants of known candidate on agarose gels and its utility in candidate gene mapping has been described. We can anticipate that the cost of EcoTilling can be significantly reduced by the adoption of such innovative strategies for allele mining (Fig. 1).

2.2 Sequencing-based allele mining

This technique involves amplification of alleles in diverse genotypes through PCR followed by identification of nucleotide variation by DNA sequencing. Sequencing-based allele mining would help to analyse individuals for haplotype structure and diversity to infer genetic association studies in plants. Unlike EcoTilling, sequencing-based allele mining does not require much

sophisticated equipment or involve tedious steps, but involves huge costs of sequencing. Despite the claim that EcoTilling can be done at a fraction of the cost of SNP/haplotyping methods, the elaborate equipment and expertise required and the need for confirmatory sequencing in EcoTilling procedure, draws reconsideration for its wide applicability. However, each technology has its strengths and weaknesses that need to be carefully considered in the light of the intended application (Fig. 1).

3. Applications of allele mining

Allele mining can be effectively used for discovery of superior alleles, through 'mining' the gene of interest from diverse genetic resources. It can also provide insight into molecular basis of novel trait variations and identify the nucleotide sequence changes associated with superior alleles. In addition, the rate of evolution of alleles; allelic similarity/dissimilarity at a candidate gene and allelic synteny with other members of the family can also be studied. Allele mining may also pave way for molecular discrimination among related species, development of allele-specific molecular markers, facilitating introgression of novel alleles through MAS or deployment through genetic engineering.

3.1 Characterization of allelic diversity

Identification and access to allelic variation that affects the plant phenotype is of utmost importance for the utilization of genetic resources in crop improvement. Exploitation of gene banks for efficient utilization depends on the knowledge of genetic diversity, in general, and allelic diversity at candidate gene of interest, in particular. Hence, characterization of genetic diversity or allelic/genetic diversity among the accessions of the collection is highly important to establish the genetic relationships among them and to assess their genetic worthiness in terms of its utility for improving a target trait. Allele

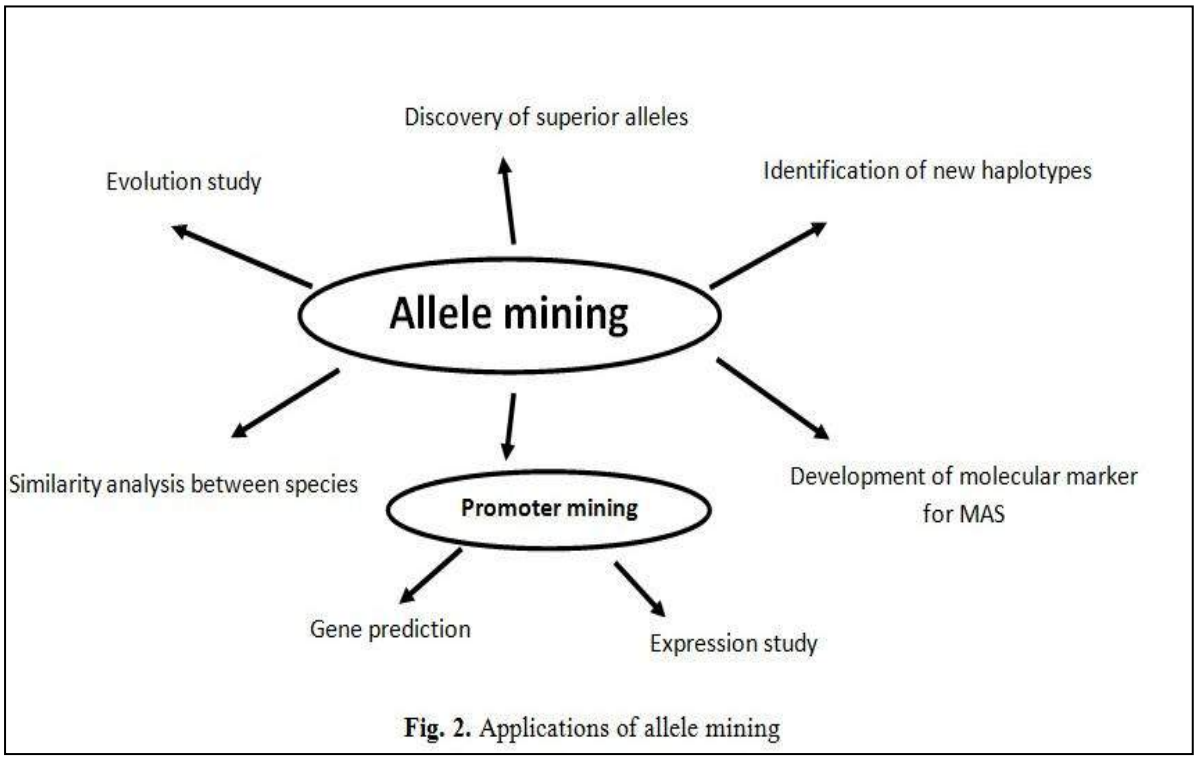

Fig. 2. Applications of allele mining

mining seems to be a promising, although largely untested method to unlock the diversity in the collections of genetic resources in the world gene banks.

3.2 Identification of new haplotypes

Allele mining can be potentially employed in the identification of nucleotide variation at a genomic region associated with phenotypic variation for a trait. Through this, one can evaluate the frequency, type and the extent of occurrence of new haplotypes and the resulting phenotypic changes. Knowledge on the most common haplotype changes and their frequency in the populations would form the basis for association mapping studies.

3.3 Development of molecular markers for MAS

Identification of sequence variation will pave the way to develop allele-specific marker assay for precise introgression of the identified 'superior and/or novel' alleles to suitable genetic background.

3.4 Allelic synteny and evolutionary relationship

Using the sequence information obtained from allele mining studies, syntenic relationships can be assessed among the identified loci/genes across the species/genera.

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Phytase Enzyme in Poultry Diets for Utilization of Unavailable Phosphorus (Phytin) In Feeds and Reducing Water Pollution (Eutrophication)

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History:

At the beginninng, phytase was developed in the Netherland to reduce pollution of environmental phosphorus and surface eutrophication in areas of intensive livestock production and intensive agriculture**.** Supplementation of exogenous phytase in monogastric diets gained recognition in the early 1990. Phytase enzyme use commercialized in 1991 (Selle, 2007).Factors responsible for increase in use of phytase enzyme in last 10 years are- 1. Increase in the raw material prices 2.Growing concern for the environmental impact of meat production.

Introduction:

Enzymes play an important role in enhancing feed utilization and reducing feed cost. Phytase is a proven technology used to release some of the non-digestible phosphorus and reduce excretion of this element, thereby reducing the cost of inorganic phosphorus supplementation. Phytase has mainly been considered to be a tool to increase phosphorus availability/digestibility from vegetable sources. The majority of broiler diets used in Indian poultry industry are corn and soybean meal based, both of which are important for the protein and energy requirements of the broiler bird. The bulk of poultry feed is plant based ingredient, comes primarily from the seeds of plant. Phosphorus is essential to meet animal's physiological requirements for maintenance and growth. However, most dietary P in vegetable raw materials exits in the form of phytate which is not digestible and available for the animals, resulting in reduced performance. Most of the phosphorus in plant is found in seeds mainly as a component of a molecular called phytin. Feeds of intensively reared poultry typically contain a high proportion of cereals, grain legumes and oilseed meals. An approximately two third of the phosphorus is present as Phytate phosphorus. In plant, phytic acid exists in its anionic form, Phytate.Phytic acid, mineral complex in a mature seed(myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate IP 6),is the

primary storage form of phosphorus naturally found in plants(seeds and grains) (Woyengo *et al*.,2009;Thavarajah *et al*.,2009).Phytate, which is present in many plant based feedstuffs is the main phosphorus store in plants (Cosgrove,1980).Phytic acid contains 28.2% P (Selle *et al.,2000*),and in mature seed,50-80% of the total phosphorus can be contained in phytic acid molecule (Lott,1984).Phytic acid is typically concentrated in seed/grain (Graf,1986) where it is found in protein bodies or specialized protein storage vacuoles (Lott,1980) bound with phytate. In mature seeds, phytic acid is highly reactive and found as a complex salt of Ca, Fe, Mg, Cu, Zn, CHO & proteins. In some cases it is bound to proteins and starches. This complexed or chelated molecule of phytic acid is known as phytin (Bohn *et. al*.2008). Phytic acid is a very stable molecule. It differs from other organophosphate molecule in having high phosphate content, which results in a high negative charge over a wide pH range. Its presence in the diet has a negative impact on the bioavailability of mineral ions such as Zn^{2+},$Fe^{2+/3+}$,Ca^{2+},Me^{2+}, Mn^{2+} and Cu^{2+} (Fredlund *et. al*. 2006).These formed complexes are substantially less soluble in the small intestine & therefore, less likely to interact with phytase (Angel *et al*.,2002).Besides, phytate has also been reported to form complexes with protein at both low and high pH values. These complex formations alter the protein structure, which might result in decrease protein solubility, enzymatic activity and proteolytic digestibility (Kumar *et. al*. 2010). Phytin phosphorus typically averages 72% & 60% of total seed phosphorus in corn and SBM resp, the two predominant feed ingredients in poultry diets in the US (Ravindran *et al*., 1995).

What is phytase?

Phytase is an enzyme which breaks down phytic acid & therefore improves P,Ca,Zn,Na,Mg & amino acid availability & digestibility in broilers. (Walk, 2012).In other way, Phytase (myo-inositol hexaphosphate hydrolase),ia an enzyme that is able to break down the phytic acid molecule by catalyzing the hydrolysis of phosphate ester bonds (Angel et al,2002).[Fig.1]

Categories of phytases:

- Fungal from-Aspergillus and Peniophora; yeast; Bacterial from E.coli
- Two types of phytases commonly used in poultry diets: 3-phytase-microbial origin A.niger & 6-phytase-plant origin- P. lycii, E.coli *(*Selle *et al., 2007)*. Characterized by the point on the inositol ring where dephosphorylation is initiated. Bacterial 6-phytase preferentially hydrolyze higher molecular weight IP i.e. those with highest anti-nutritional effects eliminating more IP6 & IP5/unit of P-release (Cowieson *et al*.,2011).

Location of phytin molecule:

Cereals - Aleurone layer

Oilseeds -Associated with storage proteins throughout the seeds

Phytate –ubiquitous in feed ingredients

Ingredients	gm/kg
1. Wheat	1.9-2.7
2. Corn	1.6-2.6
3.Sorghum	1.4-2.4
4.Rice bran	10.8-11.1

5.SBM	2.8-3.3

(Eeckhout and DePaepe (1994) AFST,47:19-20.O'Dell et al ,(1972) JAFC,20:718-808.

Phytate act as anti-nutrient for poultry in the following way:

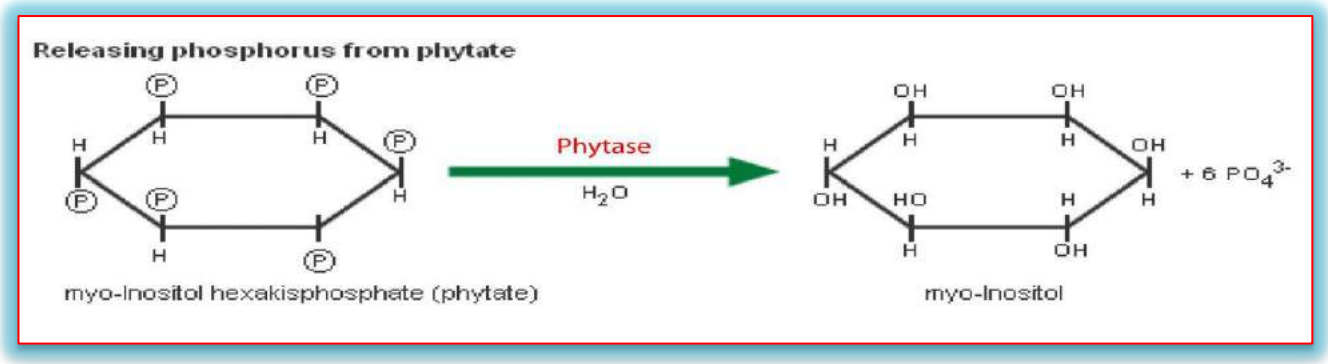
- ❖ Direct irritant: Reduces mineral availability and increasing pepsinogen & HCL secretion.
- ❖ Electrostatic aggressor: Reduces energy & protein digestibility and increases mucin secretion & endogenous losses.
- ❖ Chelator of minerals/mineral co-factors: Reduces protein solubility, pepsin & trypsin activity & reduces pH in the proximal & distal gizzard.
- ❖ Reducing the blood glucose response
- ❖ Deficiency related diseases-poor quality egg and meat in poultry
- ❖ Forms a strong complex with some protein and resist their proteolysis
- ❖ Impacts of Phytate on mineral digestibility: Increased pH leads to phytate becomes more negatively charged attract cations such as P,Ca,Zn,Cu.Fe,Mg.Stable salts are formed.Hence,complex structure formation of phytate and minerals.
- ❖ Impacts of Phytate on protein digestibility:__Presence of phytase reduces pepsin activation and educes overall protein digestibility.

Need of addition of phytase enzyme:

- ✓ Phytase reduces the need to add phosphorus to the feed diet.
- ✓ Supplementation of microbial phytase was observed to reduce the need of mineral supplementation by increasing the avaibility of cations bound to phytic acid (odetallah, 2000).
- ✓ Eliminates the anti-nutritional effect of the phytate through higher enzyme doses**.**
- ✓ Animal producers & feed manufacturers can reduce the inclusion of inorganic phosphorus & calcium source in poultry feeds (e.g. Dicalcium Phosphate and Limestone) without negatively influencing growth performance & with a reduction in the cost formulation.
- ✓ It releases phosphate groups from phytin potentially making this phosphorus available to the animal.
- ✓ It is the only recognized enzyme that can initiate the release of phosphate from phytin (IUB,1979)
- ✓ Releases phytate phosphorus more effectively in the GIT
- ✓ Improves processing stability (Conditioning & Pelleting)
- ✓ Phytase supplies phosphorus so DCP incorporation cost lessen down
- ✓ Phytase supplies more than just minerals & hence there is a space effect.
- ✓ Improves storage stabilty

Ideal properties of phytase for effective release of phytate P:

a) Excellent thermostabilty.
b) Optimum pH & pH profile.



c) Have good substrate affinity & rate of degradation under acidic condition in proximal GIT & Sustained gastric performance.
d) Resistant against endogenous proteases
e) Lowering dietary calcium contents
f) Increasing phytate solubility/accesibility
g) Application in combination with other additives.

FTU

It is an unit of expressing the activity and efficacy of phytase enzyme. One unit of phytase (FTU) is considered to be the amount of enzyme that releases 1mmole of iP/min from 0.00015 mol/1Na-phytate at pH5.5 & 37˚C (Ravindran *et a*l.,2000) One unit of phytase is defined as the amount of enzyme required to liberate 1µmol of orthophosphate from phytin per minute at pH 5.5 & 37˚C (Zylaetal.,1995).

Difference between poultry and ruminants regarding phosphorus availability:

Poultry: Have a enzymatic digestive process unable to broken down phytate .Due to lack of bacteria and less hydrolyzing property

Ruminants: Have a fermentation process. Phytate can be broken down by rumen bacteria releasing phosphorus to be absorbed by the animal.

Release of bound phosphorus by phytase:

Super-dosing:

The formulation concept with feeding higher levels {1000-2000 FTU/kg} of phytase with no nutrient sparing beyond the first 500 FTU/kg with benefits in performance, bone strength ,fat digestibility & anti-oxidant status is c/a 'superdosing'(Feedinfo.com,06.06.11).Superdosing involves the use of high doses of phytase to accommodate the replacement of expensive animal protein meals with cheaper vegetable alternatives. Latest figures suggest that 93% of the poultry feed in Asia is currently treated with a phytase feed enzyme (AB vista).In broilers, superdosing may be the addition of 1500 FTU/kg utilizing a 500 FTU/kg nutrient matrix which is applied from one day of age, the extra 1000FTU/kg is not intended to reduce diet cost, rather relax the nutrient requirements & improves the FCR & body weight gain via elimination of phytate.

Importance of superdosing:

Superdosing in broiler diets has shown to improve broiler performance (Pirgozley *et al*., 2011), while also improves the anti-oxidant status of animals (Karadase et al., 2010) which can be related to reduction in the anti-nutritive effect of phytate. Superdosing

therefore has huge potential to deliver additional financial gains to the Asian poultry industry. It results in improved digestive efficiency due to reduced endogenous losses across a range of relevant nutrients but, in particular, for, phosphorus, calcium, threonine, cysteine, serine, proline, glycine & sodium. The use of superdosing levels of microbial phytase in the diets of poultry results in increased feed intake, commensurate weight gain, improved FCR & phytate destruction. (Nutrition & Health feedstuffs, July1, 2013; 13).A higher average live weight and net profit was observed for superdosing of phytase with 1500 FTU/ Kg of feed (Raut, 2014). It indicates importance of proper selection of dose for phytase to get best result in terms of profitability.Decreased feed cost and therefore increase profitability may be the main advantages of super dosing of phytase in broiler diet (Raut, 2014) .It is recommended that superdosing of phytase enzyme at dose rate of 1500 FTU/ Kg of feed is more beneficial from bird's performance survivability and improved profitability. It offers poultry producers both economic and environmental benefit in the same package (Raut, 2014).

Mechanism of action:

The bacterial phytases preferentially target the higher molecular weight esters of inositol phosphate & thus, destroy proportionately more IP-6 & IP-5 than IP-4 & IP-3 per unit of phosphorus release in the initial reactive phase. IP-6 & IP-5 have a much greater capacity to chelate ca++,CHO, amino acid, mineral & fat than IP4 & IP3 (Luttrell,1992;Persson et al,1998).

Misconceptions of superdosing:

Ca:P ratio may become imbalanced & skeletal problems or wet litter

Nutrient matrix value of 500 FTU phytase for broiler:

Note: Limit of superdosing of phytase is 2000 FTU/kg, above this value there is no improvement in birds.

	500ftu/kg (100gm/t)
Available P (%)	1500
Ca (%)	1650
Sodium (%)	350
Lysine (%)	170
Methionine (%)	39
Cystine (%)	351
Methionine + Cystine (%)	390
Threonine (%)	330
Tryptophan (%)	190
Glysine + Serine (%)	570
Arginine (%)	130
Valine (%)	230
Isoleusine (%)	255
Crude Protein (%)	4210
ME (MJ/Kg) (%)	2170
ME (Kcal/Kg) (%)	520000

Dose: 500ftu/kg means- 100gm/ton i.e. 1gm/kg of feed (Raut, 2014).

Effect of phytase enzyme on pollution:

- Reduces the environmental phosphorus pollution from intensive agriculture (animal waste).
- Reduces P excretion by 50%.
- Avoids '*algal blooms*' in waterways.
- Avoids '*eutrophication'(fish deaths).*
- Avoids reduction in *dissolved oxygen* in water.

- Maintains *BOD* in the waterways.
- Avoids *run off land.*
- *NPK level* decreases in the soil.
- Cut *90,000 ton of P* excretion/year from animal waste
- Preserve *the non-renewable inorganic P source* (US)

CONCLUSION:

Supplemented phytase hydrolyzed phytate and consequently reduced the antinutritional effect of phytate and thereby improved bird's performance. Microbial phytase not only reduces the need for inorganic phosphorus and calcium but also served to reduce the need for protein in the diet. Supplementation of phytase resulted in hydrolysis of phytate bond and resulted in more utilization of calcium and phosphorus thereby reduced nutrient through fecal matter, resulting in reduced emission of polluting elements in the excreta of broiler. The super dosing of phytase can be used to improve overall utilization of calcium, phosphorus and other vital nutrient resulting in alleviation of heat stress decreased feed cost and therefore increased profitability .It offers poultry producers both economic and environmental benefit in the same package. Superdosing of phytase enzyme at dose level of 1500 FTU/ Kg of feed is more beneficial from improved birds performance, less excretion of vital nutrients, survivability and improved profitability.

Fig 1. Phytase enzyme

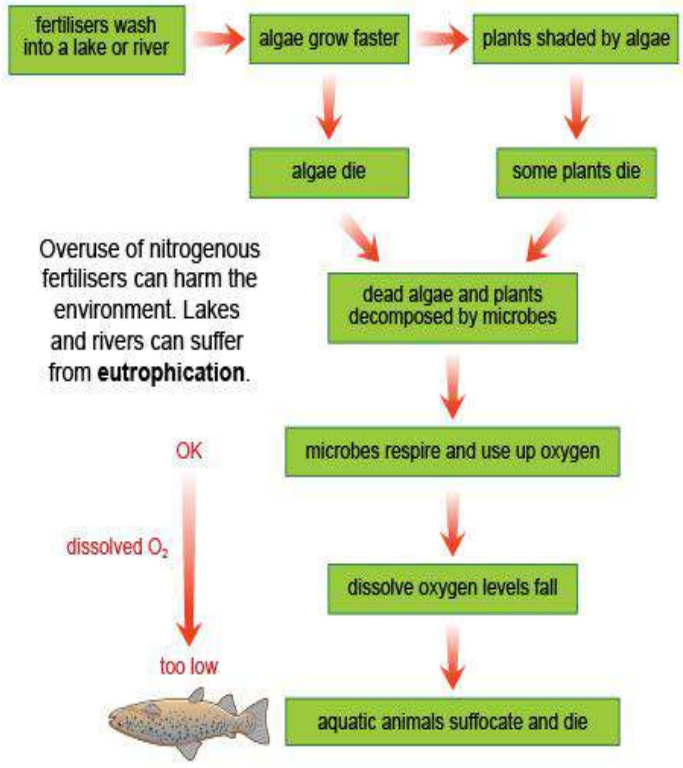


Fig 2.Eutrophication

Algal blooms

Eutrophication

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Trans-boundary animal diseases and its control- An overview

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Abstract
Livestock sector plays a pivotal role in navigating the country's developmental indices towards a better picture by providing food security, improving quality life and securing rural employment. The growth and development of livestock sector is inevitable for any country's growth trajectory.In a developing country like India, agriculture contribute significantly towards the gross domestic product (GDP) in which the livestock sector is a key component. Furthermore the growing population rate, change in live style and increasing level of income has heightened the demand for meat and egg protein consumption. Therefore the livestock farming is garnering importance owingto its role in rurallivelihood of farmers, particularly landless farmers, regular income and women empowerment in India. However, there are many diseases which affect the animal health standards and directly or indirectly affect the production as well as economy of country. Keeping in view of the above facts it is very much essential and need of the hour to minimize the disease occurrence that affects the livestock sector to achieve the much needed growth trajectory.

Keywords: Trans-boundary animal diseases, livestock sector.

INTRODUCTION

As we are entering an era of globalization and more and more connectedness, there are very possibilities that the disease causing agents spread from one country to other by crossing the geographical boundaries. With increasing human population, livestock and livestock products trade within and across countries, together with climate changes, threat from trans-boundary diseases is intensifying. Trans-boundary diseases are highly contagious and have the potential for rapid spread, irrespective of national borders, causing serious socioeconomic consequences (Otte et al., 2004). Although there are no technological constraints to checkmate major trans-boundary livestock diseases, the lack of a concretegovernment policy and robust mechanism to counter it, make the condition more precarious.

SIGNIFICANT TRANSBOUNDARY ANIMAL DISEASES

Foot-and-Mouth Disease (FMD)

Foot-and-mouth disease is highly contagious and can spread rapidly in cloven- hoofed livestock populations through movement of infected animals and animal products, contaminated objects (e.g. livestock trucks), and even by wind currents. Vaccination is complicated by a multiplicity of antigenic types and subtypes. Substantial progress has been made towards control and eradication of FMD in several regions of the world, notably Europe, and parts of South America and Asia.

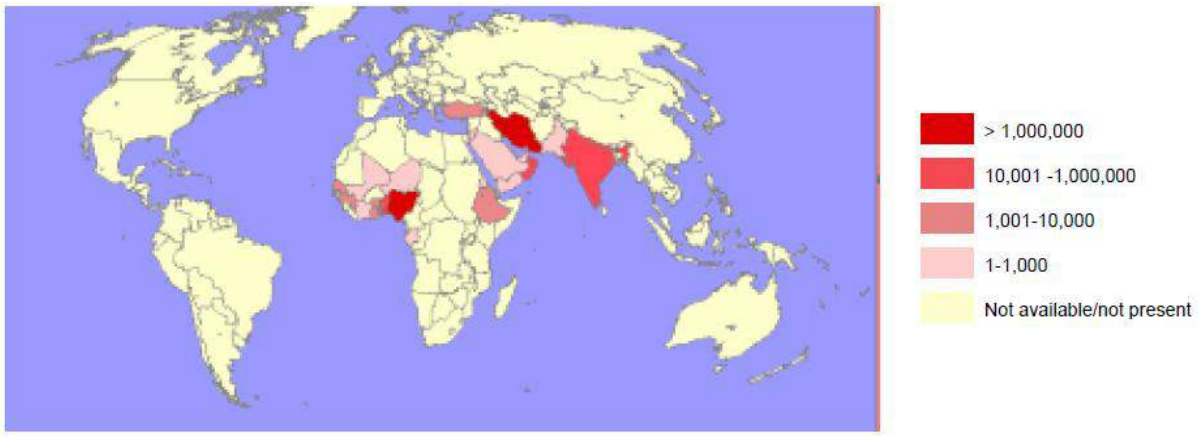

Figure 2 Total number of CBPP cases reported to OIE between 1997 to 2001 (Otte et al., 2004)

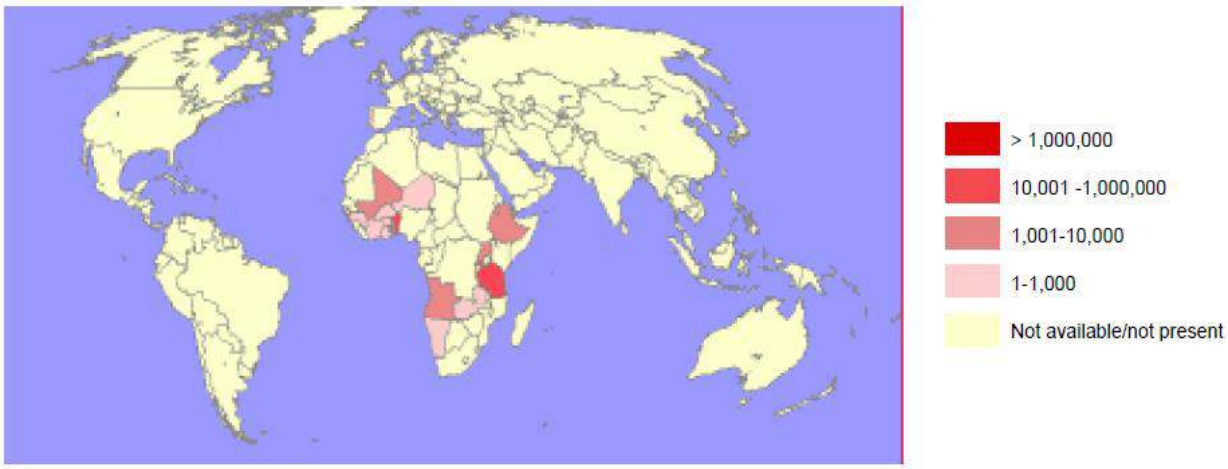

Figure 1: Total number of PPR cases reported to OIE between 1997 to 2001 (Otte et al., 2004)

Contagious bovine pleuropneumonia (CBPP)

CBPP is often regarded as an insidious, low mortality disease of cattle, but this is based on experiences in endemic areas. In susceptible cattle populations the disease can spread surprisingly rapidly and cause high mortality. The movement of infected animals (either acute cases or chronic carriers) spreads the disease. Major CBPP epidemics have been experienced in Eastern, Southern and West Africa over the last few years.

Figure 3: Total number of swine fever cases reported to OIE between 1997 to 2001 (Otte et al., 2004)

Bovine Spongiform Encephalopathy (BSE)

BSE, caused by a novel infectious agent (prions) was first recognized in the UK in 1986. The disease is thought to be transmitted among cattle through feed supplements with meat and bone meal containing infected particles from affected animals. BSE can most probably affect humans consuming infected tissues causing a fatal neurological disease called variant Creutzfeldt-Jakob Disease.

Peste des petits ruminants (PPR)

Peste des petits ruminants is a disease affecting sheep and goats. Its spread has been partly due to inadequate international availability of an effective PPR vaccine until recently, and also the fact that small ruminants have perhaps not received adequate attention in disease surveillance and quarantine programmes in some regions. The Americas, Europe and Oceania are free from PPR.

Classical swine fever (CSF)

Classical Swine Fever or hog cholera is a generalized viral disease affecting only pigs. The disease is endemic in much of South and South-East Asia, where it is a constraint to the development of the pig industry.

African swine fever (ASF)

African swine fever is the most lethal transboundary disease for pigs. It is also a viral disease which has shown a great propensity for sudden, unexpected international spread over great distances. This is often associated with transportation of contaminated pig meat products, including garbage from ships and aircraft containing food scraps. Presently, there are no vaccines against ASF. The only practical disease control measures for commercial piggeries is denial of access to wild and village pigs through fencing and other sanitary precautions.

Newcastle disease (ND)

Newcastle diseases is caused by a virus spread primarily through bird to bird contact among chickens, but it can also spread through contaminated feed, water, or clothing. It is a major constraint to the development of village chicken industries, particularly in Asia and Africa.

Avian Influenza (AI)

Avian influenza has been recognised as a highly lethal generalised viral disease of poultry since 1901. It has since been found that AI viruses cause a wide range of disease syndromes, ranging from severe to mild, in domestic poultry. AI viruses are probably ubiquitous in wild water birds. Pathogenic strains could emerge and cause disease in domestic poultry in any country at any time without warning.

International cooperation for TADs control

Cooperation in regional and international fora is vital for the control of the occurrence and severity of such animal diseases, as the efforts of a single country will go in vain to tackle and eradicate the diseases due to the trans-boundary nature of the diseases. For this concern GFTADs (Global Framework for the progressive control of Transboundary Animal Diseases) programme gives a platform for such cooperation and allianceto tackle trans-boundary animal diseases and safeguards the livestock sector from infectious diseases. Also it ensures the improvement of food security and economic growth as well as reduction of animal and human health threats.

The initiatives to control TADS in South Asian countries

There have been many steps undertaken by many intergovernmental bodies to counter trans-boundary diseases and one such counter measure is initiative under the umbrella of GF-TADs to engage with the countries in South Asia to identify diseases that affect severely and to improve control mechanism for these diseases through a regional collaboration. After much debate and deliberation three priority diseases identified were FMD, PPR and HPAI as per the severity of infection and trans-boundary nature and are collectively responsible for jeopardizing food security, threatening food safety and seriously impeding trade and market opportunities in livestock and livestock products in the region.

Preparedness for control of TADs in India

India is fighting with many exotic diseases which by virtue of trans-boundary nature have established their virulency in India. To combat with these diseases India is developing diagnostic technologies for most of them. Steps are also being taken to disseminate technology in advance areas of disease diagnosis and control for the benefit of the animal industry. The country is committed to establish a comprehensive national campaign for diagnosis and control of emergency diseases similar to that developed for the control of Rinder Pest, Foot and Mouth disease and Avian Influenza.

Laboratory facilities

In India, for diagnosis and control of such trans-boundary diseases many facilities are available from district and state levels to referral diagnostic facilities in national laboratories with advanced technologies. Keeping in mind, a containment laboratory of Bio-safety Level-4 (BSL-4) High Security Animal Disease Laboratory (HSADL) has been established with state-of-the-art facilities at Bhopal in 1998. Latest technologies like PCR, gene cloning

and sequencing have been incorporated according to the norms laid down by OIE for the diagnostic purpose. Facilities are also available for monoclonal antibody production against various exotic animal pathogens. Presently projects are being undertaken for research and diagnosis of avian influenza, rabbit hemonhagic disease, Bovine viral diarrhea, Bovine immuno-deficiency, Aujeszky's disease, porcine reproductive and respiratory syndrome, transmissible gastroenteritis, African swine fever, malignant Catarrhal fever, caprine arthritis and encephalitis. In addition, the laboratory has developed recombinant antigen and mono clonal antibody based competitive ELISA kits for diagnosis of bovine viral diarrhoea and bovine immuno-deficiency.

Livestock market vis-a- visTADs in India

There are strong social and economic rationale for public intervention and cross-border collaboration for collective action against TAD. It has been a challenge to develop and implement cost effective actions within a comprehensive framework for control of TAD. In India, the technical ability to control animal diseases has greatly advanced in recent years.

The country has harnessed the technological advances and off late launched National Animal Disease Reporting System (NADRS) and web based GIS platform (National Animal Disease Referral Expert System – NADRES) to support surveillance and control of livestock diseases. Amongst the various central sector schemes, funds earmarked for five central schemes are being directly used for control of TAD in India. These are Assistance to States for Control of Animal Disease (ASCAD), Foot andMouth disease Control Program (FMD-CP), National Control Program of PPR, National Project on Rinderpest Eradication and National Animal Disease Reporting System. The improved information exchange has facilitatedreaction to TAD with greater regional cooperation amongst SAARC countries. South Asia in general and India in particular are incurring huge losses mostly from two important transboundary animal diseases viz. Foot and mouth disease (FMD) and Peste des Petits Ruminants (PPR).

CONCLUSION

So from the facts enumerated above it can be concluded that in an era of globalization, the chance of occurrence of trans-boundary diseases is obvious owing to the mass movement of people, animal trade and the changing environment. These trans-boundary animal diseases pose a serious threat not only to the animal health and production but also to the human population in form of zoonotic diseases. So to counter the spreading of such diseases is utmost importance in regional as well as globally. Biosecurity and biosafety bears much importance at grass root levelin the farm along with at national and international level. Checking the spread of diseases within the country can reduce international spread.There is an urgent requirement for the safeguard of livestock industry from epidemics and

to uphold the safe international trade oflivestock and their products. In this regard, it isessential to develop scientific and risk-basedstandards that facilitate the international trade in animal commodities.

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Amla (Indian Gooseberry):

A Future Fruit

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Phyllanthus emblica, also known as emblic myrobalan, Indian gooseberry, Malacca tree or amla from Sanskrit amalika is a deciduous tree of the family Phyllanthaceae. It is originated in Indian sub-continent. India ranks first in the area and largest producing country of world. It is known for its edible fruit of the same name. Gooseberry is among the healthiest food due to its high nutrient content. It is a rich source of antioxidants, iron, vitamin A, C, fiber, potassium, magnesium, calcium.

Growing and Potential Belts:

State	Growing belts
Haryana	Bewal, Gurgaon
Himachal Pradesh	Palampur, Bilaspur, Hamirpur
Karnataka	Bilgiri Rangan hills in Mysore
Madhya Pradesh	Dewas, Hoshangabad, Shivani, Tikamgarh, Betul, Chindwara, Shivapurkala, Panna, Rewa, Satna
Tamil Nadu	Tirunelveli, Thoothukudi, Sivagangai, Coimbatore, Salem, Dindugal
Uttar Pradesh	Pratagarh, Rai Bareli, Varanasi, Jaunpur, Sultanpur, Kanpur, Fatehpur, Agra, Mathura

Gooseberry / Amla Health Benefits and Nutrition Facts

Gooseberries, a highly beneficial fruit and it not just said it has been proven. Many researches have found its efficacy on different health problems. They may seem to be simple fruit, but packed with dose of active antioxidants.

1. Eye

Amla is healthy for the eye. It cuts the risk and rectifies cataract or nearsightedness. Gooseberry stands higher in the list of antioxidants content compared to goji berry, blueberry, pomegranate seeds and whortleberries. It shows benefits against free radicals. Free radicals are responsible for degenerative diseases. It also protects eye retina from oxidative stress. Eating with honey is effective in increasing eye vision. Vitamin A plays a vital role in preventing eye sight problems.

2. Improve skin health

Eating it daily early in the morning improves skin health and gives a perfect glow to the skin. Since it contains antioxidants, it protects skin damage from UV rays. Amla prove beneficial in the production of blood cells due to high Vitamin C, Iron, folic acid, and other nutrients. Skin receives more oxygen with

increasing blood cells, which directly improve skin health.

3. Aging

Generally, Aging is common with the increase in age. But the phase of aging is completely reversed. Skin starts to show sign of aging at an early age. Unhealthy diet, pollution and stress are main causes of aging. Oxidative Radiance Activity Capacity (ORAC) value is 3387 micromole per 100 g. Healthy cells is damaged by free radicals. After damaging the healthy skin and it quicken the process of aging. But antioxidants fights these free radicals and decrease the process of aging.

4. Diabetes

In previous studies, it was found that emblica has dramatic result in Diabetes patients. Consuming just 3 quarter of tsp of powder showed a drop of blood sugar level from 130-140 to healthy zone to 65-75 within 21 days. While the diabetes medicines showed far less effective than Gooseberry.

5. Heart

Gooseberry is rich in antioxidants, iron, calcium, anthocyanin, flavonoids and potassium that are required for heart health. Gooseberry reduces bad cholesterol thus reduce the blockage of blood flow towards the heart. The antioxidants capacity of 1 tsp of Amla is 782 unit that makes it the healthiest fruit. These provide the fruit with efficiency to fight free radicals, recovery of damaged DNA cells and much more. It improves nerve health thus it supports the proper flow of blood. Antioxidants protect nerve cell damage from free radicals. Also, it plays major in strengthening heart muscles. Along with antioxidants, potassium plays a crucial role in strengthening the nervous system. It also balances the fluid and electrolyte level. With the better nervous system, healthy cholesterol and strong heart muscle gooseberry prove the best food for heart health.

6. Immunity

Gooseberry is the richest source of Vitamin C, that is responsible for boosting immunity. Vitamin C plays a vital role in improving immunity to fight against free radicals. With increased immune system body is resistance towards common disease like flu, cough and also prevent infections.

7. Hair

Gooseberry / Amla oil is widely used to improve hair health. It is a rich source of iron and other nutrients that increase the growth of hair. It is also effective to increase hair pigmentation. It also prevents hair loss.

8. Digestive properties

Gooseberry is a rich source of fiber that improves digestion. Low fiber diet leads many health problems. Constipation is due to the poor digestive system. A healthy diet in fiber can improve digestion by providing roughage. Due to improves in the digestion problems like hemorrhoids can be prevented.

9. Cancer

Gooseberry is a rich source of antioxidants which is effective to prevent DNA cell damage from free radicals. Also due to its high Oxidative Radiance Activity Capacity (ORAC) it protects cell damage from oxidative stress. The Best part of Amla / Gooseberry is that it doesn't lose it nutrients value even in powered form. During studies it was found that Amla / Indian Gooseberry has tremendous effect on multiple cancers.

10. Anaemia and Brain health

Being richer in iron, along with its unique combination Vitamin C that increases body capacity to absorbs nutrients required for blood production. Due to the high iron in the blood, it provides oxygen to the brain. Also, it improves memory. As it contains a high amount of antioxidants, it is effective to prevent degeneration of brain caused due to oxygen free radicals. Thus, it provides complete health benefits to the brain. Gooseberry is also effective to protect from Alzheimer's disease but not to cure it.

Amla is a proven complete fruit with medicinal properties. It has been used since the ancient civilization. Even after knowing its benefit, it is underutilized fruit but due to changing food habit of today's generation, It will definitely fulfil the demand of modern people.

Economics of Donkey Milk Production

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Abstract

An enterprise with much scope in the current Indian market that has not been ventured yet is Donkey farming. It is a well established practice in countries like Italy, France, and Russia. Donkey population in the world is about 41 million and china stands first with a population of 11 million. India has approximately population of 0.44 million, most of which are used for draught purpose. The complete potential of donkey farming is not exploited in terms of their use as a milch animal. There is an increase in demand for the donkey's milk in the local market as well as the national level in the recent years. In relative to other livestock farming, establishment and maintenance of donkey farming is considerably less expensive. Besides the conventional use of milk for consumption, donkey's milk is used in other industries, in preparation of cosmetics and also has medicinal value.

INTRODUCTION

The History of usage of Donkey milk goes back to centuries, where, Hippocrates (460 – 370 B.C.), the father of medicine, prescribed donkey' milk for numerous purposes, such as liver problems, infectious diseases, fever, edema, nose bleeds, poisoning and wounds. It is well known that Cleopatra, the Queen of Ancient Egypt, known for her stunning beauty, took her daily baths in Donkey' milk. Until the beginning of the 20th century donkeys' milk (donkey' milk) was used as a substitute for breast milk. Particularly, in France, Catalonia and Southern Italy around a hundred years ago one could find donkeys visiting houses and be milked on the spot. The properties of donkey milk were known to be highly beneficial to weak children. William Buchan's Domestic Medicine from the early 1900s refers to donkey milk as a cure for coughs and respiratory problems. In India even today donkeys' milk is given to newborn babies to boost their immune systems and give them a good, strong voice.

COMPOSITION OF DONKEY MILK

Donkey milk, along with mare's milk, is the closest to breast milk, with notably low lipid ratios and high lactose ratios. Donkey milk is similar to mare milk

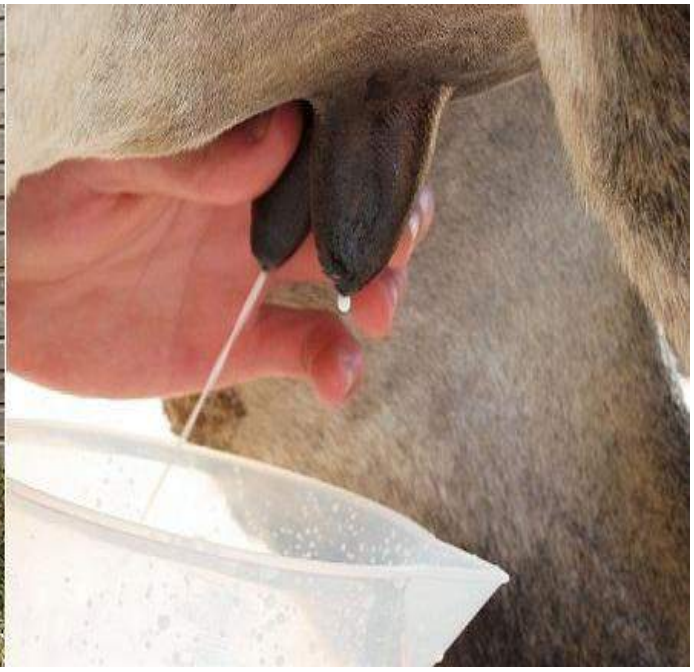
Composition of donkey's, mare's, human and cow's milk (g/100 g)				
composition	**donkey**	**mare**	**human**	**cow**
pH	7.0 – 7.2	7.18	7.0 – 7.5	6.6 – 6.8
Protein g/100g	1.5 – 1.8	1.5 – 2.8	0.9 – 1.7	3.1 – 3.8
Fat g/100g	0.3 – 1.8	0.5 – 2.0	3.5 – 4.0	3.5 – 3.9
Lactose g/100g	5.8 – 7.4	5.8 – 7.0	6.3 – 7.0	4.4 – 4.9
Total Solids (TS) g/100 g	8.8-11.7	9.3-11.6	11.7-12.9	12.5-13.0
Casein Nitrogen (CN) g/100 g	0.64-1.03	0.94-1.2	0.32-0.42	2.46-2.80
Whey protein g/100 g	0.49-0.80	0.74-0.91	0.68-0.83	0.55-0.70
NPN g/100 g	0.18-0.41	0.17-0.35	0.26-0.32	0.1-0.19
Casein Nitrogen (CN) %	47.28	50	26.06	77.23
Whey protein %	36.96	38.79	53.52	17.54
NPN %	15.76	11.21	20.42	5.23
Adapted from Guo et al., 2007, Vincenzetti et al., 2007				

and human breast milk in that it is relatively poor in protein and fat but rich in lactose. The casein to whey protein ratio is intermediate between human breast milk and cow milk. Gross composition of milk differs by the mother's lactation stage, with ash and protein content showing a declining trend, but pH, percentage of whey protein, and amino acid content remaining the same.

Donkey's milk has enzymes (lysozyme and lactoferrin) which are powerful anti-microbial agents and these are practically absent in the milk of cows, ewes and goats. It is rich in vitamins (A, B1, B2, B6, C, D, E), minerals (calcium, magnesium, phosphorus, iron, zinc), trace elements and essential fatty acids (omega 3 and 6). It contains a lot of retinol (vitamin A) which has a very significant wrinkle-fighting and tightening effect. Retinol also helps to accelerate healing and collagen production. The skin regenerates more easily and thus eliminates impurities. Donkey's milk moisturizes and is recommended for early skin aging.

USES OF DONKEY'S MILK:

- ✓ Donkey milk is considered to be the closest to woman's milk. It is very nourishing because it contains more lactose and less fat than cow's milk.
- ✓ More recently, studies have shown that that donkey's milk could serve as an alternative to cow's milk for children allergic to bovine proteins.
- ✓ It is generally believed that donkey milk effaces wrinkles in the face, renders the skin more delicate, and preserves its whiteness. Donkey milk is still used today in the manufacture of soaps and moisturizers.
- ✓ Donkey milk was also formerly used in medicine. Its healing virtues have been known since ancient times, when doctors would recommend it to cure diverse affections.
- ✓ In the South Indian state of Tamil Nadu, a popular folk belief states that donkey milk can aid infants' immune systems and voice develop.



FACTS ABOUT DONKEY AND DONKEYS MILK:

- Donkeys vary considerably in size, depending on breed and management. The weight ranges from 80 to 480 kg (180 to 1,060 lb).
- Donkeys are considered a seasonal one, but the latitude in which the animal is domesticated can greatly influence the reproduction. A jennet is normally pregnant for about 12 months, though the gestation period varies from 11 to 14 months,
- Although jennets come into heat within 9 or 10 days of giving birth, their fertility remains low and it is usual to wait one or two further estrous cycles before rebreeding. Because of this and the longer gestation period, donkey breeders do not expect to obtain a foal every year, as horse breeders often do, but may plan for three foals in four years.
- The hybrid between a jack and a mare is a mule, valued as a working and riding animal in many countries.

Milking

- The production of donkey's milk is extremely complicated, making it a rare product. As with all mammals, lactation is triggered by the birth of a child.
- The animal has only two teats and no reservoir, and therefore should be milked three times a day, in order to get between 1.5 and 2 liters of milk.
- And milking can be done only for four to five months, since all the milk is left for the foal for the first two months.
- By comparison, a donkey gives an average of 30 liters, year-round. Milking is done manually, and the foal needs to be present, otherwise the jenny will not give milk.
- Donkey's milk is very white and more fluid than cow's milk, as it contains very little fat. But it is sweet.

Marketing:

Some of the potential markets for donkey milk are:

1. Local market
2. Pharmaceutical companies

3. Cosmetic companies
4. Cheese industries
5. Exporting the milk
6. Cheese making industries.

Price of the milk depends on the local demand and the companies to which the milk is supplied. There is no standardization of the donkeys milk to fix the price. In south Indian states like Tamil nadu, donkey's milk is sold at Rs2000/liter.

The cheese, known as pules, is produced on a donkey farm in Serbia and is known to be the costliest cheese in the world.

ECONOMICS OF 10 DONKEY [JENNY] UNIT:

Assumptions:

- Cost of milk is based on the average local market price prevailing in Visakhapatnam and other local markets, which is about Rs 1,000/liter.

Non-recurring expenditure:

1. Land available.
2. Water available.
3. Purchase of 10 lactating donkeys: 10× Rs 25000= **Rs 2,50,000**
4. Housing for 10 lactating donkey and 10 foals

Space requirement = 35.5 sqft/donkey = 35.5× 10 = 355 sq.ft
Space requirement = 14 sq.ft/foal =14× 10= 140 sq.ft
Total space requirement = 495 sq.ft
Amount required for 1 sq.ft = Rs 400
For 495sq.ft = 400× 495 = Rs **1,98,000**

5. Equipments

a. Sanitary milking pail
b. Milk can
c. Chaff cutter
d. Restraining ropes and chains
e. Buckets

Equipment cost = **Rs 25,000**

Total non recurring expenditure = Rs 4,73,000

Recurring expenditure:

1. Non-variable recurring expenditure

a. Interest on capital @12% = **Rs 56,760**
b. Depreciation on donkeys value @5% = **Rs 12,500**
c. Depreciation on building values @5% = **Rs 9,900**
d. Depreciation on equipment @10% = **Rs 2,500**

2. Variable recurring expenditure :

a. Dry fodder requirement for 10 donkeys = 10× 2kg = 20kg/day
For 365 days = 20× 365=7300kg

b. Dry fodder requirement for 10 foals for 6 months (till weaning)
= 0.5kg× 10× 180days=90kg

c. Dry fodder requirement for 10 foals for 6 months (after weaning)
= 1kg× 10×180days=180kg
Total dry fodder requirement = 7570kg
Cost of dry fodder @Rs8/kg= 7570× 8= **Rs 60,560**

d. Green fodder requirement for 10 donkeys = 10× 15kg× 365 days= 54,750 kg
Green fodder requirement for foals @8kg/foal = 10× 8kg× 365 = 29,200kg
Cost of green fodder @Rs1/kg= **Rs 83,950**

e. Concentrate requirement for 10 donkeys = 10× 1.5kg× 365days= 5,475kg

Cost of concentrate @Rs22=22× 5,475=**Rs 1,20,450**

f. Labour : one milk cum attender @ rs 5000/ month
For 12 months =5000× 12=**Rs 60,000**

g. Health coverage = **Rs 10,000**

h. Miscellaneous @250/donkey = **Rs 2,500**

Total recurring expenditure = Rs **4,19,120**

RECEIPTS:

1. Sale of milk

Milk production /day/donkey = 0.5liter
For 180days @ the price of Rs 1,000/liter
=1000× 180× 0.5= Rs 90,000/donkey
For 10 donkeys =Rs 9,00,000/-

2. Sale of foal @ Rs2500= 2500 × 10= Rs25,000

3. Sale of donkeys dung @ Rs1/kg = 5 kg× 365× Rs1× 10donkeys
=Rs 18,250

Total receipts = Rs 12,23,250

Net profit = Total receipts – Total recurring expenditure

= 9,43,250 – 4,19,120
= **Rs 5,24,130.**

Pregnancy diagnosis in bitches- An overview

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Early pregnancy diagnosis is valuable to clients, allowing appropriate changes in schedule of working of show dogs and is valuable to veterinarians, allowing pregnancy termination via ovariohysterectomy or medical therapy before mid-gestation. Several methods are used for pregnancy diagnosis in bitches. Methods such as evaluation of teats, weight gain and abdominal enlargement are not reliable methods of pregnancy diagnosis. More reliable methods include abdominal palpation, measuring the level of relaxin in blood, ultrasound of abdomen and radiographs. These methods vary in their ability to positively identify the number of fetuses and fetal viability.

Different methods of pregnancy diagnosis in bitches are;

I. Abdominal palpation

Undoubtedly, the most traditional method of pregnancy diagnosis is digital palpation of the abdomen. Abdominal palpation, usually 3-4 weeks post-mating, is used commonly for the diagnosis of pregnancy in the bitch (Gradil *et al.,* 2000). Abdominal palpation of pregnancy may be possible in small or medium sized bitches which are not too obese. Abdominal palpation reportedly is accurate in a window from 24 to 35 day post-breeding. Prior to about 28 days, the individual amniotic vesicles are small and difficult to palpate, especially in obese or tense bitches. Small ovoid swellings can be palpated along the uterine horns 21-30 days post breeding. At about 30-35 days the accuracy is high (87%) and depends on the palpation of tense conceptual swellings (6 to 30 mm in diameter) within the uterine cornua but they must be differentiated from faeces in the colon and the palpator must have expertise to differentiate or identify them correctly. These swellings double in size every week until days 35 to 38. After 35 days, increased amniotic fluid volume distends the vesicles and makes them confluent, obscuring their characteristic shape and turgidity and difficult to differentiate. The bitch's foreparts must be slightly elevated. They may be palpable in the flank and also in the lower abdomen. Palpation is accurate for positive pregnancy diagnosis 87–88% during the second month of pregnancy. Palpation has been reported to be only 12% accurate in assessment of litter size.

II. Radiography

Fetal skeletons have been reported to be visible from 44 days of gestation. Because

the normal bitch may stand for breeding for a wide window of time around ovulation, this may range from 43 to 54 days post-breeding. Mineralization is evident earlier on lateral than on ventro-dorsal projections. In the last 15 days of pregnancy, radiography has been reported to be 100% accurate for pregnancy diagnosis. Foetal skeletons are visible with high accuracy only by the sixth week of pregnancy although they may be sometimes visible as early as 23-25 days of gestation (Toal *et al.,* 2005). The foetal skulls are visible by day 45 and the entire fetal skeleton is visible by the end of seventh week of gestation. The accuracy of radiographic diagnosis is dependent on the quality of radiograph obtained. Radiography is generally suggested for bitches, whenever there is a doubt about the nature of the abdominal contents at or near whelping. The number and position of the foetuses can be detected easily by radiography at this time. Mostly, a single radiograph taken with the animal in lateral recumbency is sufficient. However, sometimes a dorsal or a dorso-ventral view may be required. Signs of foetal death as seen by radiography include the spalding sign (overlapping of the cranial bones), collapse of skeleton, gas shadows in the foetal heart, stomach, around the foetus and tightly flexed spine (seen in foetuses died for long time) (Jackson *et al.,* 2004). Degree of foetal mineralization can be used to assess gestational age and predict whelping date, if foetal teeth are visible on the lateral projection radiograph. For good radiograph, kvp should be 50 and current should be 0.5 – 1 Amp.

III. Ultrasonography

Ultrasonography is a safe and accurate modality for pregnancy diagnosis. Three types of diagnostic ultrasound are described in the veterinary literature for canine pregnancy diagnosis. They are A-mode, Doppler and B-mode. A-mode or amplitude depth ultrasound, identifies presence of fluid. It cannot define the origin of the fluid as definitively uterine nor does it allow assessment of fetal viability or number. Similarly, Doppler ultrasound provides an audible signal identifying fetal heartbeats, but gives no idea of fetal number or more exact information as to fetal viability. For these reasons, these two techniques are rarely used in the dog. B-mode or real time ultrasound allows assessment of pregnancy status, fetal number and viability and investigation of the uterus and extra-reproductive abdominal structures. For most dogs, diagnostic sonograms can be achieved with a 5.0 MHz transducer. Toy breeds may require use of a 7.5 MHz transducer. The visualization of earlier pregnancy or the visualization of a non-pregnant bitches uterus necessitate the use of probes of higher frequency (7.5 to 10.0 MHz) as the uterus lie more closer to the skin. Colour Doppler ultrasonography in bitches can detect placental fetal circulation (Blanco *et al.,* 2008). Ultrasonography can be used to visualise foetal vesicles from day 16-20 of pregnancy onwards (Shille and Gontarek 1985; Yeager *et al.,* 1992). Foetal heart beats can be seen, using real time ultrasound from day 24-28 of pregnancy onwards. Amniotic vesicles have been reported to be visible as early as days 16–20 of gestation, but as these small fluid-filled structures may be obscured by intestinal gas early in

gestation, most authors recommend that dogs be assessed for pregnancy no earlier than day 25 of gestation. B-mode ultrasonography has been reported to be 94–98% accurate for pregnancy diagnosis when used after 24–25 days of gestation and 99% accurate for pregnancy diagnosis at >28 d from the last breeding . Fetal heartbeats have been reported visible from 23 to 28 days of gestation. Fetal movement has been reported to be visible from 34 to 36 days of gestation. It is not a method of choice for assessment of litter size. The restricted viewing window created by the transducer and tortuous nature of the canine uterine horns preclude continuous evaluation of the horns individually. For assessment of fetal number, ultrasound has been reported to be accurate 31.8–36.0%of the time, with overestimation of small litters and underestimation of large litters.

Hormone assays

Pregnant bitches do not produce a pregnancy-specific gonadotropin, such as human chorionic gonadotropin (hCG) in women or equine chorionic gonadotropin (eCG) in mares. In the absence of a pregnancy-specific hormone, changes in other circulating hormone concentrations must be assessed.

1. Progesterone- Serum progesterone concentrations remains high in diestrus, regardless of breeding status. Maintenance of CL occurs because of absence of release of an effective luteolysin from the uterus as in other species after pregnancy recognition. Therefore, measurement of progesterone is of no value in bitches.

2. Prolactin: Serum prolactin concentration spontaneously rise in the later half of diestrus, with a significantly greater elevation in pregnant bitch than in non-pregnant by days 30–45 of gestation . Assays for canine prolactin are not commercially available at this time.

3. Relaxin: Relaxin is a hormone produced primarily by the canine placenta and is therefore, a pregnancy-specific hormone known in bitches. Serum relaxin concentration rise significantly compared to non-pregnant bitches, beginning at 20–30 days of gestation and peak at mid-gestation. An assay for canine relaxin is commercially available (WitnessTM, Synbiotics Corporation, San Diego, CA, USA). Pregnancy may be diagnosed as early as 21-day postbreeding. Relaxin assay cannot be used to estimate litter size.

4. C-peptide: C-peptide is a portion of prohormone for canine relaxin and is detectable in urine as relaxin is formed and degraded in the second month of pregnancy. Assay of this hormone in urine may be marketable as an easy in-house assay for veterinarians or an in-home test for clients.

5. Estrogen: Total estrogen concentrations in urine have been reported to be increased 21-day post-mating in pregnant bitches as compared to non-pregnant. Perfection of this technique may create a marketable in-house assay for veterinarians or in-home test for clients.

6. Acute phase proteins: Acute phase proteins are a class of molecules released in normal physiologic conditions, such as pregnancy and in the presence of inflammatory disease. The occurrence of inflammatory uterine disease during diestrus may confound ability of these tests to differentiate pyometra from

pregnancy. Changes in serologic concentrations of several acute phase proteins including haptoglobin, ceruloplasmin, alpha-globulin, C-reactive protein and fibrinogen, have been described . C-reactive protein concentrations peak at mid-gestation, and have been reported to be significantly elevated in 78% of pregnant dogs after Day 20 of gestation. Serum fibrinogen concentrations rise to >250 mg/dL by Days 21–30 of gestation. Assay of serum fibrinogen as a pregnancy test has been reported to be 98% accurate, with a value >280 mg/dL indicative of pregnancy and nearly 100% accurate with a value of>300 mg/ dL indicative of pregnancy.

Metabolic changes:

Decreased serum **creatinine and immunoglobulin G (IgG)** concentration have been reported in pregnant bitches 21-day post-breeding compared to non-bred. Serum creatinine decreased 25–33% and serum IgG decreased 40–45%. In bitches, decrease in antithrombin III activity has been reported during pregnancy as compared to non-pregnant at 30 days of diestrus. Pregnant bitches have been documented to exhibit normocytic, normochromic anemia that begins on 25–30 day of gestation and is maximal at term, with reported hematocrits of 29–35%.

General changes in pregnant bitch: The average bodyweight gain of a pregnant bitch from oestrus to parturition is 36% (range 20-55%), with the increase being most marked in the last 15 -20 days of pregnancy. A change in body shape is usually visible by about day 56 of pregnancy and foetal movements may also be noted around this time. The nipples enlarge and mammary development occurs during the second half of pregnancy and a serous secretion may be present shortly before parturition (Christiansen, 1984).

Ultrasonographic and radiographic features of early pregnancy in bitches

Sonographic Structure appearance(days post mating)	
Fetal fluid (amniotic vesicle)	18 – 20
Fetal Heart beat	24
Fetal bones	42 - 50
Radiographic features of pregnancy in bitches	42 - 45

Hormonal metabolic features of pregnancy in bitches

Relaxin (pregnancy specific hormone in bitches)	**20 -30 days**
Progesterone	Throughout pregnancy
Prolactin	30 -45 days
Estrogen	21 days
FSH	17 days
Acute phase proteins (fibrinogen,c-reactive proteins etc.)	20 days
Creatnine and immunoglobulin G (IgG)	21 days

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System of Wheat Intensification

– A Resource Conservation and Agro-Ecological Method of Wheat Cultivation

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Wheat is the most important food grain crop of the world. Wheat contributes more calories (20%) and protein to the world's diet than any other food crop. It is grown on an area of 29.04 m ha in India, producing 104.80 m t grains with average productivity of 3117 kg ha^{-1}. It is cultivated as a cereal food crop mainly in Uttar Pradesh, Punjab, Madhya Pradesh, Maharashtra, Bihar and Rajasthan. Wheat contributes most towards public distribution system (PDS) and has become a backbone of country's food security (Prasad and Gupta, 2012). During the past two decades, productivity gains from the usual wheat technologies with their substantial input-dependence have unfortunately been declining. Due to which, the small and marginal farmers are facing difficulties in coping with the high demand of costly inputs to meet the requirement of recommended technologies for increasing productions. Under these circumstances, alternative methods of crop establishment and management that could deal with these conditions – giving higher yield at less cost, with low water requirements and more resilience to climatic stresses – are desirable and should be evaluated.

System of wheat intensification, is an alternate method of wheat cultivation, popularly known as 'Sri Vidhi Gehun' in India. It is a new practice of wheat cultivation manipulating the soil environment favourably for better root and shoot growth using principles of SRI being practiced in paddy. It demands to maintain:

- wide spacing of plants for better light and air utilization,
- increased use of compost and organic matter in the soil,
- quality seed to be selected and treated using appropriate biotic and abiotic agents, and
- better soil aeration by use of mechanical weeder.

Principles of SWI

SWI is primarily based on these two principles of crop production –

1. Principle of root development: A well-established rooting system is

necessary for the proper development of crop plant. Root development is the first step of healthy growth and development of any plant. Thus, there is requirement of proper nourishment and sufficient space around the plant for healthy root development. So, wider spacing between plants is very crucial for proper growth and development of crop plants.

2. Principle of intensive care

Intensification does not mean high number of plant population per unit area; but comparatively it is proper space maintenance and taking care of plants very closely. So, there is need of intensive care in every stage of plant growth specially management of weed, insect, disease, organic manure and irrigation to enhance productivity sustainably.

Methodologies Used in SWI

The System of Wheat Intensification involves the following modified practices for achieving higher productivity which are based on the above principles –

1. Improved Seed: It can be applied for any kind of wheat variety, however, the local varieties used under existing practice are less productive as compared to newly release improved varieties. Therefore, selection of improved varieties will be crucial in increasing the productivity of wheat crop.

2. Seed Treatment: The seeds of wheat are usually treated with fungicides like Bavistin or Vitavax to control seed borne fungal diseases including smut followed by organic mixture of well decomposed compost, jaggery and cow urine for improving microbial activity in the soil.

Procedure for Seed Treatment:

- Separate the foreign materials from 10 kg seed.
- Make 20 liters of hot water (up to 60^{o} C) in a vessel.
- Put the seed in the hot water in the vessel and remove the floating seeds.
- Add 5 kg vermi-compost, 4 kg jaggery and 4 liters of cow urine; mix the seed properly and keep for 8 hours.
- Separate the seed mixture from the solution, sieving it through a cotton cloth after 8 hours.
- Add 20 gram Bavistin to the seed mixture and keep this for 10 hours in a wet jute bag for germination and further sowing.

3. Land Preparation

Conventionally, the farmers accumulate organic manure in open field for months and apply before final land preparation which results in the loss of nutrients through leaching and evaporation. In modern agriculture, the use of chemical fertilizers takes palace instead of organic manure. While the SWI emphasizes on efficient use of organic manure rather than chemical fertilizers because it helps to improve the soil health in addition to providing nutrients to the crop. If there is lack of moisture in the field, apply pre-sowing irrigation to maintain the moisture level in the field which helps in land preparation as well as germination of the seeds.

4. Seed Rate: In traditional method of cultivation, 100-120 kg of wheat seed is required for 1 hectare but seed rate is lowered to 20-30 kg per hectare under SWI.

5. Method of Sowing: Sustaining the plant to plant distance is very important for facilitating proper root development and tillering in wheat crop. So, the treated seeds with high germination rate are sown at the rate of 2 per hill in line at 20-

25 cm apart and 8 cm between plants, which saves large amount of seed and also reduces the cost incurred on it.

Seeds are sown in lines at a depth of 2.5 – 3 cm using seed drill. If seed drill is not available, strings or ropes are used for maintaining proper spacing. Moisture should be available in the field while sowing germinated seed.

6. Gap Filling: Somewhere the seeds have not germinated in the field, the gap should be filled with germinated seeds within 10 days of sowing. If there are more than two seeds germinated in one hill they should be uprooted properly to facilitate proper growth of the plant.

7. Irrigation: First irrigation should be done when root initiation starts i.e. at 15 days after sowing, because unavailability of moisture in soil prevents root initiation. The second irrigation should be given at 25 days after sowing, as tillers start emerging during this stage and third irrigation should be given at 35 days after sowing. If there is availability of irrigation water, then, subsequent irrigations are given at 60, 80 and 100 days after sowing.

8. Fertilization: Organic manure is applied before land preparation at the rate of 10 t ha^{-1} FYM or 4 t ha^{-1} vermicompost and incorporated in the soil by ploughing immediately. About $2/3^{rd}$ or half of nitrogen and potassium, and full dose of phosphorus should be applied at the time of last ploughing as basal dose and incorporated in the soil in the form of DAP (68 kg ha^{-1}) and MOP (34 kg ha^{-1}). Rest of the nitrogen should be applied in 2 split doses: about 40 kg urea at 15 DAS as root initiation starts and 20 kg urea + 15 kg MOP at 40 DAS i.e. during maximum vegetative stage.

9. Inter-culture and Weeding: Hoeing and weeding should be done; after the first, second and third irrigations; using cono-weeder to loosen the soil and to make the wheat field free from weed. The loosened soil results in better aeration for the root zone and increases the root length by letting them take more moisture and nutrient from the soil and thus helps in bringing more tillers in the plant with more vigour.

10. Crop Rotation with Legumes for increasing productivity: In most of the wheat growing areas, it is generally cultivated in rotation with rice, maize and millets year after year. Growing the same crops in the same field for many years depletes the soil health and also helps to build pests and pathogens in the cropland. Therefore, rotation with legume crops like soybean, mung, urd bean, groundnut, cowpea, cluster bean, dhaincha, etc. will help to improve productivity of wheat by adding nutrient to the soil and improving the soil properties.

11. Management of Organic manure: During 1960s, most of the farmers rely completely on farmyard manure (FYM) for fertilizing their field. It is for this reason that every household rears cattle for income generation and thus there is availability of FYM. But after green revolution, the application of chemical fertilizers had been increased and this results in the increase in yield of the crop for a certain period, but at the same time it also spoils the soil by destroying the soil properties. On the other hand, organic manures release the nutrients slowly and steadily in small proportion, therefore, the nutrients are available to crops for a longer period of time.

Table 1: Comparison between SWI & Traditional method of wheat cultivation

Parameters	SWI	Conventional Method
Seed rate (kg ha^{-1})	20-30	100-120
Seed treatment	Required	Not necessary
Method of sowing	Line sowing	Broadcasting
Spacing	20-25 cm × 5-8 cm	No proper spacing
Weeding	2-3 mechanical weeding by hoe or cono-weeder	Mostly chemical control
Fertilization	Organic manure such as FYM or vermicompost along with chemical fertilizers	Only through chemical fertilizers
Ear length (cm)	18	12
No. of ears per hill	53-40	2-5
No. of grains per ear	60-75	40-50
Average grain yield (kg ha^{-1})	3480 (32% over Conventional)	2630
Cost of production (Rs. kg^{-1})	8.17 (26% less than Conventional)	11.05
Stem, roots and leaves	Thick stem, long roots, wide and green flag leaves	Thin stem, short and superficial roots, pale green flag leaves

Thus, organic manures help in increasing the soil health along with increase in the microbial activity in soil.

CONCLUSION

The traditional wheat cultivation system requires more chemical fertilizers and nearly 100-125 kg of seed per hectare while SWI uses only 20-30 kg of improved seed in one hectare, with 20-25 cm spacing between rows and 8 cm between plants, organic manures and seed treatment through organic mixture to ensure higher yield. Adequate spacing between the plants and sowing of two seed grains at one point enables adequate aeration, moisture, sunlight and nutrient availability to the crop roots, leading to felicitous root system development from the early stage of crop growth. It helps faster growth and development of the plants. Only 2-3 times irrigation and weeding through cono-weeder save time and expenses on labour.

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Culling criteria in farm animals: An overview

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Abstract
Culling of less productive animals is very much essential for optimum genetic improvement. However, substantial involuntary removal in a herd might hamper the genetic improvement by reducing the replacement and productive life. The decision of culling impacts future herd performance and profitability. Knowledge of the reasons of culling helpful in developing breeding and management strategies. The culling due to health and reproductive problems is high and all efforts are required by to improve health and reproductive efficiency.

INTRODUCTION

For genetic improvement in the herd, it is necessary to remove the low producing animals and retain the high producing ones for producing future generations and to obtain replacement stock only from the selected cows (Hill, 1990). Culling and mortality together constitute disposal pattern among animals. Removal of cows through culling accounted for nearly 80% of disposal. Culling is the removal of undesirable animals from the herd to facilitate the entry of replacement heifers for improving the herd performance or to keep the herd size constant. It is practice to remove the unproductive animals and to obtain phenotypic and genetic improvement by retaining the best cows for future lactations and to obtain genetic improvement by breeding replacement stocks only from the selected cows. Any individual cow should be culled, regardless of her age, if there is a heifer available which is expected to outperform her. The rate of culling as reported by various workers in different breeds under different sets of managemental and environmental conditions ranged from 11.6 to 33.2 % per year. The removal of animals from the herd is either voluntary on the basis of low milk production or involuntary removal for the reasons such as reproductive problems, teat and udder disorders, disease and poor growth (Saha et al., 2012) etc. Voluntary culling is culling of a cow for low production irrespective of her health, where a healthy cow is replaced because her replacement is more productive. In general voluntary culling on the basis of low milk production was responsible for

20 to 50 % of total culling (Wakchaure et al., 2015). Whereas, involuntary culling is culling of a cow due to disease and/or low fertility or deformity regardless of her performance relative to her herd mates (Hadley et al., 2006). Although removal of less productive animals from the herd is likely to bring genetic improvement in the progeny, but substantial removal of animals by involuntary culling hampers the genetic progress by reducing the number of replacement. So, dairy producers need to focus on the causes of involuntary culling as these are the culls that greatly hamper profitability of a farm as well as animal well being.

The disposal of animals by culling comes under two categories, i.e., from birth to first calving and in adult cows i.e., after age of first calving. Dam's low yield, low birth weights, stunted growth and poor health were the major causes of culling from birth to first calving. The major reasons of culling after age of first calving were breeding problems, low milk yields, chronic debilitating disease and teat and udder disorders. The culling due to teat and udder disorder increases significantly as the parity order increases. Breeding problems (Silent heat, repeat breeding, ovarian cysts) and acetonemia increased with increased in milk production particularly in high yielding animals. Premature disposal of female calves before reaching milch herd and undesirable disposal of lactating cows are the major constraints in achieving larger herd size which is the primary requirement for genetic improvement of any breed (Singh et al., 2002).

Culling of unfit animals carried out periodically in three ways

A. Policy culling -

Reasons for policy culling

- Not true to bred
- Parentage not known
- Genetic defect
- Poor production/reproduction
- Surplus stock
- More than 12 years of age/calved more than five times
- Animals with vices and poor body weight gain

B. Veterinary culling -

Reasons for veterinary culling

- Animal with disorder refractory to treatment
- Became unsuitable for normal production life
- Weak and debilitated and confirmed cases of animal ailing from contagious or infectious or zoonotic diseases

C. Emergency culling –

Reasons for **e**mergency culling

- Animal involved in accidents, predator attack etc, suffering from non-specific diseases whose prognosis is grave and confirmed cases of TB, Johnes disease and Brucellosis .

Reasons for culling of adult dairy animals

1. Reproductive problems such as anoestrous, repeat breeding, irregular cycle adhesion of uterus, salpingitis, abortion, prolapsed and metritis.
2. Low milk production – It may be in due to below the farm standards, decline performance and Poor performance.

3. Udder problems - Poor udder development, milk let down problems mastitis and teat defective.
4. General debility - Poor health and weakness.
5. Locomotive disorder - Fet lock joint defective, hind leg defective, joint fracture, lameness and nervous problems.
6. Miscellaneous -Old age, leg wound, blindness and respiratory problems.

Culling criteria in other farm animals

Criteria for Culling Does

- Eliminate does with poor health and higher susceptibility to nematodes (worms) as compared to others in the herd.
- Avoid does that present frequent prolapsed uterine, or the eversion of the internal uterine layer to the outside the doe's vagina. These abnormalities may be attributed to the genetic makeup of the doe, or are common among does with a history of dystocia or difficult labor.
- Cull does with poor fertility rates, such as older does that are no longer reproducing; does that fail to reproduce in a production year; or does that require several services per conception. A reproductively sound doe needs no more than two services per conception.
- Remove does that fail to maintain adequate body condition.
- Eliminate does that have poor or lower milk production and are incapable of rearing kids to wean unassisted.

Criteria for culling of Bucks

- Eliminate buckling from the herd that displays poor conformation such as cryptorchidism (a genetic malformation where only one or no testicles descend in the scrotum), buckling with hypoplastia or undeveloped testicles and orchitis (an inflammation of the testicle(s)) . These conditions can cause sterility or a permanent incapacity to reproduce.
- Bucks having abnormality of the mouth such as an undershot or overshot jaw.
- Avoid bucks with feet problems such as laminitis and arthritis, which causes pain, reduces libido, and prevents copulation.

Criteria for culling of ram

- Which are not fit for breeding.
- Which are five years old or more.
- Whose fleece weight are below the average for that particular crop of lambs in that season
- Whose fleeces are not of required type.
- Whose merit in any other required characteristics are below the standard set up for the flock.

Criteria for culling of ewes

- Which did not lambs successively.
- Which cannot nurse their lambs.
- Which are more than 7 years old.

Herd evaluation techniques

Culling	
Culling rate	Total culled /total flock **<20%**
Percentage of animals culled due to disease	Total culled due to disease / total no of cows **<5%**
Percentage of animals culled due to production	No. culled due to production /total no of cows **<10%**
Percentage of animals culled due to other reasons	No. culled due to other reasons /total no of cows **<5%**

CONCLUSIONS

The genetic improvement in the herd to maintain a high level of herd performance involves timely removal of low productive animals from the herd. The involuntary culling rate not only makes the dairy enterprises economically less profitable but also reduces the genetic improvement by lowering the selection differential for milk production because of reduced herd size. The regular intensive examination of animal health status particularly udder and teat, genital tract and for general disease could perhaps reduce the involuntary culling to a large extent. The incidence of locomotive disorders could be brought down by improving the standing place of cows and by regular trimming of hoofs. The surface should be well corrugated and should not be slippery.

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Schmallenberg virus and its effect on Livestock- An Update

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Several European countries are currently experiencing the emergence of a previously uncharacterized arbovirus of domesticated ruminants, Schmallenberg virus (SBV). SBV was detected for the first time in November 2011 in plasma samples collected from cows displaying fever and diarrhoea, farmed near the town of schmallenberg, in Germany (Hoffmann *et al.,* 2012). Infected dairy cattle exhibited fever, reduced milk yield, loss of appetite, and diarrhea. Weeks after these signs appeared, epidemic abortions, births of malformed or stillborn animals and perinatal deaths of calves, lambs, and goat kids were reported (Hahn *et al.,* 2012). Metagenomic analysis identified a novel orthobunyavirus (family *Bunyaviridae*), termed Schmallenberg virus (SBV), which is closely related to Akabane and Shamonda viruses (Hoffmann *et al.,*2012*)*. These arthropod-borne viruses are well-recognized ruminant pathogens in Africa, Asia, and Oceania; fetal infection is associated with an arthrogryposis–hydranencephaly syndrome. The virus has spread through Europe, reaching the UK in late 2011. The full extent of Schmallenberg virus spread is currently unknown. However, according to the Department for Environment, Food and Rural Affairs (Defra), 83 farms (78 sheep farms, five cattle farms) in 14 English counties are now confirmed to have animals that tested positive for the virus. The virus has been detected in wide range of animals but impact primarily domestic ruminants such as sheep and cattle. SBV is spreading rapidly and extensively throughout Europe. There have been more than 8,000 farms with confirmed cases SBV in Europe from September 2011 – April 2013. Biting midges seem to play a key role in the transmission of the infection (Rasmussen *et al.,* 2012), and this transmission led to seasonal spread of the infection in summer and autumn 2011. So far, evidence has not shown that humans are susceptible to Schmallenberg virus infection (Reusken *et al.,* 2012).

AETIOLOGY

Classification of the causative agent

The "Schmallenberg virus" (SBV) is an enveloped, negative-sense, segmented, single-stranded RNA virus. It belongs to the Bunyaviridae family, within the Orthobunyavirus genus. The

Schmallenberg virus is a member of the Simbu serogroup viruses, which includes Shamonda, Akabane, and Aino viruses. The Simbu viruses which are most related to SBV are Sathuperi and Douglas virus. Field and laboratory studies indicate a causal relationship between SBV infection and the reported clinical signs.

Resistance to physical and chemical action

From extrapolation from the California serogroup of Orthobunyaviruses:

Temperature: Infectivity lost (or significantly reduced) at 50–60°C for at least 30 minutes.

Chemicals/Disinfectants: Susceptible to common disinfectants (1 % sodium hypochlorite, 2% glutaraldehyde, 70 % ethanol, formaldehyde)

Survival: Does not survive outside the host or vector for long periods

EPIDEMIOLOGY

According to the epidemiological investigations, reinforced by what is already known about the genetically related Simbu serogroup viruses, SBV affects ruminants. Serological studies indicate that it is not zoonotic.

Acute infections of adult ruminants or malformed Schmallenberg virus–positive offspring have been detected on more than 5,000 farms in Austria, Belgium, Denmark, Finland, France, Ireland, Germany, Italy, Luxembourg, Norway, Poland, Spain, Sweden, Switzerland, the Netherlands, and the United Kingdom. Also, a high proportion of adult ruminants were seropositive for antigens of the virus in the core region affected by Schmallenberg virus in the Netherlands, Germany, and Belgium (Garigliany *et al.,* 2012; Elbers *et al.,* 2012).

1.Hosts

In cattle, sheep,goats, bison, roe deer the disease has been confirmed by PCR or virus isolation. Serological confirmation has been done in red deer, alpaca, mouflons. Epidemiological and virological studies of human populations considered to be at risk did not demonstrate evidence of zoonotic potential.

2.Transmission

Epidemiological investigations indicate insect vector transmission. In terms of transmission routes, recent entomological investigations have identified SBV in field samples of biting midges of the *Culicoides obsoletus* group. (*EFSA, 2012*). Further information is required to determine whether mosquitoes play a role. Vertical transmission across the placenta is proven. SBV has been found in bovine semen. However, the potential for transmission by this route is still under study. Direct transmission from animal to animal is very unlikely.Further research is still needed to confirm these transmission routes.

2.1.*Viraemia and incubation period*

Experimental infection in cattle and sheep showed no clinical signs or mild symptoms at 3 to 5 days post-inoculation with an incubation period of between 1 and 4 days and viraemia lasting for 1 to 5 days.

Sources of virus

Material found to be positive in virus isolation (up to February 2013): Blood from affected adults and brain from infected fetus.

Material found PCR positive (up to February 2013): Organs and blood of infected fetus, placenta, amniotic fluid, meconium.

Occurrence

Some Orthobunyaviruses had previously been reported in Europe but viruses from the Simbu serogroup had never been isolated in Europe before 2011. Schmallenberg virus was first detected in November 2011 in Germany from samples collected in summer/autumn 2011 from diseased dairy cattle showing clinical symptoms of fever and reduced milk yield. Similar clinical signs (including diarrhoea) were detected in dairy cows in the Netherlands where the presence of SBV was also confirmed in December 2011. Since early December 2011, congenital malformations were reported in newborn lambs in the Netherlands, and SBV was detected in and was isolated from the brain tissue. Up to now, The Netherlands, Belgium, Germany, United Kingdom, France, Luxembourg, Spain, Italy, Switzerland, Austria and Ireland have reported stillbirth and congenital malformations with PCR positive results. In addition, further spread of SBV to other countries like Sweden, Norway, Finland, Poland and Estonia was reported recently.

DIAGNOSIS

Clinical diagnosis:

So far, most of the clinical detection was linked with the presence of congenital malformations or stillbirth. Manifestation of clinical signs varies with species: bovine adults have shown a mild form of acute disease during the vector season, congenital malformations have affected more species of ruminants (cattle, sheep, goat and bison). Some farms have also reported sheep and cows with diarrhoea.

In adult cattle, disease is probably often inapparent, but some acute disease during the vector-active season. Symptoms reported include fever (>40°C), impaired general condition, anorexia, reduced milk yield, diarrhoea, recovery within a few days, abortion. Arthrogryposis/ Hydranencephaly, brachygnathia inferior, ankylosis, torticollis and scoliosis were observed in malformed animals and stillbirths (calves, lambs, kids). The exact rate of malformation is not known and varies depending on the stage of gestation at the time of infection (OIE, 2012).

Lesions

The most frequent macroscopic lesions in animals that were SBV positive by qRT-PCR, especially calves, were arthrogryposis, brachygnathia inferior, torticollis, kyphosis, lordosis, scoliosis, and muscle hypoplasia (Herder *et al*., 2012). The predominant CNS lesions or conditions were cerebellar and cerebral hypoplasia, hydranencephaly, porencephaly, hydrocephalus, and micromyelia. Among the 8 small ruminants that were SBV positive by ISH (in situ hybridization method) , hydrocephalus was found in five, cerebellar hypoplasia in five, hydranencephaly in one, and arthrogryposis in six. For the two calves that were SBV positive, micromyelia and arthrogryposis were found in both and cerebellar hypoplasia in one (Hahn *et al.,* 2012*).* The symptoms can be summarised as arthrogryposis and hydranencephaly syndrome (AG/HE).

In an experimental study using NIH Swiss mice, histopathology of brains collected at 72 h post-infection revealed bilateral symmetrical vacuolation and loosening of the neuropil of the superficial cerebral cortex and the mesencephalon. Small

areas of haemorrhage within large areas of malacia were also found in the cerebral cortex In brains collected at 120 h post-infection, there was random multifocal vacuolation of the white matter of the cerebrum with small amount of nuclear debris. There was a minimal, multifocal perivascular infiltrate of lymphocytes in the adjacent grey matter. The presence of SBV was confirmed by immunohistochemistry using a polyclonal antibody against the N protein (Varela, *et al*., 2013).

Differential diagnosis

For the acute infection of adults: The symptoms are not specific. All possible causes of high fever, diarrhoea, milk reduction and abortion should be taken into account.

For the malformation of calves, lambs and kids: Other orthobunyaviruses, bluetongue, pestiviruses, genetic factors,toxic substances.

Laboratory diagnosis

Samples should be transported cooled or frozen.

- *From live animals for the detection of acute infection:* EDTA blood, Serum at least 2 ml, transported cooled.
- *From stillborns and malformed calves, lambs and kids:*

Virus detection: Tissue samples of brain (cerebrum and brainstem) , spleen, amniotic fluid.

- From live newborn: amniotic fluid, placenta, meconium.
- Antibody detection: pericardial fluid, blood (preferably pre-colostral).
- Histopathology: fixed central nervous system, including spinal cord.

Procedures

Identification of the agent

- Real-time RT-PCR (Bilk et al., 2012); commercial PCR kits are available
- Cell culture isolation of the virus: insect cells (KC), hamster cells (BHK), monkey kidney cells (VERO)

Serological tests on serum samples

- ELISA: commercial kits available
- Indirect Immunofluorescence
- Neutralization test

PREVENTION AND CONTROL

Vaccination

There is currently no specific treatment for Schmallenberg virus. Cattle-derived infectious serum is a viable and robust option for a standardized SBV infection model, which is required for vaccine evaluation and pathogenesis studies (Wernike *et al.,* 2012). In May 2013, the Veterinary Medicines Directorate announced that it had licenced MSD Animal Health to sell a vaccine for SBV named 'bovolis SBV' to be available in summer 2013.To achieve immunity cattle require two doses, and sheep one dose of vaccine. It is recommended that the course is completed at least three weeks before mating.

The Animal Health Veterinary Laboratories Agency (AHVLA) issued the following list of actions which could be taken to minimise the risk of SBV.

- Delaying tupping until midge activity is reduced
- Delaying breeding from ewe lambs until 2013
- Using products that repel or control biting insects prior to tupping and in early pregnancy
- Housing ewes inside
- Removing muck heaps to deny breeding habitats

Immunity

The ability to develop long lasting immunity has been previously observed in animals infected with the Akabane virus (AV), an orthobunyavirus closely related to SBV (EFSA, 2013). Pregnant animals that have been repeatedly infected with AV possess sufficient immunity to suppress transfer of AV to developing foetus. It is not currently known whether the analogy of SBV with AV is entirely appropriate. However, some preliminary studies show that animals re-infected with SBV following previous exposure do not develop further viral infections (EFSA, 2013).

Import restrictions

Numerous countries have imposed import restrictions on ruminant-based commodities import restrictions on ruminant based commodities including live ruminants, meat, semen and embryos. Countries with import restrictions on EU products due to SBV include Russian Federation, Egypt, Morocco, the United States, Uruguay and Brazil. USA banned import of bovine germplasm collected in EU countries after June 1, 2011.

Sanitary prophylaxis

Control of potential vectors during the vector-active season may decrease the transmission of virus. Reschedule of breeding outside the vector season should decrease the number of fetal malformations.

CONCLUSION

Schmallenberg virus is a livestock disease that was first identified in Germany in November 2011. Symptoms were initially observed in cattle, with adults showing brief signs of moderate disease. But months after these initial symptoms were reported in farms acrosss Europe observing severe pathology in newborn sheep, goats, and cows, including abortions, still births and malformations. Now SBV has been reported in most countries in Europe including across all of UK and Republic of Ireland. It has been identified in animals on more than 6,000 holdings, with cattle holdings more affected than those with sheep. SBV has been detected in several species including cattle, sheep, goats, horses, alpacas, wild deer and bison. SBV specific antibodies have also been detected in wild boar, fallow deer, roe deer, red deer and mouflons. Continued infections of SBV since 2011 show that the virus is capable of overwintering, and is likely to spread in unaffected regions, or those with a low prevalence of infection previously. The Schmallenberg virus has created serious problems for farmers across Europe, whose lamb populations are significantly declining as a result. The need for an effective new vaccine against SBV is urgent, but now that the virus can be isolated, grown and studied in the laboratory, there is genuine hope that future research will lead to greater scientific understanding about the virus. This will, in turn, allow for the possibility to develop treatments to curb the spread of this new disease.

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Relevance of Agricultural Land Use Planning for Indian Farming Community

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Among the natural resources land being one of the scarce resources affected by the competition among multiple uses and is very important constituents of agriculture. Thus, it is crucial for food and nutritional security of the country. Indian economy is predominantly agricultural oriented, hence, relevance of natural resources particularly land is increasing in the face of shrinking land per capita land availability. The land holding size is declining too rapidly on account of division and fragmentation of holdings generation after generation. Per capita land availability was 0.5 ha in 1950-51, which declined to 0.15 ha by the turn of the century and a projected further decline to less than 0.1 ha by 2020. Under such situation it is imperative to develop strategies and agricultural technologies that enable adequate employment and income generation, especially for small and marginal farmers who constitute more than 80% of the farming community (Jha 2003). Land use planning (LUP), especially in developing countries like India is one such strategy cum tool to provide answers to so many emerging issues including land degradation, increasing demand for food, fodder and fibre from the existing resources.

Therefore, sustainable land use planning is the need of the hour to manage the land resources effectively so as to ensure round the year income and employment for the farming family, in addition to higher crop production. Hence, it serves the purpose of food security for the burgeoning population and also secures livelihood for millions of marginal and small farmers of our country.

LAND USE PLANNING- DEFINITION

"Land use planning is a systematic and iterative procedure carried out in order to create an enabling environment for sustainable development of land resources which meets people's needs and demands. It assesses the physical, socio-economic, institutional and legal potentials and constraints with respect to an optimal and sustainable use of land resources, and empowers people to make decisions about how to allocate those resources" (FAO/UNEP1999). In other words, it is the systematic assessment of land and water potential, alternatives for land use and

economic and social conditions in order to select and adopt the best land use options.

Aims of Land Use Planning

It aims to make the best use of limited resources by –

- Assessing present and future needs of the resources and systematically evaluating the lands ability to supply them;
- Identifying and resolving conflicts between competing uses between the needs of the present generation and those of future generations;
- Seeking sustainable options and choosing those that can best meet out the identified needs;
- It aims at panning to bring about desired changes.

PRINCIPLES OF LAND USE PLANNING

- Land use planning aims at sustainability through balancing social, economic and environmental needs;
- Land use planning is an all inclusive process.
- Land use planning is realistic and oriented to local conditions.
- Land use planning considers local knowledge
- Land use planning takes into account traditional strategies for solving problems and conflicts.
- Land use planning is a process leading to an improvement in the capacity of stakeholders.
- Land use planning is future-oriented.
- Land use planning is an iterative process.

Relevance of Land Use Planning for India *vis-a-vis* Farming Community

In a developing country like India, land is not only an important factor of production but also the basic means of subsistence for majority of the people. Agriculture contributes less than 14 percent to India's Gross Domestic Product and provides employment to nearly 62 percent of the country's population (Economic survey 2014-15). The level of urbanization in India has increased from 17 percent in 1951 to 31% in 2011. The number of urban agglomerations, having a population of more than one million has increased from 5 in 1951 to 53 in 2011. This situation calls for scientific land use planning at various levels to address the issue of urbanization. The Draft National Land Utilization Policy, 2013 of India has the broad objectives, which includes and ensures optimal utilization of the limited land resources in India for achieving sustainable development, addressing social, economic and environmental considerations. It also ensures protection of agricultural lands from land use conversions so as to ensure food security and to meet consumption needs of a growing population and to meet livelihood needs of the dependent population.

ROLE OF LAND USE PLANNING

1. Land use planning as a tool of sustainable land use

Among the strategies, LUP as a tool of sustainable land use indicates the use of agriculture lands to best suited crops and permanent fallow and to other economic uses (Das et al. 2005). The sustainable use of land resources is emerging as an important issue in natural resource

management, to this end land use planning facilitates an opportunity to control land use. Optimum utilization of soil resources for enhancing agricultural productivity is possible only through sustainable land use. Land use planning is implemented in order to associate solutions for present problems (e.g. soil erosion, low agricultural production and low farm income in rural households) with the planning towards long-term conservation and sustainable use of land resources (Naidu et al. 2014).

2. Land use planning as a tool for food security

The land use planning as a tool for achieving food security can contribute to improving the availability of food within a defined region at local or national level in a number of ways which includes delineation of areas for food production and protection from being converted into other land uses such as construction and other commercial uses. According to World Food Summit (1996) food security existed when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life. Land use planning in combination with market analysis and infrastructure planning can improve access to food.

3. Climate change and land use planning

Land use planning has an important role to play in adaptation to climate change by assessing the vulnerabilities and impacts related to climate change. It also plays a pivotal role in mitigation of climate change by way of reducing forest degradation by limiting conversion of forests to pasture lands, infrastructure development, destructive logging etc.

4. Land use planning as a tool for farmer's participation in decision making

Land use planning is done at different level viz., national, state, district, block, watershed and village level. It involves local people's in preparation and implementation of land use plans and hence, give them a sense of participation and involvements in decision making of their natural resource conservation.

5. Land use planning as a tool for socio-economic development

Land use planning done at various levels, integrates land and other natural resource base information with the socio-economic condition of the of the farmers of the area so that socially acceptable and economically viable land use plans can be formulated and implanted for the larger area representing the majority of the farming community specially marginal and small farmers.

6. Land use planning as a tool for higher land productivity and crop production

As a tool it helps in allocation of land among different components of production, ensures better land use and increases the productivity of land per unit area. It puts the land under different uses according to land capability classes. The crops are also grown as per the soil suitability class of the particular crop, which ensures higher crop yields and sustainable crop production.

7. **Land use planning as a tool for employment generation and livelihood security**

Land use planning ensures allocation of land among different enterprises in such a way that these enterprises may yield round the year income and employment for the farming family. Various production systems viz., livestock, horticulture, crops and vegetables etc. ensures better land utilization and additional man-days in comparison to crop alone and hence ensures the livelihood security for the farming family.

Priority areas for land use planning

- Fragile agriculture and forest lands
- Desert and dry land areas
- Mountain ecosystems and key watersheds
- Inland waters and wet lands
- Coastal zones, and
- Peri-urban areas

CHALLENGES TO LAND USE PLANNING

- Development of sustainable and productive land use systems
- Protection of natural resources by establishing a balance among land, water and other resources use

Figure 1: Pictures representing various land use plans (LUP)

- An enabling political, social and economic atmosphere for the adoption of sustainable land use systems.

Thrust Areas for Land Use Planning

- Monitoring and assessment of land degradation in critical areas by remote sensing, GIS and other tools;
- Increased efforts in transfer of technology, research and development, and investments to address critical land degradation problems and promote sustainable land management.
- Capacity building in policy-making and land use planning approaches and raising awareness at all levels of causes and consequences of inadequate planning and management of land resources.

CONCLUSION

The relevance of natural resources particularly land is increasing to ensure food and livelihood security under dwindling per capita land availability. Land as a system, supports on it various physical and biological entities of great significance. Increasing competition between agriculture and other sectors for land resources, conflicts over access and rights to land, water and biological resources affect food security, environmental balance and well-being of present and future generations. The challenge ahead is to develop and promote sustainable and productive land use systems. In this context, land use planning proves to be an effective tool which, not only manage the land resources sustainably but also ensures food and livelihood security by way of better land utilization, sustainable crop production and round the year employment generation.

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Vermi-technology: A need of the day

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Vermi-technology is a process by which all types of biodegradable wastes such as farm wastes, kitchen wastes, market wastes, bio wastes of agro-based industries, livestock wastes etc. are converted to nutrient rich vermicompost by using earthworms as biological agents. Vermicompost contains major and minor nutrients in plant available forms, enzymes, vitamins and plant growth hormones. It is very effective eco-friendly, cheap, and easy method of recycling biodegradable waste using selected species of earthworms.

NEED OF VERMITECHNOLOGY-

The need of vermitechnology in the respect of Indian conditions are-

1. India spend about Rs. 230 million per year for waste disposal alone, this expenditure includes the cost of collection, transportation and disposal.
2. Despite spending money on waste disposal, air and water pollution remain unabated in India.
3. In India atleast 60% of the solid wastes are organic in nature.
4. Casting of earthworm contains as much as 5 times more nitrogen, 14 times more calcium, 3 times more magnesium and 11 times more potassium than that of 15 cm. top soil.
5. Large amount of humic substances are produced during vermicomposting and these have been reported to have positive effects on plant growth independent of nutrition.

Suitable species & desirable attributes of worms for vermitechnology-

There are four species of earthworms available in India, which are called manure worm. 1. *Eisenia foetida* 2. *Eudrilus eugeniae* 3. *Lumbrieus rubellus* 4. *Lumbrieus terrestris.* These can be cultured on animal dung, poultry, droppings and vegetables and other kinds of biodegradable wastes.

Desirable attributes of worms suitable for vermicomposting are-

- Worm should exhibit high biomass consumption together with a high efficiency of conversion of ingested biomass to body proteins, a physiological trait required for achieving high growth rate.
- Worm should have wider range of tolerance to environmental factors including adaptation to feed on a variety of organic residues
- Worm should produce large numbers of cocoons with short hatching time enabling rapid population growth and, linked to this rapid growth, faster composting of organic residues.

- Life cycle of the worm should be such that mature/adult phase is quickly reached.
- Using a mixture of species is likely to be more useful than use of single species.
- Worm should be disease resistant.

VEMICOMPOSTING

Vermicomposting is the biological degradation and stabilization of organic waste by earthworms and microorganisms to form vermicompost. This is an essential part in organic farming today. It can be easily prepared, has excellent properties, and is harmless to plants. The earthworms fragment the organic waste substrates, stimulate microbial activity greatly and increase rates of mineralization.

It has been estimated that earthworms add 230 kg N/ ha/ year in grasslands and 165 kg N/ha/year in woodland sites. Earthworms increase the nitrate production by stimulating bacterial activity and through their own decomposition. There are reports that concentrations of exchangeable cations such as Ca, Mg, Na, K, available P and Mo in the worm casts are higher than those in the surrounding soil.

Procedure to prepare vermicompost-

It is an aerobic, bio-oxidation, non-thermophilic process of organic waste decomposition that depends upon earthworms to fragment, mix and promote microbial activity. The basic requirements during the process of vermicomposting are

- **Bedding** - Bedding is any material that provides a relatively stable habitat to worms. For good vermicomposting, this habitat should have the following characteristics- high absorbency, good bulking potential and low nitrogen content (high Carbon: Nitrogen ratio).
- **Food Source -** Regular input of feed materials for the earthworms is most essential step in the vermicomposting process. Under ideal conditions, worms can consume amount of food higher than their body weights, the general rule-of-thumb is consumption of food weighing half of their body weight per day. Livestock excreta, viz., goat manure, cattle dung or pig manure are the most commonly used worm feedstock as these materials have higher nitrogen content. When the material with higher carbon content is used with C: N ratio exceeding 40: 1, it is advisable to add nitrogen supplements to ensure effective decomposition.
- **Moisture -** Perhaps the most important requirement of earthworms is adequate moisture. They require moisture in the range of 60-70%. The feed stock should not be too wet otherwise it may create anaerobic conditions which may be fatal to earthworms.
- **Aeration -** Factors such as high levels of fatty/oily substances in the feedstock or excessive moisture combined with poor aeration may render anaerobic conditions in vermicomposting system. Worms suffer severe mortality partly because they are deprived of oxygen and partly because of toxic substances (e.g. ammonia) produced under such conditions. This is one of the main reasons for not including meat or other fatty/oily wastes in worm feedstock

unless they have been pre-composted to break down the oils and fats.

- **Temperature -**
- The activity, metabolism, growth, respiration and reproduction of earthworms are greatly influenced by temperature. Most earthworm species used in vermicomposting require moderate temperatures from 10 – 35°C.
- **pH -** Worms can survive in a pH range of 5 to 9, but a range of 7.5 to 8.0 is considered to be the optimum. In general, the pH of worm beds tends to drop over time due to the fragmentation of organic matter under series of chemical reactions. Thus, if the food sources are alkaline, the effect is a moderating one, tending to neutral or slightly acidic, and if acidic (e.g., coffee grounds, peat moss); pH of the beds can drop well below 7. In such acidic conditions, pests like mites may become abundant. The pH can be adjusted upwards by adding calcium carbonate

METHOD OF PRODUCTION

1. Pit Method
2. Windrows Method

Pit Method-

- Pit method is commonly used for small scale production of vermicompost.
- Construct a pit of 3 x 2 x 1 m size over ground surface using bricks. Size of pit may vary as per availability of raw materials.
- Fill the pit with following four layers:

1st layer – sand or sandy soil of 5-6 cm. This layer helps to drain excess water from the pit

2nd layer - paddy straw or other crop residue of 30 cm above 1st layer which will be used for providing aeration to the pit.

3rd layer - 15 to 30 days old dung over paddy straw layer at a thickness of 20-30 cm. This helps in initiating microbial activity.

4th layer - pre-digested material about 50 cm.

Inoculate earthworm @ 1000 worms per square meter area or 10 kg earthworm in 100 kg of organic matter. Spray water on the bed and gunny bag. Maintain 50-60% moisture of the pit by periodical water spraying.

WINDROWS METHOD-

This method is widely used for large scale production of vermicompost. Important steps are-

- Load the organic wastes in the form of bed (preferably 10 feet L x 3 feet W x 1.5 feet H). Size of bed may vary as per availability of organic waste.
- After loading, the fresh bed should be covered with jute mate or dry agriculture wastes such as rice-bran, banana-leaf, maize residue etc.
- Sprinkle water over the covered vermibed to maintain 40% moisture in bed.
- Moisture percent can be checked by forming lump of organic waste using hand. it should easily form lump.

Watering of bed should be stopped for at least 2-3 days before harvesting. Earthworms go down in the moist soil and the compost is collected from the top without disturbing the lower layers of vermibed having earthworm. Vermicompost harvested will be of dark brown colour and free flowing.

Figure 1: Pit Method

Figure 2: Windrows Method

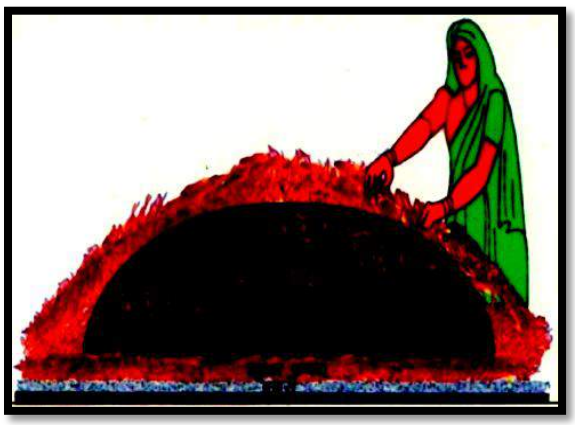

Figure 3: Loading of wastes

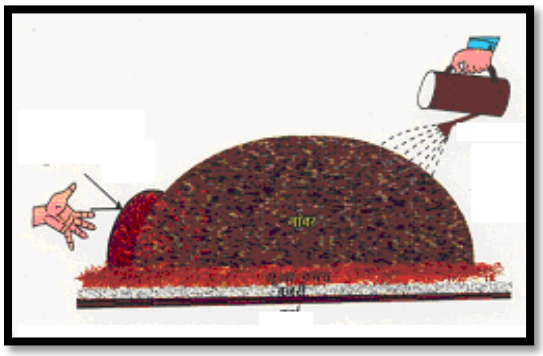

Figure 4: Watering of beds

Figure 5: Collection of vermicompost

Chemical composition of worm cast

Organic carbon	9.15 to 17.88%
Total Nitrogen	0.5 to 0.9%
Phosphorus	0.1 to 0.2 6
Potassium	0.15 to 0.256%
Sodium	0.055 to 0.3%
Calcium magnesium	22.67 to 47.6 Meq/100 g
Copper	2.0 to 9.5 mg L-1
Iron	2.0 to 9.3 mg L-1
Zinc	5.7 to 9.3 mg L-1
Sulphur	128.0 to 548.0 mg L-1

Vermiwash

Vermiwash is a liquid that is collected after the passage of water through a column of worm action and is very useful as a foliar spray. It is a collection of excretory products and mucus secretion of earthworms along with micronutrients from the soil organic molecules. These are transported to the leaf, shoots and other parts of the plants in the natural ecosystem. Vermiwash, if collected properly, is a clear and transparent, pale yellow coloured fluid. Vermiwash seems to possess an inherent property of acting not only as a fertilizer but also as a mild biocide.

Chemical composition of vermiwash-

pH	7.48 ± 0.03
Electro conductivity	0.25 ± 0.03 dS/m
Organic Carbon	0.008 ± 0.001%
Total Kjeldhal Nitrogen	0.01±0.005 %
Available Phosphate	1.69 ± 0.05%
Potassium	25 ± 2(ppm)
Sodium	8 ± 1(ppm)
Calcium	3 ± 1(ppm)
Copper	0.01± 0.001(ppm)

ADVANTAGES OF VERMITECHNOLOGY

- It restores microbial population which includes nitrogen fixers, phosphate solubilizers etc.
- Provides major and micro- nutrients to the plants.
- Improves soil texture and water holding capacity of the soil.
- Provides good aeration to soil, thereby improving root growth and proliferation of beneficial soil microorganisms.
- Decreases the use of pesticides for controlling plant pathogens.
- Improves structural stability of the soil, thereby preventing soil erosion.
- Enhances the quality of grains/ fruits due to increased sugar content.

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Synseed Technology In Fruit Crops- A Brief Note

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In fruit crops, seed propagation is not as successful and possesses various limitations. Limitations through seed propagation may be due to heterozygosity in the seed, small size of seed, non-endospermic seed, low germination rate, and also because in some fruits, there is no seed formation (pineapple, banana, mulberry *etc.*). These fruit crops are conventionally propagated through cutting, budding, grafting, layering *etc*. but these techniques are time consuming and cumbersome. Many species are desiccation sensitive (papaya, banana, citrus, *etc.*) or having recalcitrant seeds (mango, avocado, litchi, jack fruit, *etc.*). Distribution and exchange from field gene banks are difficult in this context. Also, genetic resources of tropical and subtropical fruit crops are difficult to conserve for further utilization. Plant tissue culture provides an effective alternative for such problem of fruit crop multiplication and their conservation. There is need of efficient technology, which facilitates faster multiplication of propagules with potent regeneration. In recent years, synseed or synthetic seed technology using encapsulation of non-embryogenic propagules derived under *in vitro* condition has become an important asset to micropropagation. It is a novel technique for mass multiplication of propagules and to conserve them for long duration. For commercial production of artificial seeds, enhanced production of propagules is mandatory. Current tissue culture practices are not able to produce adequate quantity of propagules for commercial exploitation of synthetic seeds technology. So, the problems related to synseed technology needs to be resolved in future research for proper utilization of this technique.

SYNSEED CONCEPT

Synseed or Synthetic seeds are defined as artificially encapsulated shoot buds, cell aggregates, somatic embryos or any other plant parts, which are having potential to become a plant as like normal seeds when sown in the growing media under optimum condition also it should retain this ability even after storage. Synthetic seed contains an embryo produced by somatic embryogenesis embedded within a synthetic medium that supplies nutrients for developing embryo and is packed up with artificial covering. This technique is designed to combine the

benefit of asexual propagation with those of seed (sexual) propagation and storage. The concept of synthetic seed was given by Murashige (1977), but Kitto and Janick (1982), published the first report on the development of artificial seeds. They described the production of synthetic seeds (desiccated) by using carrot somatic embryo which was coated with a mixture of polyoxyethylene glycol (Polyox) and water-soluble resin. Later in alfalfa Redenbaugh *et al.* (1984) successfully produced synthetic seeds by encapsulating somatic embryos with alginate hydrogel (hydrated).

CHARACTERISTICS OF SYNSEEDS

1. High volume, large scale propagation method
2. Maintains genetic uniformity of plants
3. Direct delivery of propagules to the field, thus eliminating transplants
4. Less cost per plantlets
5. Rapid multiplication of plants

IMPORTANCE OF SYNSEED TECHNOLOGY

Encapsulation technology is an exciting and rapidly growing area of seed biotechnological research. It has considerable impact on conservation and delivery of tissue cultured plants in a more economical and convenient way. It is a novel technique for propagation of elite genotypes, rare hybrids, genetically engineered plant, and threatened, rare and endangered plants for which seed availability is either difficult or expensive. The potential scope of synthetic seeds has shown in Figure 1. Synthetic seeds possess potential advantages as they are easy to handle (small structure), having genetic uniformity of plants and direct sowing to the field or green house and also fascinates economical mass multiplication of elite plant varieties.

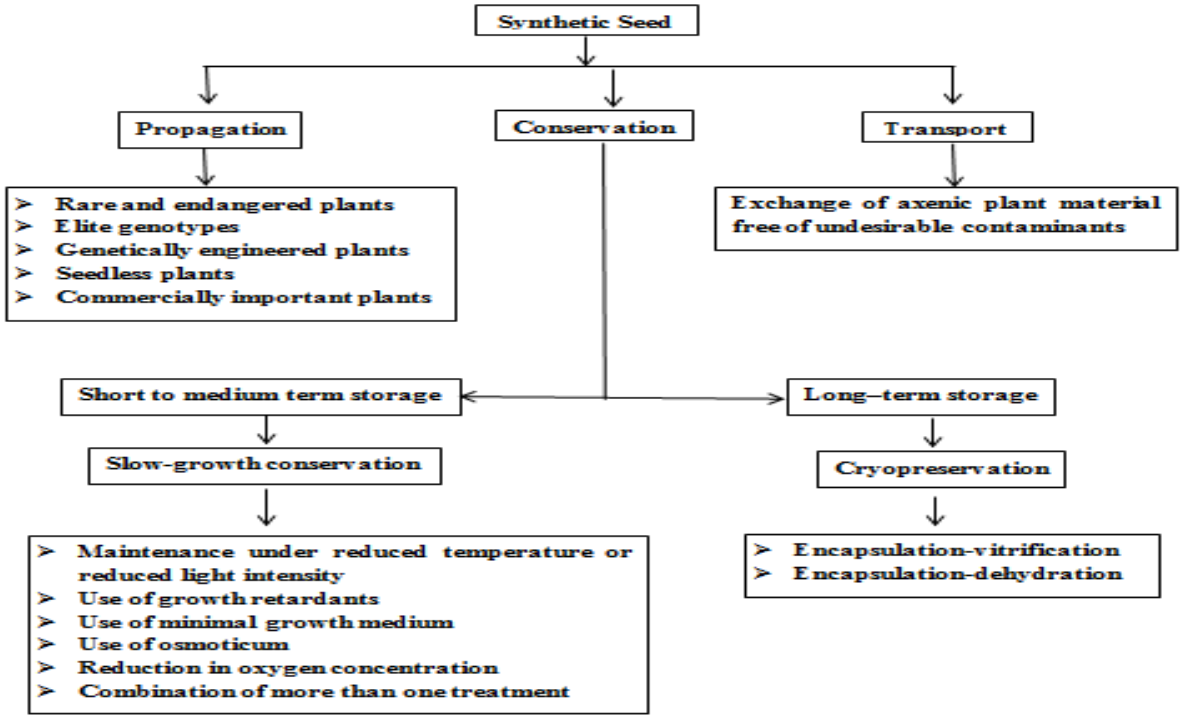


Figure 1: Potential uses of synthetic seeds.

Throughout the year availability of seed has become possible with synthetic seed technology whereas, most of tree plants produce seeds only in certain months of the year.

Conservation of germplasm is an important aspect of encapsulation technology. Propagules can be stored for a longer duration under *in vitro* condition in pathogen free environment. For short and medium term storage, the aim is to increase the interval between subcultures by reducing growth, which can be achieved by changing the storage condition or culture medium.

In recent years, new cryopreservation techniques like encapsulation–vitrification and encapsulation–dehydration has been developed for the production of artificial seeds. In several fruit crops the encapsulation dehydration technique has been tried.

TYPES OF SYNTHETIC SEED

It can be hydrated or dehydrated seeds depending on their desiccation tolerance.

1. Desiccated synthetic seed

The seeds are produced from somatic embryos either encapsulated polyoxyethylene glycol or naked followed by their desiccation. Propagules can be desiccated by reducing relative humidity in a chamber (slow process) or by unsealing the petri dishes and leaving them overnight to dry. So, this type of seed can be produced in plant species whose tissue (or embryo) can tolerate desiccation.

2. Hydrated synthetic seed:

In Hydrated synthetic seeds embryos are embedded in hydrogel capsules. Hydrated synthetic seeds are produced in those plant species where the somatic embryos are sensitive to desiccation i.e., recalcitrant. Different coating agents such as sodium alginate, potassium alginate, agar, sodium pectate, carrageenan, carboxymethyl cellulose with sodium alginate gelatin, guar gum, gelrite, *etc*. have been tried as hydrogels. Commonly sodium alginate is commercial in practice as it is having less cost, moderate viscosity, low spin ability of solution with quick gelation and no toxicity for propagules.

Steps involved in synthetic seed production:

The somatic embryos for synthetic seeds are produced in the lab through culturing of somatic cells and treating with different hormones to produce root and shoot through cell differentiation. The following are the different steps involved in artificial seeds production;

1) Establish somatic embryogenesis
2) Mature somatic embryos
3) Synchronize and singulate somatic embryos
4) Mass multiplication of embryos
5) Encapsulation of matured somatic embryos
6) Desiccation process
7) Field sowing

Steps: Somatic embryogenesis and synthetic seed production

A. Early stages of globular embryo development from embryogenic calli
B. Same as in **a** stage, embryos were further developed into torpedo shaped structures
C. Development of various stages of embryos
D. Two well-developed embryos
E. Synthetic seeds produced using torpedo shaped somatic embryos
F. Germination of synthetic seed

Synthetic seeds in some fruit plant species: case studies

A large numbers of reports were published regarding the development of synthetic seeds and their application in propagation and conservation of several fruit crops (Tables 1 and 2).

Table.1. Development of synthetic seed in some fruit plant species.

Plant species	Propagules encapsulated
Actinidia deliciosa	Apical and axillary buds
Carica papaya	Somatic embryos
Ananas comosus	Axillary buds
Citrus sp.	Somatic embryos
Malus pumila	Apical and axillary buds, Nodes, Shoot tips
Mangifera indica	Somatic embryos
Musa sp.	Shoot tips, Shoot apices, Somatic embryos
Psidium guajava	Somatic embryos, Shoot tips, Nodal segments
Punica granatum	Nodal segments
Pyrus communis	Shoot tips
Vitis vinifera	Somatic embryos
Pistacia vera	Somatic embryos and embryogenic mass

APPLICATION OF SYNTHETIC SEEDS

By combining the benefits of a vegetative propagation system with the capability of long-term storage and with the clonal multiplication, synthetic seeds have many diverse applications in the field.

1) Multiplication of non-seed producing plants, ornamental hybrids or polyploid plants

2) Propagation of male or female sterile plants for hybrid seed production

3) Germplasm conservation of recalcitrant species

4) Multiplication of transgenic

Table.2. Reports on synthetic seed based *in vitro* conservation of some fruit plants.

Plant species	Plant material	Conservation method (Slow growth/cryopreservation)
Actinidia deliciosa	ST	Encapsulation–dehydration
Ananas comosus	SBs ST SA	Low temp. Storage Encapsulation–dehydration Encapsulation–vitrification
Citrus sp.	SEs SEs EA SEs	Low temp. Storage Encapsulation–dehydration Encapsulation–dehydration Low-temp. storage
Fragaria ananassa	M ST	Encapsulation–vitrification Low-temp. Storage
Malus domestica	ST	Encapsulation–dehydration
Malus domestica (Rootstock M.26)	ST	Encapsulation–dehydration
Mangifera indica	SEs	Encapsulation–dehydration
Morus indica	Abs ST	Low-temp. Storage Encapsulation–dehydration
Psidium guajava	SEs, ST	Use of growth retardant (ABA), Use of minimal growth medium, Low-temp. storage,
Punica granatum	NS	Low-temp. Storage
Pyrus communis	ST	Encapsulation–dehydration
Rubus idaeus	ST	Low-temp. Storage
Vitis vinifera	ST Abs ECS SEs	Encapsulation–dehydration Encapsulation–dehydration Encapsulation–dehydration, Encapsulation–vitrification Use of growth retardant (ABA)

ABs—axillary buds, EA—embryonic axes, ECS—embryogenic cell suspension, M—meristem, NS—nodal segments, SA—shoot apices, SBs—shoot buds, SEs—somatic embryos, ST—shoot tips.

Synthetic seeds technology- Limitations and future prospects

- Limited production of viable micropropagules that are useful in synthetic seed production.
- There is loss of embryogenic potential with aging cultures.
- Asynchrous development of somatic embryos.
- Improper maturation of somatic embryos that makes them inefficient for germination and conversion in to normal plants.
- There is lack of dormancy and stress tolerance in somatic embryos that limit their storability.
- Soma clonal variations which may alter the genetic constituent of the embryos.
- Large-scale production of high quality viable micropropagules in a cost-effective manner.

FUTURE THRUSTS

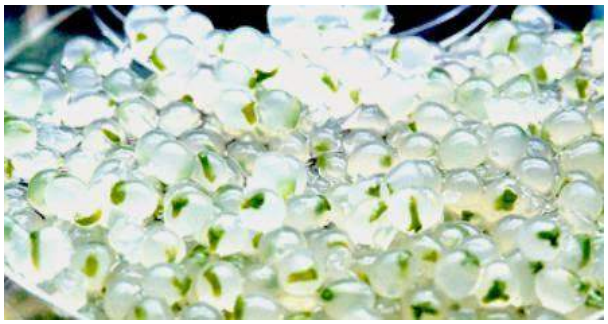
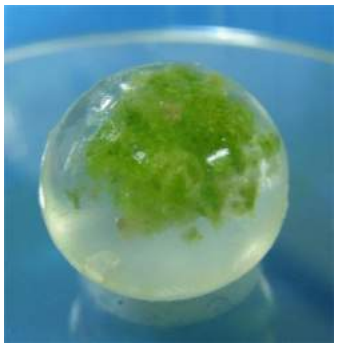
The problems related to synseed technology needs to be resolved in future research for proper utilization of this technique. Aside from the cost of developing encapsulating units, additional investments are necessary to develop methods and machinery for handling synseed, both during production and sowing. Although little progress has been made to demonstrate the feasibility of synseeds, their successful implementation can only be accomplished on a small scale while commercial use is still more of a concept than a reality.

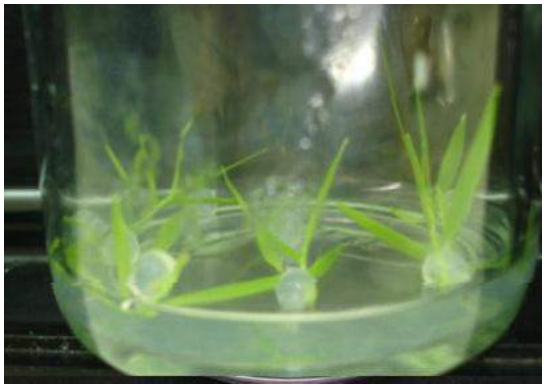
For mass multiplication of artificial seeds, enhanced production of propagules is mandatory. There is need to standardize methods for synchronization propagules development and subsequently automation of the whole process of sorting, harvesting, encapsulation and germination of the seed can enhance the foot step towards synthetic seeds production.

CONCLUSION

Synthetic seed technology is a new hope for fruit crop propagation and their long time conservation. As fruit crops being perennial in nature need large area to conserve them under field condition that also needs regular management practices and also vulnerable to genetic erosion, pest and diseases attack. A faster multiplication rate, easy handling and *in vitro* conservation led to develop synseeds in a number of fruit crops. For commercial exploitation of synseed technique of multiplication and conservation, there is need to standardize tissue culture protocol properly with synchronous embryo maturity and storage condition for synseed to conserve for long period with efficient regeneration potential.

Hereunder pictures are given in respect of synseed

Participatory land use planning for sustainable agriculture- A community centric approach

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Participatory land-use planning (PLUP) is a process of land-use planning that is carried out with the active participation of the community at large. The role of participatory approaches has recognized worldwide for the land management and conservation studies (Hoang Fagerstrom et al. 2003). Thus, participation should guarantee that the beneficiaries own interests has been considered while formulate the plan for local area. The intention of participatory approaches is to treat the people as central to development by involving them in interventions that affect them and over which they had limited control before (Bill Cooke 2001). The participatory development aims to enhance the involvement of people who are socially and economically marginalized in decision-making (Guijt and Shah 1998) ranging from planning, execution and implementation of the projects and programmes. Past few decades observed a shift in the rural development approaches i.e. reversal of approaches from top-down to bottom-up, centralized to local diversity and blueprint approaches to learning processes (Chambers 1994). The changes include from survey questionnaires to new participatory approaches involving outside agencies along with the local rural or urban communities.

Now a day's participatory land use planning is widely used approach to find out best possible solution for alternative land uses that would contribute to a balanced socio-economic development and livelihood of the local communities (Darabant 2013). ICAR-NBSS&LUP in its vision 2050 document laid greater emphasis on the increased applications of contemporarily relevant anticipatory and participatory tools and machine learning techniques in land use planning particularly the use of participatory rural appraisal (PRA) tools for farm level land use planning. The land use planning approaches involving local people's participation at grass root level ensures equity, gender sensitivity and social cohesion of the various communities in decision making over the use of natural resources such as soil, water and plants to ensure their optimum use. All these component of participatory land use planning works in unison with the sustainable development of the resources

and hence, it could rightly be considered as a tool for sustainable agriculture.

Participatory land-use planning (PLUP) – Definition and concept

Participatory land use planning is an iterative process based on the dialogue amongst all stakeholders aiming at the negotiation and decision for a sustainable form of land use in rural areas as well as initiating and monitoring its implementation. The objective of participatory land use planning is to achieve sustainable land use, that is, a type of land use which is socially just and desirable, economically viable, environmentally sound and culturally and technically compatible. It sets in motion social processes of decision-making and consensus-building concerning the use and protection of private, communal or public land (GTZ, 1999).

It is also defined as the process of land-use planning carried out with the active participation of the concerned community. It evaluates and proposes the best possible uses for land resources of the area in order to improve the livelihoods of the local population. Important land resources in a village include soil, water and plants, which are used for producing crops, livestock, timber, housing, drinking water, etc. Their optimal use depends on the biophysical conditions of the land, people's ability to utilize the land, people's socio-economic conditions and their expectations. It ensures suggest sustainable land-use options and thereby improved land use through systematic analysis of the potential and constraints of the natural resource base of the area. The implementation of PLUP is ensured through ownership over the process by the community and through reliance on local institutions.

Concept of PLUP

If land use planning teams works with the villagers, the community will be involved in the process of land use planning, the villagers will feel more committed to managing and protecting their natural resources particularly the land for agricultural. If the community at large is neglected in the process then they will probably view the exercise merely as a "government activity" and may hardly be involved in managing the natural resource base of the area. The implementing agencies of the PLUP should therefore, provide every opportunities for the village communities to participate in the land use planning activities. The participation of various agencies and village communities particularly farmers and other stakeholders those involved in the land use planning exercise is the basis of PLUP.

Need for participatory land-use planning

PLUP is considered an appropriate strategy to identify optimal solutions for land uses and conflicts among different land uses. In fact the need to enact land-use planning along with the implementation of the process is largely driven by the community. Therefore, participation of various socio-economic groups is necessary to ensure that the interests and expectations of all sections have considered in the planning process. Local community participation is essential in the land use planning process for the balanced socio-economic development of the area in general and the community at large in particular. It strengthens local

governance by ensuring the participation of local community from initiation of the planning process to its execution and follow-up, hence, it is considered as a powerful social tool for sustainable land management, capacity building and empowerment.

Participatory land-use planning is one of the most potent tool in terms of implementing the real research knowledge and technical intervention into a socially relevant context. Therefore, it assumes greater significance under present day problems and challenges faced by the farming community on account of dwindling land resources as well as their degradation. It proved to be effective in managing the natural resources sustainably, equitably and in technologically acceptable level with the active participation of the local communities and is considered as a pillar of sustainable agricultural development.

Methods used in PLUP

- GIS based participatory land use planning (Hessel et al. 2009).
- Pair-wise ranking matrix and sensitization of communities.
- Individual interviews and focus group discussion.
- Transect walks and village resource mapping were used for creating awareness and to collect primary data for participatory land use planning (Chaturvedi et al. 2015). Participatory resource mapping, historical timelines and semi-structured interviews were also suggested for PLUP.
- Group meetings and brainstorming
- Problem identification and ranking
- Village and households mapping
- Soil, vegetation and topographic surveys
- Community area delineation
- Participatory and result-based monitoring

Participatory land use planning ensures

- Interdisciplinary team approach for problem solving
- Participatory approach for targeting the community based issues related to land use
- Gender sensitization and participation in land use planning decisions

Advantages of PLUP

- Land-use plan prevents and solves conflicts over land resource,
- Facilitates discussion among social groups,
- Documents traditional land use rules and regulations,
- Ensures that interests of entire community are reflected,
- Improves ecological condition of land resources,
- Helps to develop new sources of income,
- Secures the resource base,
- Improves and empowers local governance,
- Improves accountability of the local administration

CONCLUSION

Participatory land-use planning involves local community hence, it is considered as a bottom up approach to find out best possible solution for land uses contributing to a balanced socio-economic development. It aims at involvement of people who are socially and economically marginalized in decision-making over

their resources. In fact implementation of the land-use planning process is largely driven by the community therefore, participation of various socio-economic groups is necessary to ensure that their interests and expectations are being served properly in the planning process. It is a powerful tool for sustainable land management, capacity building, gender sensitization and empowerment using effective techniques such as participatory rural appraisal (PRA) for farm level land use planning. In the years to come participatory land use planning approaches will be a potential tool for conflict resolution among multiple uses of land, effective and efficient utilization of local community resources for the benefit of the society at large (gender neutral, pro-poor) on sustainable basis, thereby ensuring a sustainable agricultural development.

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