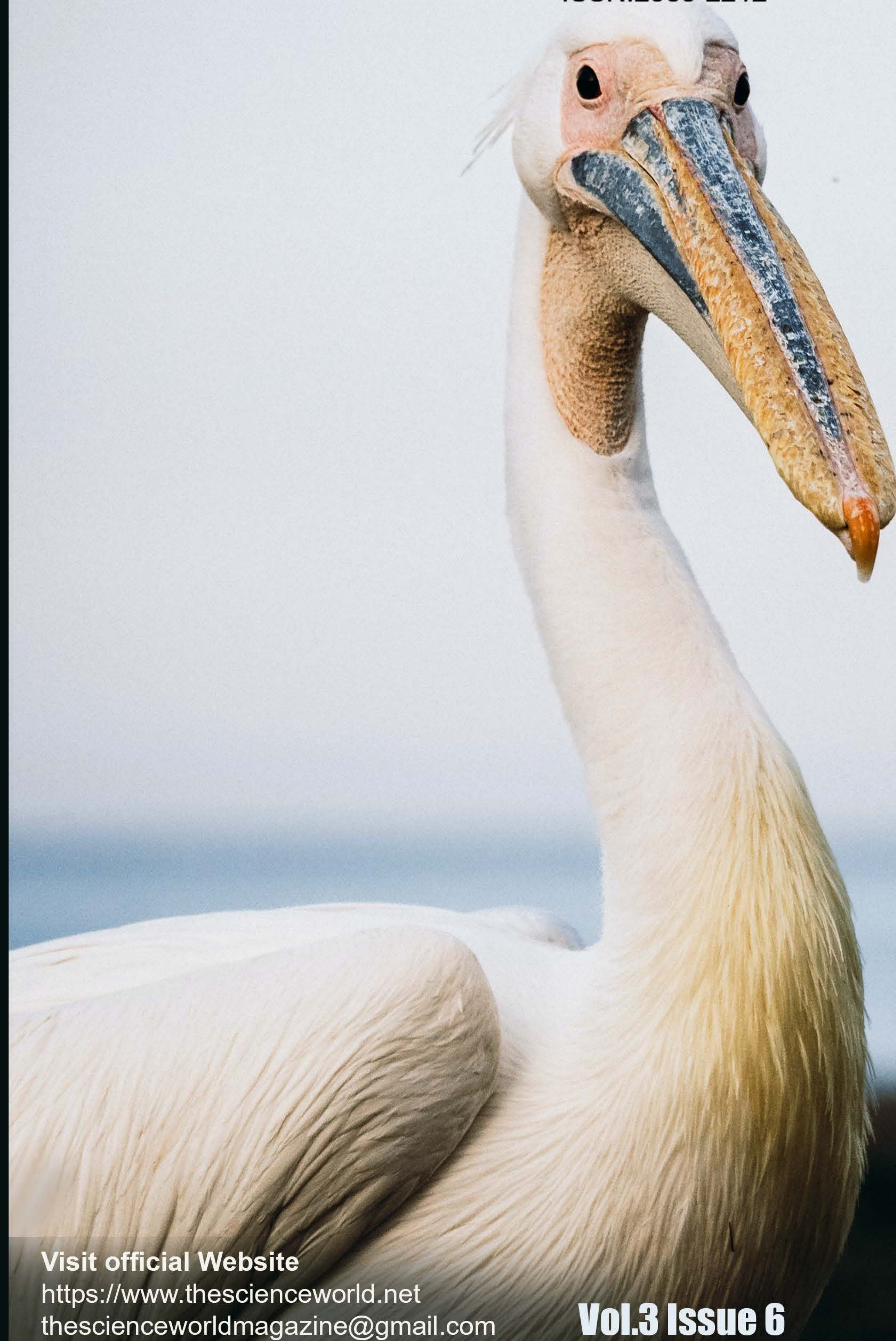


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**June, 2023**

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Best Article/Success Story/Original Article/ Caser study

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## Effect of estrogen and progesterone from cattle milk: Reason of early puberty in Female human

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Milk contains a variety of essential minerals, including calcium, protein, and vitamin D. Many people believe that it is necessary for a balanced diet. Others, however, have a number of reasons why they choose not to consume it. Milk and milk products come from a range of animals, including cows, sheep, camels, goats, and others. All of the following are appropriate milk substitutes: soy milk, almond milk, flax milk, coconut milk, and hemp milk. The customer and the type of milk they drink determine how healthy milk is. The high protein, low fat, and additive-free makeup of pasteurized milk makes it beneficial to a wide range of people. On the other hand, some flavored milks contain as much sugar as a can of soda. These choices are not intelligent. Today, cow's milk is more than just one product. In addition to other options, it can be raw, organic, hormone-free, fat-free, lactose-free, fortified with extra omega-3s, or long-lasting. Despite the fact that milk contains numerous hormones that affect female humans.

### **Nutrition**

The nutritional composition of milk depends on the amount of fat and whether or not it has been improved. Many US manufacturers now supplement their milk products with added vitamins. dependable source The 149 calories in one 244 gramme (g) cup of whole milk with a fat content of 3.5% to 3.8% come from 7.9 g of fat, 7.7 g of protein, 12.3 g of carbs, 276 mg of calcium, 205 mg of phosphorus, 322 mg of potassium, and 3.2 mcg of vitamin D. One 244 g serving of food has 83 calories, 0.2 g of fat, 8.2 g of protein, 12.4 g of carbs, 298 g of calcium, 246 mg of phosphorus, 381

mg of potassium, and 2.9 mcg of vitamin D. Both varieties of milk contain choline, magnesium, vitamin A, riboflavin, vitamin B-6, vitamin B-12, folate, and a host of other nutrients.

### **Health benefits**

The body can benefit greatly from the minerals found in milk. The sections below go into more detail about the special health benefits of milk. Milk provides calcium and vitamin D, both of which are good for bones. In fact, it might help prevent osteoporosis. A powerful antioxidant called glutathione is found in greater amounts in the brains of older people who consume more dairy products, according to many studies. Adults who had three daily servings of milk and milk products had antioxidant levels that were about 30% greater than those of adults who consumed less than half a serving.

### **Blood pressure and heart health**

The AHA states that consuming more potassium and less salt are essential for reducing the risk of cardiovascular disease. Researchers analyzed the data of almost 90,000 postmenopausal women, and their conclusions were published in 2014 Trusted Source. Around 25% of the women who consumed the most potassium showed a 21% lower risk of any type of stroke and a 27% lower risk of ischemic stroke. However, the saturated fat included in full-fat dairy products increases your risk of atherosclerosis and heart disease. For this reason, people who are at risk for stroke or cardiovascular disease should choose skim or low-fat milk.

### **Depression**

When vitamin D levels are sufficient, serotonin, a hormone connected to mood, hunger, and sleep, is created. A 2019 meta-analysis found that supplementing with vitamin D may help those with severe depression better manage their symptoms. However, the scientists wanted more studies to back up these findings.

### **Muscle building and weight loss**

With about 8 g of protein per cup, milk is a good source of protein. Protein is crucial for maintaining or gaining lean muscle mass as well as repairing bodily tissues. Getting enough protein in your diet can increase your muscle mass and hasten the healing of Trusted Source wounds. More research is needed to determine whether it can help people lose weight. If someone wants to lose weight, they should select skim or low-fat milk; if they decide to go with full-fat milk, they should be mindful of their overall daily caloric consumption.



## **Risks**

Some people do not drink dairy products like milk. These people may choose to adhere to a particular diet, such as veganism or paleo, in an effort to treat acne or other conditions, have a sensitivity to or allergy to lactose (the sugar found in milk) or casein (the protein found in milk), are concerned about the environmental effects of dairy farming practices, or believe that milk may contain oestrogen. Some people argue that since humans are the only species that continues to drink milk after weaning, milk drinking is unnecessary and unnatural.

## **Hormones in milk**

Some individuals worry that the oestrogen and growth hormones present in dairy milk can be harmful to them. Scientists found that mice who drank milk with high levels of oestrogen experienced hormonal changes in a study that was published in 2016<sup>Trusted Source</sup>. They do note that the amounts of oestrogen were far higher than those generally found in cow's milk. The assumption that the hormones in dairy milk can harm humans has not yet been supported by science in any way.

## **Cancer and Allergies, intolerances, and sensitivities**

Drinking a lot of milk may increase your risk of developing ovarian cancer, per certain <sup>Trusted Source</sup> studies. But the reasons behind this remain a mystery. For people who don't want to consume milk, there are dairy substitutes available. Many people consume milk without experiencing any unwanted effects. However, some people could react negatively. A milk allergy is distinct from lactose intolerance. If a person with a milk allergy consumes milk, they may have an abnormal immunologic reaction, in which case their immune system may produce allergic antibodies like immunoglobulin E. Some people may have nausea, diarrhoea, rashes, or passing blood in their stool after consuming milk. Anaphylactic shock, which can be abrupt and fatal, can result from a severe allergic reaction. If after touching or consuming milk, a person starts to bloat or has difficulties breathing, they require medical help right away. If you are allergic to milk, you should avoid dairy products including butter, whey, yoghurt, and cheese.

## **Lactose intolerance**

Some people have trouble digesting milk because their bodies do not create enough lactase. Lactase is an enzyme that breaks down lactose, a sugar present in milk. Occasionally, this might simply be a momentary problem, such if you have an infection. A person with lactose intolerance may experience bloating, gas, or diarrhea after consuming milk. However, the degree of lactose



intolerance varies from person to person. While yoghurt and hard cheeses, which contain trace levels of lactose, may be suitable for some people to consume, others may not be able to consume even a drop of milk in their coffee. Lactose-free milk has enzymes added to help with lactose digestion. This might lessen or eliminate these symptoms.

### **Casein sensitivity**

Casein is a protein found in milk. When drinking milk, those who are sensitive to casein may have irritation. Throughout the entire body, including the digestive system, trusted source. Anyone who suspects dairy products may be the cause of their issues might consult a dietitian. You could try an exclusion diet or be tested for food sensitivity if you're not sure whether a dairy-free diet is good for you. Low-fat dairy products without added sugar can be a nutritious addition to any diet, provided the consumer does not have an allergy or intolerance to them. In addition to calcium and other vitamins and minerals, milk also contains protein, which is crucial for healthy bones. However, other foods also contain these nutrients. Full-fat milk is not recommended for people who are trying to reduce weight or who are at high risk of cardiovascular disease. Flavored milks often contain significant amounts of sugar, artificial sweeteners, binders, coloring, and other ingredients. These are usually not good options. A dietitian or a doctor can be consulted by anyone who wants to drink milk but is unsure of whether it's a good idea.

### **Increased milk production in cattle**

The animal drug bovine somatotropin (BST), also referred to as bovine growth hormone, is permitted by the FDA to be used to increase milk production in dairy cows. Based on the somatotropin that cattle naturally make, this medicine. Milkmen administer larger oestrogen and progesterone doses to their calves in order to increase production for increased consumption. Their negative impact on your ward's puberty is stronger. When a child reaches sexual maturity sooner than is expected for its breed or population, it is referred to as early puberty.

The development of reproductive organs and the beginning of the estrous cycle are both significantly aided by oestrogen. Growing follicles in the ovaries mostly produce it. It promotes the development of secondary sexual characteristics including the genitalia, mammary glands, and the reproductive system. We can better grasp what controls when heifers become sexually mature by understanding how oestrogen effects early puberty.

Progesterone, which is essential for maintaining pregnancy and preparing the uterus for embryo



implantation, is released by the corpus luteum after ovulation. Another system that progesterone affects is the hypothalamic-pituitary-gonad axis, which controls the release of reproductive hormones. To comprehend how progesterone affects the time of cattle reproductive development, it is being examined how it affects early puberty.

Oxytocin, also known as the "love hormone," is widely known for its role in uterine contraction during breastfeeding and milk ejection following delivery. Recent studies have revealed that, among other aspects of reproductive physiology, oxytocin regulates follicular growth and the onset of puberty. Examining how oxytocin influences early puberty in cattle can provide information about its possible role as a signaling molecule in reproductive maturation.

### **Early adolescence in human female**

This type of early puberty is brought on by your child's body producing too much oestrogen or testosterone. The less common peripheral precocious puberty occurs in the absence of the brain hormone (GnRH), which typically signals the start of puberty. Between the ages of 8 and 13, girls often go through puberty, which lasts for several years. During this time, your body grows and develops. Early periods are typically brought on by hormonal changes, notably those that take place throughout puberty and menopause. In addition, a range of underlying medical conditions like PCOS and endometriosis can cause irregular menstruation.

### **Conclusion**

The hormone oestrogen, which is generated from cow milk, plays a crucial role in the onset and progression of puberty in human females by having an impact on the hypothalamic-pituitary-gonadal axis. Progesterone controls the timing of puberty by suppressing the release of gonadotropin-releasing hormone (GnRH) and delaying the activation of the reproductive axis. Despite being less well understood, oxytocin may affect the onset and timing of puberty in cattle, perhaps changing the growth and maturation of follicles. Early puberty is also regulated by the complex interactions between these hormones. The hormone oestrogen plays a crucial role in the onset and progression of puberty in female humans descended from cows through its effects on the hypothalamic-pituitary-gonadal axis. Progesterone regulates the onset of puberty by preventing the release of gonadotropin-releasing hormone (GnRH) and postponing the activation of the reproductive axis. Oxytocin, however it is less well understood, may affect the onset and timing of puberty in cattle, possibly affecting the growth and maturation of follicles. Early puberty can be managed in part thanks to the



complex interactions between these hormones. However, there are a few areas that require further research. Future study should focus on understanding the long-term implications of early puberty, understanding the role of environmental factors, integrating omics technology, and undertaking comparative studies across different breeds and species. In conclusion, there is a great deal of potential for managing cattle reproduction in the study of how oestrogen, progesterone, and oxytocin regulate early puberty in cattle. Breeding techniques, reproductive technologies, and our understanding of reproductive development will all benefit from more research in this area.





## A brief overview on toxicity effect of mercury in animals

Jay K. Desai and Gunjan

<https://doi.org/10.5281/zenodo.7998212>

### Poisonous Compounds

Mercuric oxide, mercuric chloride, mercuric iodide, mercuric cyanide, mercuric nitrate, mercurous chloride (calomel) and mercurous nitrate.

### Organic Preparations

Merbromin (mercuochrome), thimersol (merthiolate), nitromersol (metaphen), phenyl mercuric acetate, phenyl mercuric nitrate, mercurial diuretics (neptal, thiomersin sodium and mercurphylline), phenyl mercuric chloride (PMC), phenyl mercuric acetate (PMA), ethyl mercuric chloride, iodide and phosphate and methyl mercuric hydroxide.

### Occurrence

- I. Mercurial poisoning is relatively infrequent.
- II. Ingestion of seed grains treated with antifungal preparation containing mercury.
- III. Licking of organic mercurial antiseptics, accidental mercury poisoning in a mare with red mercuric iodide blister.
- IV. Inhalation of mercury vapor.
- V. Accidental administration.
- VI. observed mercury accumulation in tissues of pigs fed fish meat diet. Mercury concentration was highest in fresh tuna (0.153 mg/kg dry weight). calories, 0.2 g of fat, 8.2 g of protein, 12.4 g of carbs, 298 g of calcium, 246 mg of phosphorus, 381 mg of potassium, and 2.9 mcg of vitamin D. Both varieties of milk contain choline, magnesium, vitamin A, riboflavin, vitamin B-6, vitamin B-12, folate, and a host of other nutrients.

## **Pathogenesis**

Inorganic mercuric compounds are irritant to skin and corrosive mucous membranes. They cause extensive damage to mucosa of GIT leading to severe gastroenteritis. The absorbed compounds cause damage to the capillaries of the organs from where they are excreted, viz., kidney, colon and buccal cavity leading to nephrosis, colitis and stomatitis. Organic mercurial cause degenerative changes in central and peripheral nervous system. Primary lesions are segmental demyelination. Mercury showed a time dependent demethylation of methyl mercury in dogs with uniform distribution of converted inorganic mercury (up to 93%) in central nervous system, whereas muscles contained only 30% of inorganic mercury. Mercury was deposited in all areas of cerebral hemispheres, the brain stem and the spinal cord including nerve cells, astrocytes, microglial cells and vessel walls. There are no effects on -reproduction, unborn offspring, and semen. However, placental transfer of methyl mercury to the foetus in minks. Extensive degenerative changes in the seminiferous epithelium of ducks fed methyl mercury chloride. The intensity of the cytotoxic changes in the different organs is proportional to the amount of mercury accumulated.

## **Symptoms**

### **ACUTE FORM**

- I. Very sudden onset.
- II. Severe bloody diarrhea.
- III. Nausea and vomiting.
- IV. Anorexia, salivation, coughing and a foetid breath.
- V. Depression and muscular weakness.
- VI. Increased pulse and respiration rate.
- VII. Subnormal body temperature.
- VIII. Polyuria followed by anuria.
- IX. An ash grey color of oral mucosa.
- X. Death within a few hours from shock and dehydration.

### **CHRONIC FORM**

- I. Slow onset.



- II. Diarrhea, anorexia and salivation.
- III. Loss of body weight, weakness and anemia.
- IV. Muscular tremors, incoordination and head pressing.
- V. Blindness and posterior paralysis.
- VI. Slight rise in body temperature.
- VII. Weak, rapid pulse and respiration.
- VIII. Loosening of teeth and soreness of the gums.
- IX. Itching, loss of hair, formation of scabs around the anus, vulva and on the udder. Sometimes eczematous lesions on the skin.
- X. Tonic spasms in advanced stage.

#### **Post-mortem Appearance**

- I. Cooked appearance of the mucosa of mouth, tongue, pharynx and esophagus.
- II. Hemorrhagic gastro-enteritis and ulceration of the stomach.
- III. Perforation of the caecum and colon.
- IV. Swollen liver and kidneys.
- V. Catarrhal nephritis.
- VI. Congestion and oedema of the lungs.
- VII. Dark red blood which coagulates slowly.
- VIII. Hydrothorax and hydropericardium.
- IX. Formation of diphtheritic membrane in the large intestine.
- X. Hemorrhages in epicardium and endocardium.

#### **Chemical Tests**

- 1) **Reinsch test:** The procedure is the same as for arsenic. A bright silvery coating forms on the copper foil. On heating, mercury will volatilize and deposit as round globules of metal on the cooler part of the tube.
- 2) **Stannous chloride test:** Stannous chloride solution gives with mercuric salts a white precipitate of mercurous chloride. On adding more solution and heating, a grey precipitate of metallic mercury is formed.
- 3) **Potassium iodide** produces a yellow, then red, precipitate of mercuric iodide, soluble in



excess of the reagent.

- 4) **Diphenyl carbazone** gives a violet-blue color with mercuric salts in neutral or faintly alkaline solution.

### **Treatment**

- 1) Remove the poison from the digestive tract. A gastric lavage with 5 per cent sodium formaldehyde sulphoxalate.
- 2) Milk or white of egg should be given,
- 3) Sodium thiosulphate, 20 per cent solution, @ 25 ml/100 kg body weight.
- 4) BAL (Dimercaprol), 5-8 mg/kg body weight, I.M.
- 5) Normal saline solution, I.V. to check dehydration.



## Value Addition of Meat: A Push to Entrepreneurship

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While India has an abundant supply of meat, the meat processing industry is still emerging. Meat processing covers a spectrum of products from sub-sectors comprising animal husbandry and poultry farms, to bulk frozen meat, chilled and deli meat, packaged meat, and ready-to-eat processed meat products. In the present scenario, there is a large scope for meat processing in poultry as well as in red meat. In fact, the poultry industry has made considerable progress by developing and marketing value-added products.

Meat is an excellent source of high-quality protein and is considered as a vital part of human diet. Constituting around 20–24% protein, most of which is of high biological value, supplying essential amino acids, meat is gaining position in India food basket. Meat is also an important source of the vitamins, particularly B1 (thiamine), niacin (nicotinic acid), B2 (riboflavin), B6 and B12 (cyanocobalamin), and vitamin A (retinol). It is a major source of valuable minerals like iron, copper, zinc and selenium. Fresh meat provides key avenues for the producers and manufacturers, however, for boosting their income and profitability, value addition has been looked upon as a promising solution.

## Export potential

- The meat industry is slowly yet steadily catching pace on the global front also as India exports both frozen and fresh chilled meat to more than 60 countries of the world.
- Meat exported from India is risk-free, lean, nutritious and competitively priced meat.
- It has resulted in a consistent, high compound growth rate in export volumes.
- The major item of export includes de-boned and de-glanded frozen buffalo meat, which accounts for 97 per cent of the total meat export.
- The major market for Indian buffalo meat is Malaysia and Egypt and for sheep and goat meat are UAE, Iran and Jordan.
- India also exports a small quantity of processed meat to Thailand, Yemen, and Japan and poultry products to Saudi Arabia, Oman, Kuwait and Qatar.
- Uttar Pradesh state has emerged as the major exporter of buffalo meat followed by Punjab and Maharashtra.
- The value addition to the slaughterhouse by-products generates additional income as well as the costs of disposing of by-products can be minimized.
- There is huge potential in this sector for economic development of country through increasing exports so the policy makers should adopt critical measures at every stage to encourage and support this vital segment of the Indian agriculture.

The term "value addition of meat" refers to the process of enhancing the nutritional value, economic benefits, and health advantages of meat through the processing into a variety of designer and health-oriented meat products by incorporating a variety of functional ingredients and with the use of other techniques like tenderising the tough meat, preparing cut of parts, and utilising abattoir byproducts to develop valuable products. By replacing the current location, time, and form features of a product with ones that are more valued by consumers, its economic worth is boosted. The demand for value-added goods has been rising as a result of increased urbanisation, industrialization, income growth, nuclear families, education, and awareness. Due to these market factors, there are more options for product differentiation and value addition to raw materials, resulting in enhanced



industry profitability and viability as well as the potential to meet rising customer demands for convenience, convenience, and health.

**Advantages of value addition to meat:**

- Reduced preparation time and fewer processing stages, increasing consumer convenience.
- Sustainable product demand, enhancements to product safety and characteristics like flavour and appearance. reduction of costs, use of by-products, and competitive pricing.
- To the meat industry's sustainability.
- A rise in the product's worth overall.

**Various common procedures adopted for value addition to meat and meat products:**

**1. Combining Meats:** Combining meats is desirable to make goods with added value since it complements and supplements the quality and availability of various meats and their by-products.

**2. Using non-meat ingredients:** When processing meat, various non-meat components are used for various purposes. While some nutrients help retain moisture and offer qualities of texture, flavour, and colour, others improve the performance of muscle proteins. These ingredients are selected based on economic, raw materials availability, consumer preferences, food safety concerns etc. The most common non-meat ingredients utilized in meat products preparation are salt, phosphate, nitrite, ascorbate, sugar, soya, whole egg liquid, refined wheat flour, skimmed milk powder etc. to impart different properties in developed products.

**3. Incorporating Vegetables in Meat Products:** Incorporation of seasonal vegetables such as cabbage, cauliflower, carrot, bottle guard, pumpkin, etc. in meat products would be advantageous to reduce cost of meat products, to provide fiber and flavonoids in meat products, to facilitate consumption of vegetables and to provide balanced and healthful diet meat products.

**4. Emulsion technology for meat products:** Emulsion type meat products are the most popular processed meat products. When lean muscle tissue, fat, water and salt are mixed together and subjected to high-speed cutting and shearing action, a batter is formed. This batter is called emulsion. Meat emulsions are prepared using lean meat, water, other curing ingredients and fat. Different popular emulsion-based meat products include value added products such as meat balls/koftas, meat burger patties and meat nuggets etc.



**5. Restructuring of meat:** Restructured meat products are generally prepared from less expensive cuts, tough cuts, meat trimmings or combination of these. Restructured meat products are becoming an important component of the meat industry due to benefits like convenience in preparation, less demanded meat trimmings, different shapes product with improved tenderness, juiciness and flavour characteristics at economic cost.

**6. Curing and Smoking of Meat:** Curing process involves addition of curing agents to the meat cuts for enhancement of colour, flavour and preservation. It is the treatment of meat with preservative chemicals that restrict or prevent the growth of spoilage bacteria and food poisoning bacteria. It is used together with processes that use heat, smoke or low temperatures to give the required shelf life of cured meats.

**7. Retorting:** Thermal processing of meat destroys microorganisms and enzymes responsible for food spoilage. Thermal processing of foods refers to application of heat to improve digestibility, texture, flavour and destruction of enzymes and microbial population there by increasing the storage life. Thermal processing in metal cans or retort pouches increases shelf life of products and decreases 50% processing time.

**8. Tenderness of meat:** Tenderness of spent animal's meat can be improved by electrical stimulation, proper hanging of carcass, use of chemicals and artificial tenderizers. A variety of products such as sausage, patties, nuggets, balls, slices can be developed utilizing meat and by-products from spent animals and birds. Electrical stimulation (ES) of carcass muscles soon after slaughter accelerates their normal decline in pH and may enhance tenderization during conditioning. A number of organic acids such as acetic, citric and lactic have been used to tenderize meat.

**9. Fermentation of meat:** Meat fermentation is a preservation method which results in unique and distinctive meat properties such as flavour and palatability, colour, microbiological safety, tenderness, and a host of other desirable attributes of this specialized meat item. It is influenced by many environmental pressures that need to be controlled to produce a consistent product. Fermented meat products rely on microbial fermentation and dehydration to develop their specified flavour and texture.

**10. Utilization of slaughterhouse by-products:** Non-utilization or underutilization of by- products not only lead to loss of potential revenues but also lead to the added and increasing cost of disposal





of these products. Besides pollution and hazardous aspects, in many cases, meat, poultry and fish processing wastes have a potential for recycling raw materials or for conversion into useful products of higher value. By-products such as blood, liver, lung, kidney, brain, spleen and tripe have good nutritive value. Other important products that can be prepared include leather, carcass meal, rendered fat, sausage casings, blood meal, horn and hoof meal etc.

### **Conclusions**

The meat processing industry in India is a burgeoning one with enormous potential. The total development of this industry requires enough support at all levels and in diverse forms. For the promotion of the meat processing industry, adequate financial and technical help from government and non-government organisations is crucial. Any of the aforementioned approaches can increase the value of meat. Making the business of producing and processing beef economically viable by making more money is urgently needed. Value addition to meat and animal products also encourages youngsters to have an entrepreneurial mindset so they can produce their own resources.





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Popular Article

## Clinical sign and post-mortem findings in the poisoning of phosphorus in animals

Jay K. Desai and Seema Kumari Bishnoi  
<https://doi.org/10.5281/zenodo.7998522>

Phosphorus along with calcium is an integral constituent of the animal body and is universally distributed in soil and plants. About 80% of the total body phosphorus is deposited in bones and teeth in combination with calcium while rest is primarily in organic combinations. Deficiency of phosphorus in diet result into rickets and osteomalacia is a common syndrome.

### Sources of poisoning

Though phosphorus poisoning is of rare occurrence in farm animals because of lack of exposure, but accidental ingestion of fertilizer whilst still in sacks or immediately after spreading (from clumps of fertilizer on cultivated land), fireworks. Baits containing lumps of white phosphorus for rats, pets or ants kept on the pastures or ingestion of rats poisoned with rodent baits or grazing of animals on the battle fields where certain explosives have been used. Phosphorus is occasionally added to fats and oils in finely divided form to aid in absorption; however, excessive ingestion of these fats and oils can also cause phosphorus toxicity. White or yellow phosphorus is hazardous, while red phosphorus is toxic yet inert. The potential for toxicity in phosphates is relatively minimal. Before poisoning symptoms show, these must be consumed in large amounts.

### Mechanism of toxicity

There is no precise mechanism for phosphorus-induced toxicity. However, phosphorus causes significant irritation of the gastrointestinal mucosa and causes gastroenteritis and diarrhea due



to its local caustic effect. Any phosphorus that is ingested circulates in the bloodstream as an element first before being oxidized to phosphate, which leads to hepatic degeneration.

### **Clinical signs**

In acute poisoning, the chief symptoms include salivation, nausea, vomiting, severe diarrhea with minor abdominal pain, fever, polydipsia, and polyuria. In per-acute toxicity, animals die after exhibiting strong abdominal pain, violent convulsions, severe CNS depression, and coma. The majority of poisoned animals also show signs of jaundice, hematuria, and oliguria before passing away from delirium, convulsions, and coma. Indicators of gastroenteritis occur within 1-2 hours, and depending on the level of toxicity, the disease lasts for 3–5 days. Pigs vomit wildly, and the vomitus is brilliant in the dark and emits a distinctive garlic aroma. However, without any other clear indicators, salivation in horses and paralytic-like paralysis in birds are seen. Chronic poisoning in animals is uncommon, but exposure to phosphorus-containing gases in industrial plants poses a serious occupational risk to people.

### **Post-mortem lesions**

1. Congestion and hemorrhagic inflammation of gastrointestinal tract.
2. In some of the cases, hydrothorax and oedema of other parts of the body.
3. Fatty degenerative changes with centrilobular necrosis in liver.
4. Fatty changes are also observed in kidneys and heart.
5. Inflammation of the mucus membrane of stomach and intestines.
6. Garlic like odor of the gastrointestinal tract contents,
7. Liver is enlarged, pale and yellowish in color.
8. Spleen is small and atrophied.
9. Extravasation of blood into subcutaneous tissues and muscles.
10. In birds, visible fumes of phosphorus can be appreciated on opening the gizzard.

### **Diagnosis**

1. History
2. Clinical symptoms particularly acute gastroenteritis
3. Post-mortem findings
4. Garlic odor of the vomitus and intestinal contents



5. Estimation of phosphorus in the blood, vomitus, intestinal contents and faeces.

Note: The specimens that will be sent for laboratory analysis shouldn't have any preservative added.

### **Differential diagnosis**

1. Inorganic poisonings (arsenic, lead, mercury) causing gastroenteritis and diarrhea.
2. Organophosphate compounds.

### **Treatment**

There isn't a known treatment for phosphorus poisoning. However, treatment involves administering activated charcoal, emetics, or purgatives in an effort to remove the toxin from the body. Saline purgatives should be used instead of oily ones because they do not favor phosphorus absorption. Give symptomatic treatment as well, such as gastrointestinal demulcents and astringents for gastroenteritis and excess fluid therapy to replace lost electrolytes and fluids. Give cardiac stimulants and an intravenous glucose infusion in shock- like circumstances.



## A brief overview on various types of snakes and their clinical management

Jay K. Desai and Seema Kumari Bishnoi

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**Venom:** It is the poison or toxin secreted by specialized glands of an animal.

**Venomous animals:** These are the species or animals that have a highly developed secretory gland or clump of cells that may produce a poison and distribute it through stinging or biting. Snake bite in animals generally occurs while grazing or hunting. Most of the cases of snake bite have been reported in dogs and horses, however, other species of animals are also affected.

Snake venom contains a complex cocktail of toxins, including cations like  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $Ni^{2+}$  as well as amino acids, polypeptides, glycopeptides, and biogenic amines. A peptide or polypeptide that operates mostly enzymatically and occasionally nonenzymatically is the most active part of the venom. In general, the majority of snake venoms cause either neurotoxicity, cardiotoxicity, or chemotoxicity. The enzymatic component of the venom is what mostly causes neurotoxicity.

There are more than 3500 different species of snakes out of which more than 400 have been found to be poisonous and dangerous. Most of the poisonous snakes have been found to belong to mainly six families:

1. Elapidae: Coral snakes, cobras, kraits, mombas.
2. Crotalidae: Rattle snakes, water moccasins, copper heads, bush master, pitvipers.
3. Viperidae: The old-world vipers and adders.
4. Hydrophidae: The true sea snakes.

5. Laticaudidae: Sea kraits.
6. Colubridae: Booms Jang, bird snake, red necked, keelback snake.

Toxicity due to snake bite generally depends on:

- a) Toxicity of the venom and the quantity of venom injected
- b) Ratio of animal i.e., size of the animal and venom injected
- c) Species of snake
- d) Location of bite
- e) Species of animal involved. On the basis of body weight, horses are most susceptible, followed by sheep >cattle >goat >dog >pig >cat.
- f) Prompt availability of the appropriate therapy

Venoms of snakes contain different fractions-necrotizing, anticoagulant, coagulant, neurotoxic, cardiotoxic and hemolytic fractions. The venoms of cobra and krait are mainly neurotoxic while that of vipers and rattle snakes are hemotoxic in nature.

**Clinical signs:** Salivation, hyperexcitability, mydriasis, hypoxia, gasping, recumbency, convulsions, and death within 2-4 hours are some of the clinical indications of a snake bite containing a neurotoxic. Calves also exhibit regurgitation of ruminal contents and paralysis of the tongue, esophagus, and larynx.

**Diagnosis:** A snake bite can be identified based on the history of a sudden death, by looking for fang marks, by localized swelling, by blood flowing from the bite site, by cyanosis, etc. Making a diagnosis is aided by identifying any snakes in the area.

### **General management of snake bite**

- I. Keep the animal undisturbed.
- II. Apply a tight tourniquet above the bite location to prevent the spread and additional absorption of venom.
- III. Cut the snake bite site in the direction of the blood artery, use suction, and inject 5% soap solution into the wound.
- IV. Inject antivenin, antibiotics and antitoxins.
- V. Give polyvalent antivenin intravenously if the snake has not been recognized, and also locally at the bite location.
- VI. Depending on the animal's weight, polyvalent anti-snake venom should be delivered intravenously at a rate of 0-20 ml/animal.



VII. Provide corticosteroids to manage shock and cardio-pulmonary abnormalities as supportive treatment.

Fatty degenerative changes with centrilobular necrosis in liver.

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## Tearless Onions

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### Introduction

Onion is now the world's second most grown vegetable after tomato. The market for low-pungency onions is escalating in the United States and the United Kingdom. The use of low-pungency onions now accounts for 15 % to -25 % of total onion consumption in the United States. Pungency has been correlated with the production of lachrymatory factor (LF) upon cutting or chopping. High pungency and LF-induced tearing generally provide a detrimental experience for consumers who prefer low-pungency types. Onions produce the irritating volatile sulphur compound known as syn-propanethial-S-oxide (allyl sulphate) and release it when ruptured onion cells are exposed to air. It stimulates the eye's lachrymal glands so they release tears.

The lachrymatory sensation is caused by a lachrymatory factor (LF), identified as propanthial S-oxide. The LF had long been believed to be spontaneously produced in the disrupted tissues of the onion from 1-propenyl sulfenic acid (1-PS), a breakdown product of *trans*-(+)-S-1 propenyl-L-cysteine sulfoxide. (PRENCSO) by alliinase. The discovery of lachrymatory factor synthase (LFS), an enzyme obligatorily necessary for converting 1-PS to the LF, raised the possibility of developing tearless onions by suppressing the LFS rather than alliinase. The LFS-suppressed tearless onions are expected to have



additional values because of the availability of 1-PS as a precursor of thiosulfinates, their derivatives associated with the characteristic flavor and the health benefits of onions.

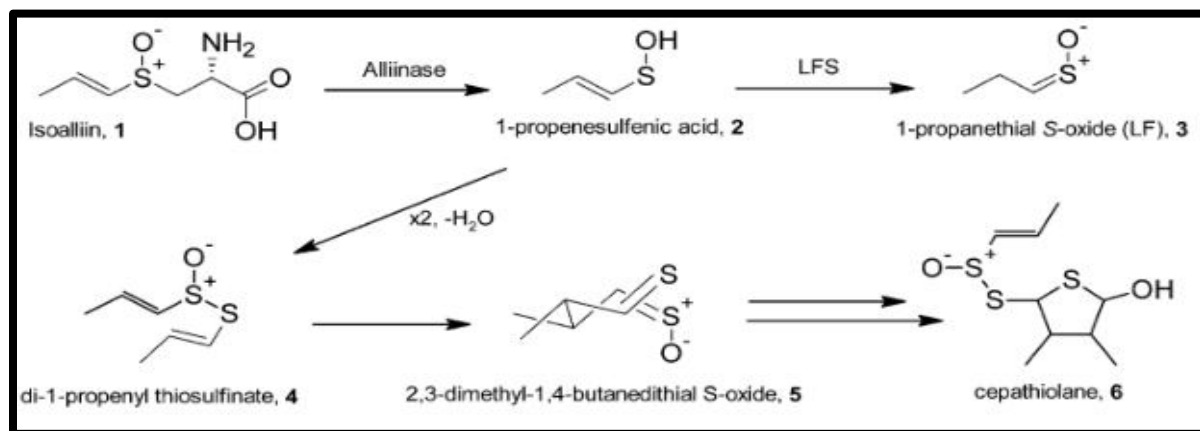
### Why do we need tear-less onions?

Onions are used daily in cooking and are an integral part of numerous dishes; despite this, everyone shies away from cutting onions.

- ✓ There is a huge demand for techniques and tricks to cut onions without tearing up (Wearing sealed goggles with a foam seal, chopping onion under water, chilling the onion)
- ✓ Extreme eye sensitivity to onions
- ✓ Sometimes, onion allergy symptoms may be observed, requiring medical attention.

### Tear-less onions

When LFS-suppressed onion is crushed, it produces increased amounts of thiosulfinates and several compounds called cepathiolanes. In tearless onion, isoallin would be predicted to produce **di-1-propenyl thiosulfinate** instead of propanethial S-oxide. Because the lfs enzyme is suppressed using different technology, resulting in increased thiosulfinates and disulfides.



**Figure 1. The main sulphur pathway following tissue disruption in tearless onions**

In garlic, similar reactions occur as in tearless onions, due to which tearless onions have some attributes and health benefits that are more similar to garlic.

National Onion Association recommends the best method to reduce tearing while cutting an onion, *i.e.*,

- ✓ Chilling the onions for 30 minutes
- ✓ Then, cut off the top and peel the outer layers leaving the root end intact.
- ✓ It is the root end that has the highest concentration of the sulphuric compounds



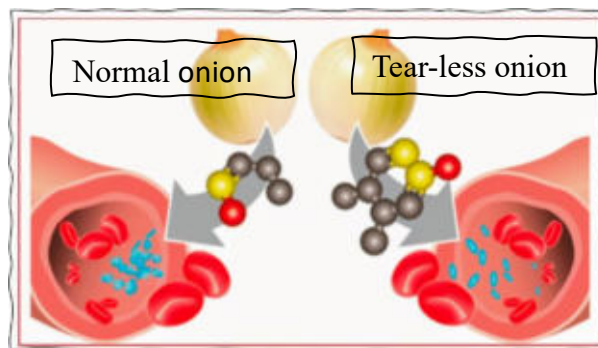
**Some of the other methods include**

- ✓ Using a very sharp knife while cutting onions. Because it causes the least amount of damage to cells
- ✓ Cutting under a kitchen vent/fan
- ✓ Freezing the onion
- ✓ Wearing goggles
- ✓ Soaking the onion under water while cutting
- ✓ Cutting off the base of the onion and throwing it away
- ✓ Microwaving onions before cutting



**Medicinal use of tearless onion**

- ✓ Reduce collagen-induced platelet aggregation
- ✓ Hypocholesterolemic effect
- ✓ Anti-Carcinogenic effect
- ✓ Anti-Asthmatic effect
- ✓ Anti-Pulmonary disorders
- ✓ Anti-Inflammatory effect
- ✓ Inhibit Cox enzyme activity



**Approaches**

Strategies for silencing LFS enzyme activity. There are three approaches to silencing LFS enzyme activity

**A. Gene silencing**

- *lfs*-gene knockout by homologous recombination-dependent gene targeting or mutagenesis
- *lfs*-gene transcript degradation by RNA interference



**B. By irradiating**

- Tearless onions can be produced non-transgenically by irradiating seeds



### C. Growing in sulphur deficient soils

- The onion should be grown in sulphur deficient soils so that they accumulate fewer secondary sulphur metabolites leading to decreased lachrymatory due to allinase mutation



### Achievements

#### Sunions- The onion that will not make you cry

- Sunions were introduced to the world in December 2017, bred by plant breeder Rick Watson
- They are not genetically modified products and were grown through an all-natural cross-breeding program over three decades by selecting less pungent strains of onion and selfing it
- Sunions have a sweet taste, mild flavor and crunchy texture and can be eaten raw
- Sunions become sweeter every day because the amounts of volatile compounds decrease over time
- Pyruvate levels fall from 5-6 to 3 or less during storage



#### Alibaug white onion gets GI tag for health benefits

- White onion of Alibaug in Maharashtra's Raigad district was given GI tag to its unique sweet taste, no-tears factor and for its medicinal properties
- Have low pungency, low pyruvic acid, high protein, fiber & high antioxidant compounds

#### Health benefits

- It boosts immunity
- Helps with insomnia
- Blood cleaning
- Blood pressure
- Heat-related ailments



#### Hindrances

- Reduced antimicrobial, antifungal and wound healing properties along with the reduction in pungency and lachrymatory effect
- Field tests of tearless onions show that they are more prone to diseases and pest
- Currently low, pungency onions have poor storability



## Conclusion

With increasing consumer preference, the future must produce tearless onions to avoid the irritating pungent onion chopping. Gene silencing plays a great role in the production of tearless onion with the use of RNA interference and irradiation with desirable mutagen LFS enzyme inhibition by competition with LFS inactivating protein is another way to achieve the LFS silenced bulbs. We can get tearless onion and chop it happily with the above-mentioned techniques.



# Effect of Heat Stress on Female Reproduction in Dairy Animals

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## Abstract

Heat stress impairs fertility and reproductive function in livestock by impairing the physiology of the reproductive tract, resulting in hormonal imbalances, lower oocyte quality, and decreased embryo development and survival. Heat stress reduces luteinizing hormone and estradiol release, resulting in shorter and weaker estrus expression, an increased incidence of anoestrus, and silent heat in agricultural animals. Thermally stressed oocytes lose their ability to fertilise and grow into the blastocyst stage, resulting in lower fertility due to the creation of poor-quality oocytes and embryos. Low progesterone secretion also inhibits endometrial processes and, as a result, embryo development. Furthermore, increased release of endometrial prostaglandin F2 alpha under heat stress jeopardises pregnancy maintenance. Heat stress in animals can be reduced by implementing scientifically sound measures that include environmental change and nutritional management. Furthermore, advanced reproductive technologies such as hormone therapies, timed artificial insemination, and embryo transfer may be used to combat summer infertility in farm animals, potentially increasing the chances of establishing pregnancy.

**Keywords:** Heat stress, dairy animals

## Introduction

The female reproductive process is hampered by high environmental temperatures at various stages during pubertal development, conception, and embryonic death. Stress decreases livestock species' reproductive function by activating the hypothalamic-pituitary-adrenal (HPA) axis, which then activates the pituitary gland, causing it to release adrenocorticotrophic hormone (ACTH). The ACTH hormone induces the release of glucocorticoids and catecholamines, which help to relieve



stress. The reproductive axis, on the other hand, is inhibited by ACTH-stimulated glucocorticoid production.

In the antral and pre-antral follicles, heat stress lowers the length and intensity of estrus, changes follicular development, and raises the rate of apoptosis. Furthermore, heat stress during follicular recruitment inhibits subsequent follicular growth and development leading to delayed ovulation. Changes in follicular development obstruct the oocytes' ability to proceed and function. Chronic ACTH release, such as that caused by heat stress, reduces ovulation and follicular development by affecting the efficiency of follicular selection and dominance, and glucocorticoids are important mediators of this reproductive inhibition. Furthermore, a high level of glucocorticoids during heat stress directly slows oocyte meiotic maturation, and corticotropin releasing hormone (CRH) suppresses ovarian steroidogenesis, which is caused by a decrease in luteinizing hormone release (LH). As a result of the drop in estradiol, the length and intensity of estrus expression is reduced.

### **Effect of heat stress on reproductive hormones in female livestock**

Reproductive hormones play an important part in the female reproductive system, since they govern various stages of growth and function. The hypothalamic-hypophyseal-ovarian axis may be influenced by high ambient temperature and solar radiation as a result of climate change. Several studies have also discovered a substantial negative relationship between environmental temperature and reproductive hormone concentration, which results in reduced reproductive efficiency in farm animals. The gonadotropin-releasing hormone (GnRH) from the hypothalamus and the gonadotropins (FSH and LH) from the anterior hypophysis are the two most essential components that govern ovarian activity. GnRH-induced FSH secretion is found to be significantly reduced in cattle after a 16-hour exposure to a higher temperature (40°C) on day 12 of the estrous cycle, although tonic FSH secretion was raised, likely due to reduced inhibition of negative feedback from smaller follicles. Low tonic LH levels also affect luteal development by reducing follicular growth, whereas heat stress reduces LH pulse amplitude and frequency in cattle with low estradiol, compromising the maturation and ovulation of dominant follicles. Furthermore, during heat stress, a decrease in pre-ovulatory LH secretion reduced the expression of estrus behaviour and delayed ovulation.



### **Effect of heat stress on follicular growth and development**

When the core body temperature rises above 40°C, it causes harm to the developing follicles. Heat stress interrupts oocyte growth, lowers the growth of dominant follicles, and increases the growth of subordinate follicles by lowering steroid hormone release, which disrupts oocyte growth. Furthermore, heat stress is also linked to reduction in follicular dominance by causing the formation of multiple big follicles with diameters greater than 10 mm, as well as a prolonged dominance of ovulatory follicles. As a result, high tonic follicular stimulating hormone (FSH) availability may disrupt the selection and dominance of normal follicles. The formation of small dominant follicles under higher temperatures results in ovulation of the infertile oocyte or sub-functional corpora lutea, as prolonged follicular dominance impairs normal oocyte maturation and lowers developmental competence. The premature dominant follicle's regression before it reaches the greater size results in a significant fall in ovulation percentage.

### **Effects of heat stress on estrus incidences**

The photoperiod controls the seasonal cycle of reproduction in female animals, and it has been discovered to be greatly influenced by climate change. Heat stress has been shown to have a deleterious impact on estrus incidence and duration, and thus on estrus detection in several investigations. The duration and severity of estrus are inversely related to ambient temperatures, with greater temperatures causing an increase in anestrus and silent heat in farm animals. When compared to winter (23.4 days), the inter-estrous interval in Japanese black cattle was found to be significantly shorter during the summer (21.5 days).

### **Embryonic growth and development**

Another key factor affecting cattle fertility is embryonic loss, and bovine embryos are susceptible to maternal heat stress during the first two weeks following breeding. The negative effects of increased body temperatures on developing zygotes and embryos may be a primary source for a loss in embryonic survival caused by heat stress. During egg maturation and ovulation, as well as the first 3–7 days of pregnancy, high ambient temperatures affected embryonic viability and development. Although increased temperatures have an effect on embryos in the pre-attachment stage, the effect diminishes as the embryo develops. Heat stress induces embryonic death through



interfering with protein synthesis, oxidative cell damage, a decrease in effective pregnancy detection, and the production of apoptosis-related stress genes.

### **Pre-partum period and days open**

The dry period is a crucial time for mammary gland involution, rapid foetal growth, and lactation induction, as well as subsequent mammary development. Heat stress in cows inhibits the release of placental hormones, which can have a deleterious impact on intrauterine foetal growth and milk output. Heat stress during pregnancy can alter endocrine responses, which can lead to an increase in foetal abortions, a shorter gestation period, a lower calf birth weight, and a reduction in follicular and oocyte maturation during postpartum estrous cycles. Pre-partum heat stress may lower thyroid hormones and placental oestrogen levels while raising non-esterified fatty acid levels in the blood, affecting udder and placenta growth, placental angiogenesis, food supply to the unborn calf, and milk production.

### **Mitigation strategies to ameliorate the impact of heat stress**

The consequences of heat stress on cattle result in significant economic losses for farmers, but there are few opportunities to recoup some of these losses by using appropriate heat stress mitigation methods. Physical adaptations of the environment, nutritional management, and genetic creation of breeds that are less sensitive to heat stress are three important critical components for maintaining the productivity of animals in hot environments. These tactics can be used singly or in combination to improve performance by providing the best possible environment for farm animals to work in. In addition, modern reproductive technologies such as gonadotropins, timed artificial insemination, and embryo transfer may be used to address summer infertility.

In general, livestock environmental management is a growing field in animal science that is receiving more attention in the face of climate change. It aims to provide an appropriate microclimate for optimum productivity by minimising negative environmental impacts on animal production systems. The primary methods of modifying the environment can be classified into two categories: (i) providing shade and (ii) using evaporative cooling techniques. During heat stress, environmental adjustments like as shade and cooling systems are crucial to maintaining milk production, milk component levels, reproductive function, and animal welfare in arid and semi-arid zones. The principles of providing shade may be traced back to efforts to reduce heat load in animals caused by





direct solar irradiation. Natural or artificial shade structures could be used. Because they protect from the sun and collect sunlight through evaporation of humidity in the leaves, trees are said to be the most cost-effective technique of providing shade. The cooling systems reduce the heat burden on livestock by combining water misting and forced ventilation through the use of spray and fans, and are commonly used within free-stall barns or under shade in open area. The adoption of an evaporative cooling system improves milk output and reproductive function in dairy cattle.

Amid order to maximise livestock output in changing climatic conditions, it is critical to ensure optimal nutritional levels for the animals. The need of providing adequate nutrition to enable optimum reproduction in animals should not be underestimated, as their energy balance is intimately linked to their fertility. Environmental temperatures are highest in arid and semi-arid locations, where available feed resources are both low in quality and quantity, negatively impacting livestock species' reproductive success. Combating the metabolic effects of heat stress is consequently critical, as animals suffering from mild to severe heat stress require an additional 7–25% maintenance requirement. Feeding feed additives promotes energy use while also stabilising the rumen environment. In addition, lipid content in the diet has a positive impact on cholesterol, progesterone, PGF<sub>2</sub> synthesis and metabolism, follicle growth, and pregnancy rates in dairy herds. Vitamin, trace element, and mineral supplements can also help to reduce the negative effects of heat stress. Injections of vitamin E and selenium lower rectal temperature and body weight loss in sheep during the summer. Inorganic chromium supplementation in the feed of buffalo calves reared in hot environments increased heat tolerance and immunological status without influencing nutritional intake or growth performance.

Hormonal treatments have the ability to reduce the consequences of heat stress in animals. The administration of GnRH during the early phases of estrus corresponds with the release of endogenous LH, resulting in a successful increase in the conception rate. On day 5 of the estrous cycle, a GnRH agonist or hCG injection causes ovulation or luteinization of the first wave dominant follicle, as well as the formation of an auxiliary corpus luteum (CL) that boosts plasma progesterone levels to compensate for persistent heat stress. When combined with a GnRH injection to promote a controlled recruitment of the ovulatory follicle, the timed artificial insemination (AI) programme increases summer fertility. This approach should be followed by a PGF<sub>2</sub> injection 7 days later to regress the CL and allow the ovulatory follicles to mature fully. Furthermore, 48 hours after PGF<sub>2</sub>,



a second dose of GnRH may induce ovulation and cow insemination at 16 hours, ensuring successful pregnancy. When paired with timed AI, the Ovsynch procedure successfully synchronised ovulation in buffaloes and boosted conception rate. Embryo transfer (ET) improves pregnancy rates in the summer because embryos are transferred after they are more susceptible to heat stress. Transfer of either frozen or unfrozen embryos produced by superovulation has enhanced pregnancy rates in cows exposed to heat stress when compared to AI.

## **Conclusion**

Increased temperature and relative humidity, as a result of climate change, will undoubtedly cause heat stress in all livestock species, affecting their reproductive potential. Fortunately, there are tried-and-true methods for reducing the effects of heat stress on animal reproduction. These include maintaining animals in climate-controlled environments, using scheduled AI algorithms to overcome poor estrus detection, and implementing embryo transfer programmes to avoid heat-related harm to the oocyte and early embryo. To help livestock cope with severe weather, management options such as strategic use of shade, wind protection, sprinklers, and ventilation in the heat should be explored. There are also some interesting research pathways that could lead to novel ways to improve reproduction during heat stress. Antioxidants are given and the growth axis is manipulated, among other things.



## The trend in change of saponification value during deep frying of ghee added with curry leaf (*Murraya koenigii*)

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### *Abstract*

The results showed a reduction in the saponification value of all ghee samples during frying. The control ghee decreased from 232.18 to 220.25, the BHA-added ghee decreased from 233.29 to 223.15, and the CLE-added ghee decreased from 232.88 to 226.46. These findings suggest that adding 1% CLE effectively prevents the decrease in saponification value during frying. Previous research also reported a decrease in the saponification value of ghee during frying, which can be attributed to the conversion of fatty acids into carbonyl compounds and the subsequent decrease in free fatty acid content.

### **Introduction**

Ghee, or clarified butterfat, is often made from cow or buffalo milk, or a combination of the two. Ghee ranks second in India's milk consumption habits behind fluid milk due to its superior frying and cooking capabilities (GAIN 2011). One of the most common culinary techniques used in both commercial and home food preparation is frying (Casal et al. 2010). Deep fat frying includes submerging food for a predetermined amount of time in heated edible oil that is above the boiling point of water (Farkas et al. 1996). The oil/fat undergoes significant changes during frying due to oxidation, polymerization, cyclization, and hydrolysis (Saguy and Dana 2003), all of which inevitably shorten the shelf life and negatively impact the final fried food's quality (Kochhar 2001).

Natural antioxidants are preferred over the legally permissible synthetic BHA, according to recent studies on ghee (Pawar et al. 2012 and Patel et al. 2013). According to previous reports, BHA does not function as a potential antioxidant at higher temperatures, which means that oils and fats containing it frequently degrade quickly when fried (Tsaknis et al. 2002). When ghee is deep-fried, Patel et al. (2013) already demonstrated that BHA is less stable than coriander extract. In order to ascertain the potential of curry leaf extract in ghee during deep frying, the current study was conducted.

### **Materials and Methods**

Ghee was cooled to 60°C to allow for the addition of individual antioxidants. The level of CLE added was determined through optimization trial and chosen 1 percent as optimum. A control sample without any added antioxidants was also prepared, while another sample was supplemented with 0.02% BHA, serving as a reference for comparison in the study. Using a ready-made gulabjamun dough, 100 balls of 6-7 g weight were fried in 1500 g of ghee samples at 180°C in an AGARO - 33390 Marvel 1700-Watt Deep Fryer made of stainless steel. After every 15 minutes of deep-frying, samples were taken and physical and chemical characteristics were examined.

### ***Saponification value***

The saponification value was determined as per FSSAI Lab Manual 2, (2015). The sample was melted and filtered through filter paper to remove any impurities and the last traces of moisture. It was ensured that the sample was completely dry. The sample was thoroughly mixed, and approximately 1.5 to 2.0 grams of the dry sample were weighed into a 250 ml Erlenmeyer flask. Then, 25 ml of the alcoholic potassium hydroxide solution was pipetted into the flask. A blank determination was conducted along with the sample. The sample flasks and the blank flask related to air condensers, were kept in a water bath, and boiled gently but steadily until saponification was complete. Saponification was considered complete when there was an absence of any oily matter and the appearance of a clear solution. Typically, clarity was achieved within one hour of boiling. After the flask and condenser had cooled somewhat, the inside of the condenser was washed down with about 10 ml of hot ethyl alcohol neutral to phenolphthalein. The excess potassium hydroxide was titrated with 0.5N hydrochloric acid, using approximately 1.0 ml of phenolphthalein indicator.

3.3.4.5.3 Calculation:



$$\text{Saponification Value} = \frac{56.1 (B-S)N}{w}$$

Were,

B = Volume in ml of standard hydrochloric acid required for the blank.

S = Volume in ml of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid and

W = Weight in gm of the oil/fat taken for the test.

## Result and Discussion

Table 1: Saponification value of ghee added with antioxidants

Saponification value			
Frying interval (min)	Ghee added with		
	Control	BHA	CLE
0	232.18	233.29	232.88
15	228.99	231.94	232.28
30	228.57	227.33	232.03
45	227.78	225.59	229.06
60	220.25	223.15	226.46

The saponification value serves as an indicator of the average molecular weight of a substance, and it demonstrates an inverse relationship with the mean molecular mass. The effects of CLE and BHA on the Saponification Value of ghee during frying were evaluated, and the results are shown in Table.3. Throughout the frying process, the saponification value of all ghee samples exhibited a reduction. For the control ghee, it decreased from 232.18 to 220.25, while for the ghee supplemented with BHA, it decreased from 233.29 to 223.15, and for the ghee added with CLE, it decreased from 232.88 to 226.46. Based on these findings, it can be concluded that the addition of 1% CLE to ghee effectively prevents the decrease in saponification value during frying. Another study conducted by



Aleena in 2020 reported a decrease in the saponification value of ghee from 231.25 to 227.53 during the frying of paneer in ghee at 180°C. This reduction in saponification value may be attributed to the conversion of fatty acids into carbonyl compounds, resulting in a decrease in the free fatty acid content of the oils and subsequently lowering the saponification value, as explained by Ngassapa et al. in 2012.

## Conclusion

In conclusion, ghee, known for its superior frying and cooking capabilities, is widely consumed in India. Frying, a common culinary technique, leads to significant changes in the oil/fat used, affecting the shelf life and quality of fried foods. Recent studies prefer natural antioxidants over synthetic ones like BHA due to their stability at higher temperatures. The current study evaluated the potential of curry leaf extract (CLE) as an antioxidant in ghee during deep frying. The saponification value, which reflects the average molecular weight, showed a reduction in all ghee samples during frying. However, the addition of 1% CLE effectively prevented this decrease, highlighting its potential as a protective agent. These findings are consistent with previous research demonstrating a decrease in saponification value during frying, attributed to the conversion of fatty acids into carbonyl compounds.

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# Artificial Chromosomes: Unlocking the Potential of Genetic Engineering

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## What is meant by ‘Artificial chromosome’?

Artificial chromosomes, also known as human-made or synthetic chromosomes, represent a groundbreaking innovation in the field of genetic engineering. These engineered structures mimic the natural chromosomes found in living organisms, offering researchers an unprecedented level of control over the genetic material they carry. With their ability to manipulate and deliver genes efficiently, artificial chromosomes hold immense promise in various areas, including gene therapy, agricultural biotechnology, and synthetic biology.

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## What is meant by ‘Artificial chromosome’?

Artificial chromosomes, also known as human-made or synthetic chromosomes, represent a groundbreaking innovation in the field of genetic engineering. These engineered structures mimic the natural chromosomes found in living organisms, offering researchers an unprecedented level of control over the genetic material they carry. With their ability to manipulate and deliver genes efficiently, artificial chromosomes hold immense promise in various areas, including gene therapy, agricultural biotechnology, and synthetic biology.

## Parts of Artificial chromosomes:

Artificial chromosomes are designed to replicate the structure and function of natural chromosomes. They consist of three essential components:

1. Centromere: ensures proper segregation during cell division
2. Telomeres: protect the ends of the chromosome from degradation
3. DNA sequence of interest: can be customized to carry specific genes or genetic elements.

By incorporating these components, researchers can create stable and self-replicating structures that can be introduced into cells or organisms.

### **Importance of artificial chromosome in research areas:**

1. **Gene Therapy:** One of the most significant applications of artificial chromosomes lies in gene therapy. Traditional gene therapy methods often rely on viral vectors to deliver therapeutic genes into target cells. However, these vectors have limitations, such as limited cargo capacity and the potential for immunogenic responses. Artificial chromosomes provide an alternative approach, allowing for the delivery of larger genetic payloads and reducing the risk of adverse immune reactions. They can be engineered to carry multiple genes, regulatory elements, and even large DNA fragments, opening up new possibilities for treating genetic disorders.
2. **Agricultural Biotechnology:** Artificial chromosomes have great potential in agricultural biotechnology. They can be utilized to introduce desirable traits into crops, such as disease resistance, improved yield, or enhanced nutritional content. By incorporating specific genes into artificial chromosomes and introducing them into plant cells, scientists can achieve more precise and controlled genetic modifications compared to traditional methods. This technology has the potential to revolutionize crop breeding and contribute to sustainable agriculture by developing crops with increased productivity and resilience.
3. **Synthetic Biology:** Artificial chromosomes are also invaluable tools in synthetic biology, a rapidly evolving field that aims to design and construct new biological systems. These chromosomes can be engineered to carry not only genes from the same species but also genes from other organisms or even entirely synthetic genes. This flexibility allows researchers to create novel genetic pathways, metabolic networks, or cellular functions, leading to the production of valuable compounds, biofuels, or pharmaceuticals. Artificial chromosomes offer a robust platform for constructing synthetic organisms and manipulating biological systems with precision.

### **Applications of Artificial Chromosomes:**

Artificial chromosomes have been developed and used in genetic engineering for various applications. Some of the major applications are discussed here:

- **Genetic Diseases:** Artificial chromosomes can be used as vectors to deliver therapeutic genes into cells for gene therapy. These artificial chromosomes can carry large DNA fragments,



including entire genes or gene clusters, allowing for the introduction of functional genes into target cells.

- **Transgenic Animal Generation:** Artificial chromosomes can be used to create transgenic animals with specific genetic modifications. By introducing artificial chromosomes containing desired genes into animal embryos, researchers can generate animals with enhanced traits or models for studying specific diseases.
- **Synthetic Biology:** Artificial chromosomes can be designed and constructed to serve as platforms for synthetic biology applications. By engineering artificial chromosomes, researchers can create artificial gene networks, biosynthetic pathways, or cellular systems with specific functions.
- **Biopharmaceutical Production:** Artificial chromosomes can be used to optimize the production of biopharmaceuticals in cell lines. By introducing artificial chromosomes carrying genes for the production of therapeutic proteins, researchers can increase the efficiency and stability of protein production in cells, leading to improved biopharmaceutical manufacturing processes.
- **Crop Improvement:** Artificial chromosomes can be used to introduce desirable traits into crops through genetic engineering. By incorporating artificial chromosomes containing genes for disease resistance, improved yield, or enhanced nutritional value, researchers can develop genetically modified crops with improved traits and characteristics.
- **Functional Genomics:** Artificial chromosomes can be utilized to study gene function on a larger scale. Researchers can introduce artificial chromosomes carrying specific genes or gene libraries into cells or model organisms to investigate the effects of gene expression or gene knockout on cellular processes and organismal development.

It's worth noting that while artificial chromosomes have shown promise in these applications, their development and implementation are still active areas of research, and further advancements are needed to fully harness their potential.

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# Transforming Aquatic Health Management: The Power of Artificial Intelligence

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## Introduction

Aquatic ecosystem health and well-being are crucial for environmental preservation, sustainable fisheries and human welfare. There is a rising need for effective and efficient management solutions due to the problems that are being posed by pollution, climate change, and overexploitation. Artificial intelligence (AI) has become a potent tool in recent years, providing creative approaches for tracking, evaluating, and defending aquatic ecosystems. It is an area of computer science that tries to give robots the ability to learn to emulate the cognitive processes of living creatures and make decisions based on prior experiences. Machine learning, which is a branch of AI, mimics natural behavior. Studying algorithms and statistical models that computer systems employ to carry out certain tasks with little human assistance is one of the specialized scientific methodologies now used for AI research (Mustapha et al., 2021). The present article examines the diverse uses of AI and scrutinizes how it has the potential to revolutionize aquatic health management.

**Keywords:** AI, Aquatic Health, Data Collaboration, Smart Technology.

## Enhanced Monitoring and Early Detection

The manual observation used in traditional monitoring of aquatic ecosystems may be time-consuming, labor-intensive and chances of human error. AI technologies, such as machine learning

and computer vision, have revolutionized monitoring capacities by enabling the automatic analysis of massive volumes of data gathered from sensors, satellites and underwater cameras. This makes it possible to continuously and in real-time monitor changes in ecosystem dynamics, biodiversity and also water quality parameters. Artificial intelligence (AI) systems can recognize minute variations in variables like temperature, pH, dissolved oxygen (DO) and nutrient concentrations, giving early notice of possible ecological disturbances or dangerous algal blooms. Future results can be predicted using AI algorithms that analyze based on previous data to find patterns and correlations that are difficult for humans to see or observe since AI offers the chance to resolve problems that are based on expertise and background knowledge to get pertinent information and make discoveries that previously depend on professional's experience. To provide high accuracy (99.99%) in prediction by removing external effects, research improvement is required to enhance statistical models and algorithms under AI and machine learning (Mustapha et al., 2021).

### **Predictive Modelling and Decision Support Systems**

AI is essential for creating prediction models that mimic how aquatic systems will behave in certain conditions. The financial damage brought on by floods from excessive rainfall and improper water quality parameters, such as DO, pH, etc., may be avoided with proper meteorological and water environment forecasting. Even the smallest increase may significantly improve decision-making in weather prediction. By increasing accuracy and efficiency, AI and data mining close the gap between real-time guidance and prediction utilizing numerical models (Mustapha et al., 2021). Understanding the effects of numerous stressors, such as pollution, climate change, and habitat loss, on aquatic ecosystems is made easier with the use of these models. They support decision-makers and resource managers in their efforts to sustainably manage fisheries, marine protected areas, and water resources.

### **Disease Detection and Aquaculture Management**

Maintaining the health and productivity of confined populations is a big concern for aquaculture, which involves culturing fish, shellfish and aquatic plants. In aquaculture settings, AI-based systems provide creative approaches to disease detection, early diagnosis and treatment monitoring. Machine learning algorithms can analyze vast datasets of fish behavior, physiological characteristics, and environmental circumstances to spot symptoms of stress, disease outbreaks, or poor water quality. Aquaculturists can quickly take preventative action, which minimizes financial



losses and lowers the need for the addition of antibiotics or pesticides.

### **Ecological Restoration and Conservation**

The support of AI technologies is crucial for conservation and ecological restoration projects. AI systems can detect important species, map habitats, and evaluate biodiversity trends by analyzing intricate ecological datasets. This information is essential and helps develop successful restoration methods and identify regions needing conservation efforts. Additionally, AI-based solutions support more effective conservation efforts by assisting in discovering and tracking endangered species, illicit fishing, and enforcing marine protected zones.

### **Data Integration and Collaboration**

AI provide a way to combine data from many sources, including satellites, underwater drones, buoys, and citizen science projects. AI enables multidisciplinary cooperation and improves our knowledge of complicated aquatic ecosystems by combining and analyzing disparate datasets. Yang et al. (2021) suggested a method for establishing operational limitations in aquaculture utilizing multi-source data to give businesses, particularly service providers, assistance for safe operational planning and choices for both coastal and offshore fish farms. Furthermore, AI-powered platforms make it possible for multiple stakeholders, such as scientists, politicians, resource managers and local people, to share real-time data and insights. This encourages a more thorough and organized response to problems with aquatic health.

### **Conclusion**

Artificial intelligence (AI) in aquatic health management has enormous potential for resolving the imperative environmental problems affecting the world's water bodies. AI provides scientists, decision-makers and stakeholders with insightful information and useful tools for sustainable management and conservation, from real-time monitoring and predictive modeling to disease identification and conservation initiatives. However, while using AI technology, it is crucial to take into account ethical issues, data quality and the necessity for human knowledge. We can ensure that our aquatic ecosystems have a healthier and more resilient future by utilizing AI's transformational potential.



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## Role of construction of major irrigation projects on emergence of zoonotic diseases

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### *Abstract*

Dams play a crucial role in providing water for agricultural purposes, but they can also have unintended consequences on the emergence of zoonotic diseases. Irrigation dams create new habitats for wildlife by altering the natural hydrology of rivers and streams. These new habitats can provide ideal breeding grounds for disease vectors such as mosquitoes and ticks, which can carry and transmit zoonotic pathogens. Another unintended consequence of irrigation dams is the displacement of wildlife and the destruction of natural habitats. Some major disease related to irrigation projects are Schistosomiasis, Malaria, Leptospirosis, Rift valley fever, Japanese encephalitis and etc.

### **Introduction**

Dams play a crucial role in providing water for agricultural purposes, but they can also have unintended consequences on the emergence of zoonotic diseases. Zoonotic diseases are infectious diseases that can be transmitted from animals to humans, and they pose a significant threat to global public health. The emergence of zoonotic diseases is influenced by several factors, including climate change, land-use change, and the construction of irrigation dams.

One of the unintended consequences of irrigation dams is the creation of new habitats for wildlife by altering the natural hydrology of rivers and streams. These new habitats can provide ideal breeding grounds for disease vectors such as mosquitoes and ticks, which can carry and transmit zoonotic pathogens. The construction of the Aswan High Dam in Egypt, for example, created an ideal habitat for schistosomiasis-transmitting snails, leading to a significant increase in the prevalence of the disease.

Another unintended consequence of irrigation dams is the displacement of wildlife and the destruction of natural habitats. This disruption can cause wildlife to migrate to new areas or come into closer contact with humans and domesticated animals, increasing the risk of zoonotic disease transmission. The construction of the Three Gorges Dam in China, for instance, resulted in the displacement of millions of people and the loss of critical habitat for wildlife, including bats that can carry zoonotic viruses such as SARS and Nipah.

Additionally, the water stored in irrigation dams can create ideal conditions for the growth of certain zoonotic pathogens. The water in irrigation canals and reservoirs can become contaminated with fecal matter from animals, providing a breeding ground for bacteria such as *E. coli* and *Salmonella*.

### **Other disease related to major irrigation projects**

There are several examples of zoonotic diseases that have been linked to the construction and use of irrigation dams. Here are a few:

**Schistosomiasis:** This is a parasitic disease caused by flatworms that live in freshwater snails. The disease is transmitted to humans through contact with contaminated water, such as that found in irrigation canals and reservoirs. Irrigation dams can create new habitats for the snails that carry the parasite, increasing the risk of transmission to humans (Hotez, 2009).

**Malaria:** This mosquito-borne disease is more common in areas with standing water, which can be created by irrigation dams. Irrigated agricultural fields can also provide breeding sites for mosquitoes, increasing the risk of malaria transmission to humans (Bouma & Dye 1997).

**Leptospirosis:** This bacterial disease is spread through contact with urine from infected animals, such as rodents. Irrigation dams can create new habitats for rodents, increasing the risk of transmission to humans (Bharti *et al.*, 2003).

**Rift Valley fever:** This viral disease is spread by mosquitoes and can be transmitted to humans through contact with infected animals, such as livestock. Irrigation dams can create new habitats for mosquitoes and increase the risk of transmission to humans and animals. (Woods *et al.*, 1997)

**Japanese encephalitis:** This viral disease is transmitted to humans through the bite of infected mosquitoes. Irrigation dams can create new habitats for the mosquitoes that carry the virus, increasing the risk of transmission to humans (World Health Organization 2015)



**Some examples of emerging zoonotic diseases that have been linked to dam construction:**

S.No	Name of disease	Dam and place	Reference
1	Schistosomiasis	Three gorges dam in China	Yang GJ, Vounatsou P, Zhou XN, Utzinger J, Tanner M. A review of geographic information system and remote sensing with applications to the epidemiology and control of schistosomiasis in China. <i>Acta Trop.</i> 2005;96(2-3):117-129.
2	Rift valley fever	Akaosombo dam in Ghana	Anyamba A, Chretien JP, Small J, Tucker CJ, Linthicum KJ. Developing global climate anomalies suggest potential disease risks for 2006-2007. <i>Int J Health Geogr.</i> 2006; 5:60.
3	Hanta virus	Three gorges dam in China	Li Y, Kang Y, Yu G, et al. Study on the correlation between hantavirus infection and construction of large-scale water conservancy project--take the Three Gorges Project as an example. <i>Wei Sheng Yan Jiu.</i> 2014; 43(4):587-591.
4	Malaria	Kariba dam in zimbabwe	Smith T, Killeen GF, Lengeler C, et al. Mathematical modeling of the impact of malaria vaccines on the clinical epidemiology and natural history of <i>Plasmodium falciparum</i> malaria: Overview. <i>Am J Trop Med Hyg.</i> 2006; 75(2_suppl):1-10.

**Socio economic impacts on indigenous tribes and other marginalized communities**

- Health impacts:** Indigenous tribes and other marginalized communities may be more vulnerable to zoonotic diseases due to factors such as poor sanitation, lack of access to healthcare, and traditional livelihoods that involve close contact with animals. The emergence of zoonotic diseases related to dam construction can therefore have a significant impact on the health of these communities, leading to increased morbidity and mortality.
- Livelihood impacts:** Many indigenous tribes and other marginalized communities rely on traditional livelihoods such as hunting, fishing, and agriculture for their subsistence. The emergence of zoonotic diseases related to dam construction can disrupt these livelihoods by reducing the availability of wild game or fish, or by making it unsafe to work in fields or other outdoor environments.



3. **Economic impacts:** The emergence of zoonotic diseases related to dam construction can also have broader economic impacts on indigenous tribes and other marginalized communities. For example, if tourism is an important source of income, an outbreak of disease could lead to a decline in visitors and a loss of revenue. Additionally, if the government or international aid agencies respond to the outbreak by implementing measures such as quarantine or culling of animals, this could further disrupt local economies.
4. **Cultural impacts:** Indigenous tribes and other marginalized communities often have close relationships with the natural environment, which can be disrupted by the construction of dams and the emergence of zoonotic diseases. The loss of traditional knowledge related to hunting or fishing, or the disruption of sacred sites or cultural practices, can have a profound impact on the cultural identity and well-being of these communities (Jobin, 2003 & World Health Organization, 2015)

### **Mitigation methods**

To mitigate the risk of zoonotic disease emergence associated with irrigation dams, several strategies can be employed. These include regular monitoring of water quality and the implementation of measures to control disease vectors. In addition, the management of water levels in irrigation reservoirs can be optimized to minimize the creation of ideal breeding grounds for disease vectors. Finally, conservation efforts to protect natural habitats and wildlife can help reduce the risk of zoonotic disease emergence associated with displacement and migration.

One important consideration is the need for interdisciplinary collaboration among different sectors such as public health, environmental conservation, and agriculture. By working together, these sectors can develop more effective strategies to address the complex challenges associated with zoonotic disease emergence. The One Health approach is an example of successful interdisciplinary collaboration that recognizes the interconnectedness of human, animal, and environmental health.

Another essential consideration is the potential impact of climate change on zoonotic disease emergence. Climate change can alter the distribution and behavior of disease vectors and increase the frequency and intensity of extreme weather events such as floods and droughts, which can create ideal conditions for zoonotic disease transmission. Therefore, it is essential to consider the potential impact of climate change when developing strategies to address zoonotic disease emergence associated with irrigation dams.

In conclusion, irrigation dams can have unintended consequences on the emergence of



zoonotic diseases by creating new habitats for disease vectors, displacing wildlife, and creating ideal conditions for the growth of certain pathogens. However, by understanding the risks associated with irrigation dams and implementing appropriate mitigation strategies, we can minimize the risk of zoonotic disease emergence and protect global public health. Interdisciplinary collaboration and consideration of the potential impact of climate change are essential to developing effective strategies to address this complex challenge.

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# Synthetic Viruses: A Double-Edged Sword in Scientific Advancement

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## *Abstract*

The field of synthetic biology has made remarkable advancements in recent years, enabling the construction of artificial organisms and genetic circuits. Among these achievements, the creation of synthetic viruses has emerged as a prominent area of study. Synthetic viruses are designed and engineered to possess specific properties and functions, ranging from therapeutic applications to understanding fundamental biological processes. This article provides an overview of synthetic viruses, highlighting their design, principles, diverse applications, and the ethical considerations associated with their development and use.

## **Introduction**

In recent years, the field of biotechnology has witnessed groundbreaking advancements that have revolutionized various industries, including medicine, agriculture, and environmental science. One of the most intriguing and controversial developments in this domain is the creation of synthetic viruses. Synthetic viruses, also known as designer viruses or viral nanobots, are artificially engineered viruses designed to carry out specific tasks, ranging from targeted gene delivery to disease eradication (Guenther, CM et al., 2014) This technological frontier holds immense potential for scientific and medical progress, but it also raises profound ethical and safety concerns.

A synthetic virus is a product of the convergence of biotechnology, genetic engineering, and virology (Collins, L. T. et al., 2023). Researchers are able to manipulate the genetic material of viruses by altering their DNA or RNA sequences, enabling them to create customized viral structures and functionalities. These synthetic viruses can be designed to infect specific cell types, deliver therapeutic payloads, or even reprogram cellular machinery for various purposes.

## Design and principles of Synthetic virus:

The design of synthetic viruses involves following steps

- **Identification of target:** The first step in designing synthetic virus is to identify the specific target or purpose for which the synthetic virus is being designed (Wimmer E, et al., 2009). This could include gene therapy, vaccine development, or other therapeutic applications.
- **Selection of viral backbone:** select a viral backbone or template from existing viruses that possess certain desirable characteristics, such as efficient replication, cellular tropism (ability to infect specific cell types), or stability. Commonly used viral backbones include adenoviruses, lentiviruses, retroviruses, or adeno-associated viruses (AAVs).
- **Genetic modification:** Once the viral backbone is selected, genetic modifications are made to the viral genome. This involves manipulating the DNA or RNA sequences of the virus to achieve the desired functionalities. Techniques such as gene insertion, deletion, or substitution are employed to introduce or remove specific genes, regulatory elements, or target-specific features, gene editing, by modification of viral capsids and incorporation of genetic circuits.
- ✚ **Gene editing using programmable nucleases:** Programmable nucleases, such as CRISPR-Cas9, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs), are molecular tools that can be used to introduce targeted double-strand breaks in specific genomic regions (Li, H., et al., 2020). These breaks can then be repaired by the cell's DNA repair machinery, resulting in precise modifications to the DNA sequence. Gene editing using programmable nucleases has revolutionized genome engineering due to its simplicity, efficiency, and versatility.
- ✚ **DNA synthesis and assembly:** With advancements in DNA synthesis technologies, it is now possible to chemically synthesize DNA sequences of varying lengths, including entire viral genomes (Hughes RA, et al., 2017) These synthetic DNA fragments can be assembled using techniques such as polymerase chain reaction (PCR), restriction enzyme digestion, and ligation, allowing the creation of custom-designed genetic constructs.
- ✚ **Site-directed mutagenesis:** Site-directed mutagenesis involves introducing specific mutations at desired locations within a DNA sequence. This technique can be used to study the effects of specific genetic changes or to engineer proteins with improved or altered functions.
- ✚ **Homologous recombination:** Homologous recombination is a natural DNA repair mechanism that can be exploited for genome engineering. By designing DNA fragments with homology to



specific genomic regions, scientists can use recombination to replace or insert genetic material into the target genome. This technique is particularly useful for introducing large DNA fragments or precise modifications.

- ✚ **RNA interference (RNAi):** RNAi is a technique that involves introducing small RNA molecules, called small interfering RNAs (siRNAs), into cells to selectively silence or knockdown specific genes (Dana H, et al., 2017). RNAi can be used to study gene function or to manipulate gene expression levels.
- **Payload incorporation:** Synthetic viruses are often designed to carry therapeutic payloads, such as therapeutic genes, drugs, or other therapeutic agents. The payload is incorporated into the viral genome or as a separate entity within the viral particle. This payload can be designed to target specific cells, deliver therapeutic molecules, or induce desired biological effects.
- **Optimization and testing:** The modified viral genome is then optimized to enhance viral replication, infectivity, and target specificity. This may involve fine-tuning viral gene expression, modifying viral surface proteins, or optimizing the packaging efficiency of the viral particles. The engineered synthetic virus is tested extensively in vitro and in animal models to assess its safety, efficacy, and functionality.
- **Safety considerations:** Safety is a critical aspect of synthetic virus design. Researchers must carefully consider and address potential risks associated with the use of synthetic viruses, including unintended effects, toxicity, and potential for uncontrolled spread. Safety features such as self-limiting replication, controlled payload release, or inducible viral inactivation systems may be incorporated to mitigate these risks.
- **Ethical and regulatory considerations:** The design and use of synthetic viruses also involve ethical and regulatory considerations. Research involving synthetic viruses is subject to rigorous oversight and compliance with applicable guidelines and regulations. Ethical considerations include ensuring responsible research practices, minimizing risks, and avoiding the misuse or intentional design of synthetic viruses for harmful purposes.

## Applications

- ✓ **Research and understanding of viral diseases:** Synthetic viruses can be used to study the mechanisms of viral infections, replication, and pathogenesis. By manipulating specific viral genes or components, researchers can gain insights into how viruses function and interact with host cells, leading to a better understanding of viral diseases.





- ✓ **Vaccine development:** Synthetic viruses can serve as tools for vaccine development. Scientists can engineer attenuated or non-pathogenic versions of viruses to create vaccines that stimulate an immune response without causing disease. By modifying viral proteins or genetic material, synthetic viruses can potentially be used as safer and more targeted vaccine candidates.
- ✓ **Gene therapy:** Synthetic viruses, particularly viral vectors, can be employed as delivery systems for gene therapy. These viruses can be modified to carry therapeutic genes into target cells, helping to correct genetic disorders or treat diseases. Viral vectors derived from synthetic viruses have shown promise in early clinical trials for conditions such as cancer, genetic disorders, and certain viral infections.
- ✓ **Drug Discovery:** Synthetic viruses offer a promising avenue for drug discovery and testing. By designing viruses with specific genetic modifications, researchers can identify potential drug targets, test the efficacy of antiviral compounds, and develop new therapeutic strategies to combat viral infections.
- ✓ **Biosensing and Diagnostic Tools:** Synthetic viruses can be engineered to act as biosensors for detecting specific pathogens or markers of disease. These engineered viruses can provide rapid and accurate diagnostic capabilities, enabling early detection and containment of viral outbreaks.
- ✓ **Biotechnology and industrial applications:** Synthetic viruses have potential applications in various biotechnological and industrial processes. For instance, they can be used to produce viral vectors for gene delivery in biopharmaceutical manufacturing or to engineer viruses for targeted gene editing techniques like CRISPR-Cas9. Additionally, synthetic viruses can be utilized in the production of vaccines, viral vectors for gene therapy, and other biotechnological products.

## Conclusion

Synthetic viruses mark a significant milestone in scientific advancement. However, their creation also poses ethical and safety challenges that must be addressed through rigorous regulation and responsible research practices. These engineered pathogens offer unprecedented opportunities for understanding viral mechanisms, developing vaccines, and advancing research in various fields. They hold the potential to revolutionize disease prevention, treatment, and diagnostics.

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# Role Of New Generation Phytohormones on Abiotic Stress

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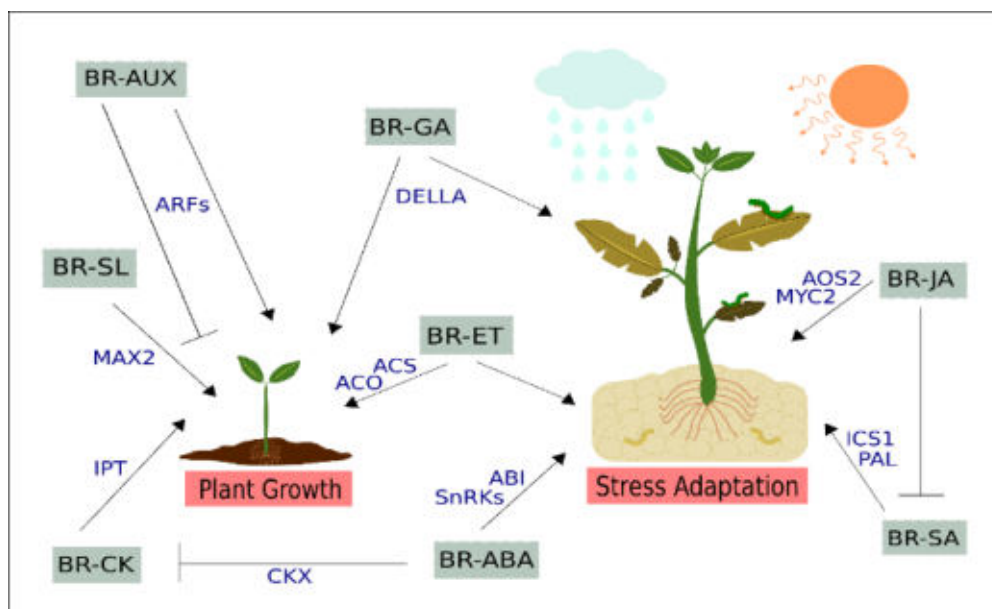
## Introduction

Plants are subjected to stress due to a large number of factors that reduce the productivity and economic instability of farmers. Due to an increase in the population, the use of automobiles and other electronics which emit toxic gases into the atmosphere also increases. It will lead to a condition called global warming. Global warming increases the temperature by 2-4°C. A rise in temperature results in a climatic change on the planet. Climatic change causes various abiotic stresses to the plants, and it is caused due to factors like temperature, water, air, light, etc. Abiotic stress gains more attention and care than biotic stress. The natural defense mechanism of plants can regulate abiotic stress. Phytohormones generate the natural defense. Phytohormones are chemical messengers that regulate plants' growth, development and metabolism. Phytohormones help in abiotic stress like drought, frost, flooding, heat, salinity, etc., by their signal transduction pathways. It also activates internal and external stimuli to avoid abiotic stress conditions. The new generation of phytohormones like brassinosteroids, salicylic acid, jasmonic acid, peptides, polyamines, prohexadiene-ca, 1-methyl cyclopropane plays a vital role in abiotic stress.

## Role of Brassinosteroids on abiotic stress

Brassinosteroids (BRs) are polyhydroxy steroid hormones isolated from rapeseed. Brassinolide is the first isolated brassinosteroid. It promotes plants' growth and development, including root development, seed germination and dormancy. Brassinosteroids increases cell division when the auxin and cytokinin are in limited amount. In addition, it also gives defense against

abiotic stress like heat, cold, drought, heavy metals, salinity and pesticides. Brassinosteroids are present in the cell surface by brassinosteroids receptors, leading to phosphorylation which activates the central transcription factor called "Brassinazole-Resistant1(BZR1)" that controls the transcription of genes that are responsive to brassinosteroids in the nucleus. It increases the activity of photosynthesis when the plants are prone to stress, resulting in increased growth and biomass. It enhances carbon dioxide assimilation and increases the chlorophyll content. Brassinosteroids also mitigate abiotic stress by increasing the production of osmoprotectants and antioxidants. It also mitigates salt stress by ethylene biosynthesis, reducing the production of antioxidants and regulates the chilling stress by the factor BZR1 which produces Respiratory burst oxidase homolog 1(RBOH1) and  $H_2O_2$  production in the apoplast. This results in increased sensitivity to photoinhibition, which gives resistance against chilling stress.



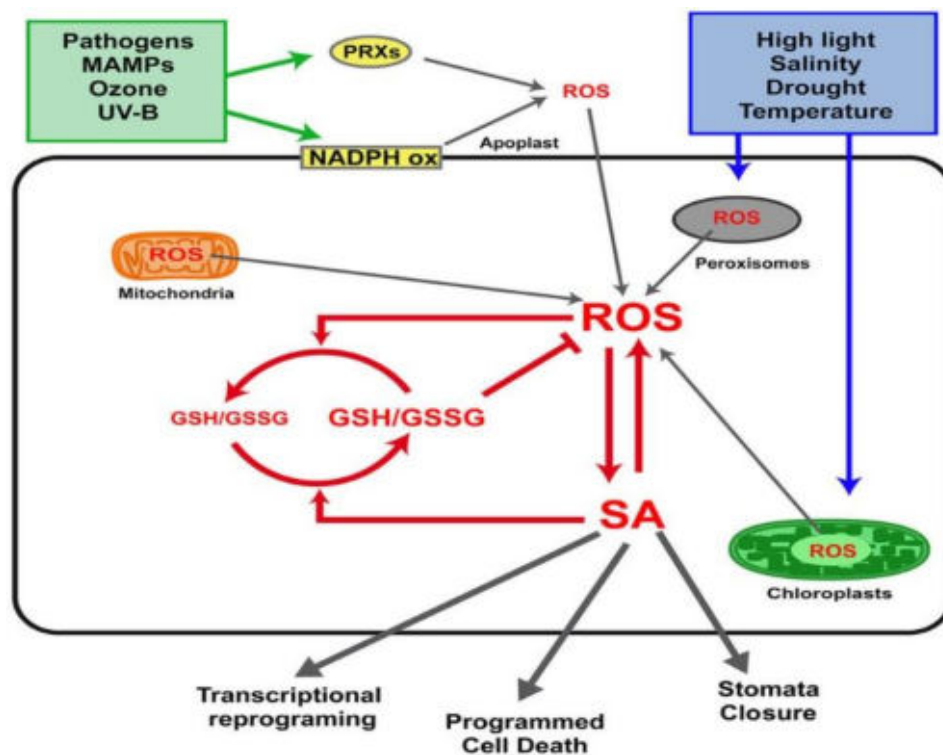
**Mechanism of Brassinosteroids to abiotic stress**

### Role of Salicylic acid on abiotic stress

Salicylic acid plays an essential role in plant growth and development. It is ortho-hydroxybenzoic acid and extracted from white willow as salicine. The stress tolerance level of plants ranges from 0.1 mM to 0.5 mM. The mechanism involved is redox regulation in plant cells. They increased the antioxidant involved in ROS scavenging, which is involved in  $H_2O_2$  detoxifying. The endogenous form of salicylic acid enhances the conductivity of stomata, photosynthesis, the efficiency of carboxylation and photosynthesis oxygen evaluation. It involves cell expansion and



elongation during stress conditions leading to increased growth of root cells of the meristem. It contributes to the increased germination rate and shoots and root elongation during stress levels. There is a direct relationship between GA3 synthesis and Salicylic acid which gives resistance against abiotic stress. It mainly deals with heavy metal toxicity like cadmium with signalling mechanisms of reactive oxygen species (ROS). It involves cell wall construction, balancing the uptake of Cd and other ions, refining the antioxidant defence system, and regulating photosynthesis, glutathione synthesis and senescence.



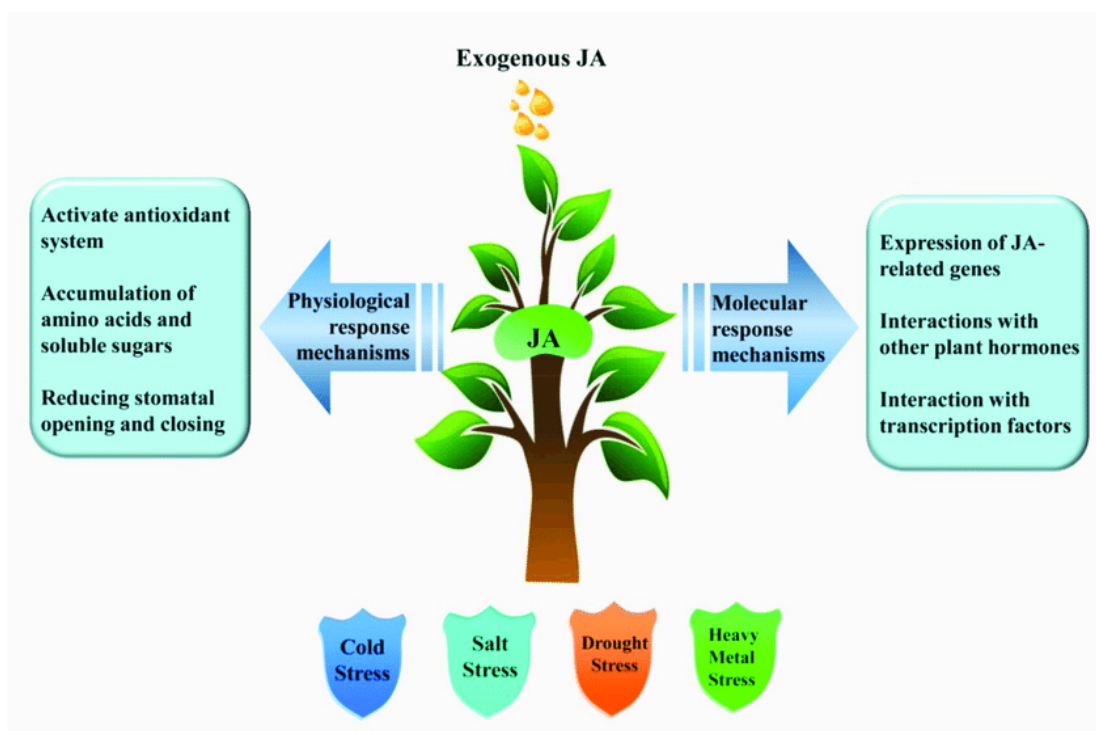
### Mechanism of Salicylic acid to abiotic stress

#### Role of Jasmonic acid on abiotic stress

Jasmonic acid is a derivative of linoleic acid and can be converted into many derivatives like methyl jasmonate. It is a plant immune hormone naturally synthesized by plants. It involves root development and the developing of reproductive organs in plants. The signalling mechanism of jasmonic acid is mainly by gene expression and coordinates with other phytohormones during stress. It regulates the antioxidant metabolism, synthesis of osmolytes and accumulation of metabolites. It also increases the Ascorbate peroxidases, glutathione reductase and ascorbic oxidase, which results in stress mitigation. Jasmonic acid decreases the salinity stress by reducing the uptake of Sodium with an increase in the uptake of Calcium and Magnesium levels. It also eliminates the toxicity of



heavy metals by increasing the osmolytes accumulation while increasing the antioxidant concentration, preventing plants' damage by heavy metals and minimizes cadmium toxicity. It reduces plants' chilling and heat stress and regulates the accumulation of amino acids and soluble sugars. It controls the stomatal opening and closing, thereby causing osmotic stress. It also regulates drought by increasing the activity of enzymes catalase, superoxide dismutases, peroxidase, chloramphenicol acetyltransferase, glutathione reductase and non-enzymatic antioxidants.



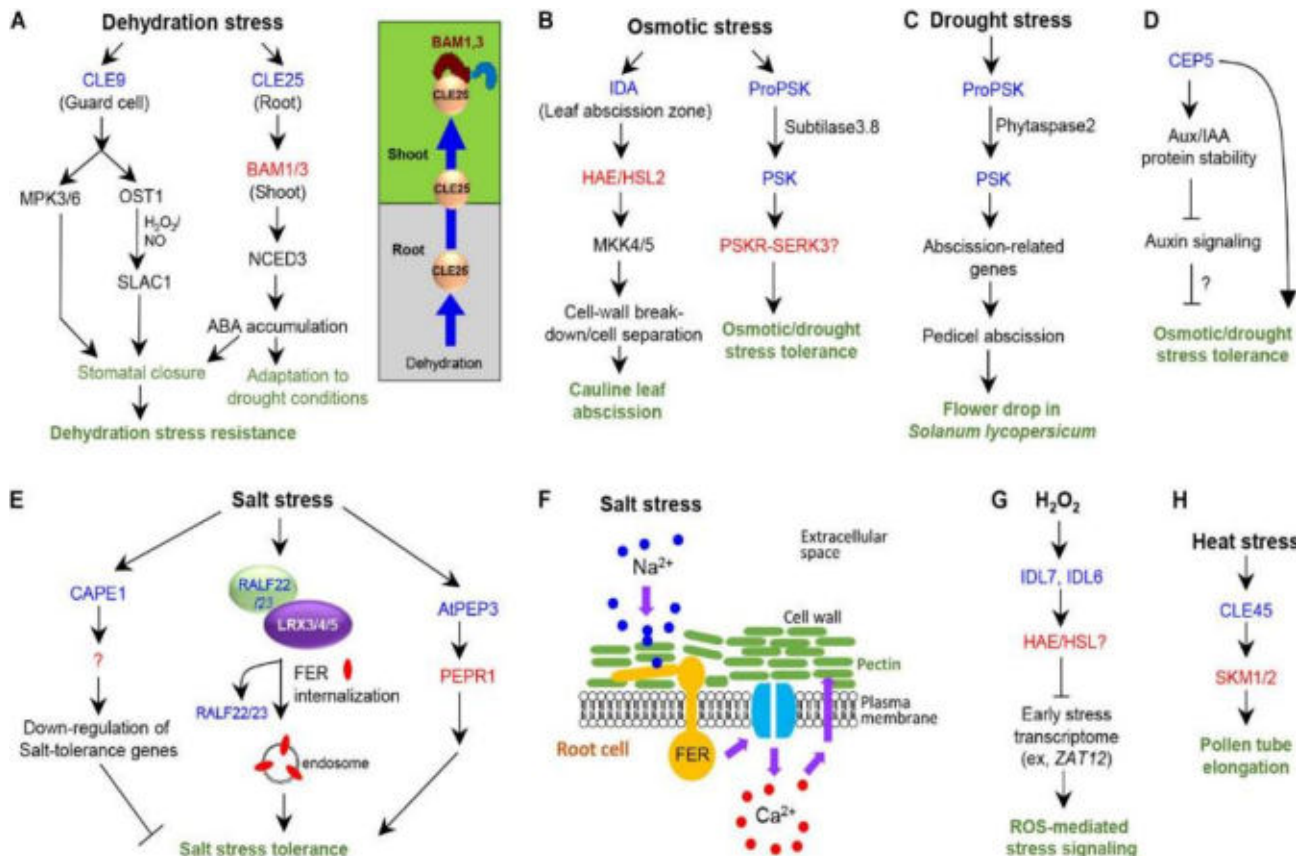
**Mechanism of Jasmonic acid to abiotic stress**

### Role of Peptides on abiotic stress

Nowadays, peptides can act as phytohormones that regulate plant growth and development. The signalling peptides are small peptides of 5-20 amino acids. These mobile peptides are recognized by leucine-rich repeat (LRR)-receptor-like kinases. It also controls the adaptation and tolerance against abiotic stress. These peptides mitigate dehydration, heat, salinity, etc. CLE peptides are involved in root cell maintenance, meristem tissue, vascular tissue, shoot elongation and stomatal opening and closing and regulate the water deficiency by gene expression to reduce the dehydration stress. IDA (Inflorescence Deficient in Abscission) regulates the abscission of the floral organ and lateral root emergence. PSK peptide (Phytosulfokine- $\alpha$ ) regulates osmotic stress tolerance. CAPE Peptides, RALF Peptides, and AtPEP3 are against Salinity stress. IDA-LIKE (IDL) peptides are involved in



ROS-mediated stress signalling. CLE45 peptides against heat stress response. Peptides also give nutrient deficiency stress response against nitrogen and phosphate deficiency.

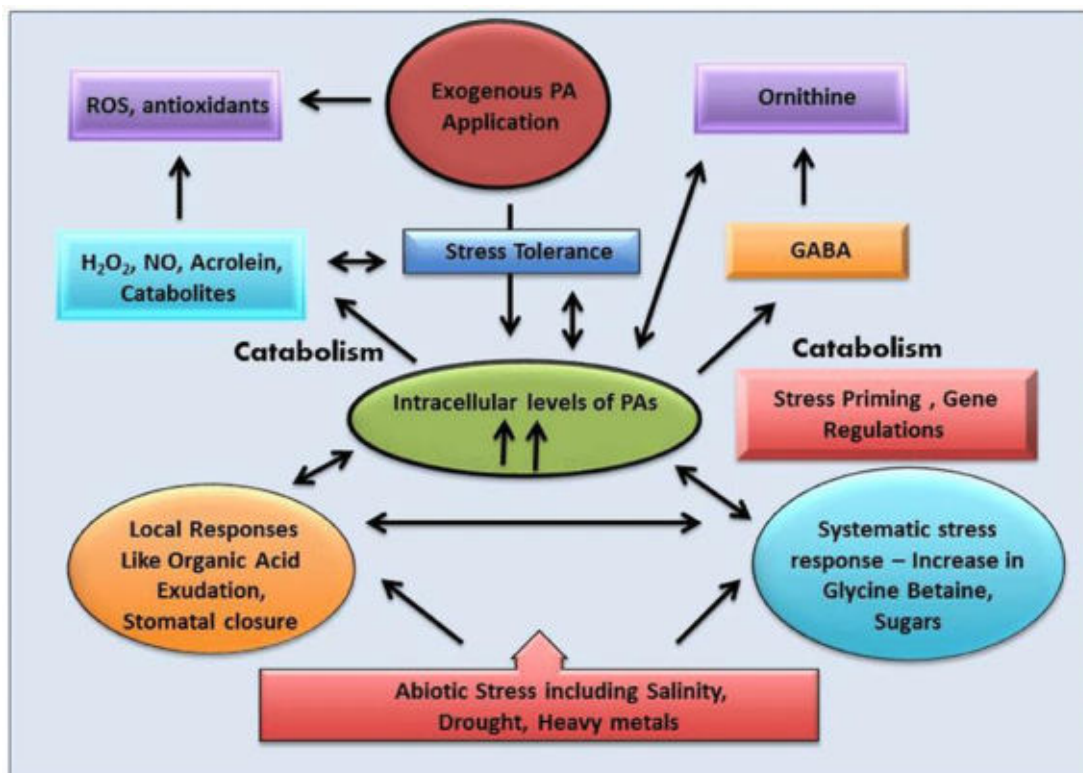


**Mechanism of Peptides to abiotic stress**

**Role of Polyamines on abiotic stress**

Polyamines are low-molecular-weight polycationic amines present in all living being. In addition to plant growth and development of plants, it also mitigates abiotic stress. The antistress property of polyamines is attributed to their acid-neutralizing, antioxidant, and cell-wall-stabilizing abilities. The level of polyamines like putrescine, spermidine, and spermine increases during stress. Through the ion channels of the plant, it regulates ion homeostasis. It also regulates the stomatal opening and closure, thereby mitigating water stress. It increases the production of antioxidants, and signalling occurs by the Reactive oxygen species mechanism. It produces compatible solutes and maintains the osmotic balance. Other abiotic stress factors are done by modulating the gene expression. Polyamine-triggered Nitrous Oxide modulation and polyamine-dependent nitrosoproteome modulation are contributed mainly to polyamine-mediated stress responses.





**Mechanism of Polyamines to abiotic stress**

### Role of Prohexadiene-Ca on abiotic stress

Prohexadiene-calcium (Pro-Ca) has been used as a plant growth regulator. This compound inhibits gibberellin (GA) biosynthesis by blocking 3,  $\beta$ -hydroxylation of GA20 to GA1, which plays a vital role in overcoming abiotic stress. It mainly contributes to plant salinity-alkali stress by modifying morphological parameters such as shoot and root growth, gas exchange parameters such as carbon dioxide concentration, transpiration rate, chlorophyll fluorescence, membrane damage, ROS accumulation, antioxidant production, soluble sugars and proline content.

### Conclusion

In the era of fertilizers, pesticides and fungicides, the naturally producing phytohormones which mitigate abiotic stress is adorable and have a specific place in organic farming techniques. The usage cost is relatively lower than the conventional protection method against abiotic stress. It is an economically and environmentally effective technique. It not only plays a vital role in the mitigation of abiotic stress. It is also involved in plants' growth, development, metabolism and physiological process. It regulates seed germination, root and shoot elongation, vegetative growth, flowering, pod development, senescence etc. Especially the new generation of phytohormones plays a vital role in





giving resistance against abiotic stress. It is essential to encourage the use of phytohormones against abiotic stress.

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## Meat Alternatives

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Over the previous forty years, the world's meat production has tripled and increased 20%. Compared to poor countries, developed or industrialized countries consume twice as much. The excessive animal rearing necessary to supply the world's demand for meat negatively affects the entire environment, public health, animal genetic variety, and the general economy. Animal feces releases methane, nitrous oxide, and carbon dioxide, whereas ruminants produce methane through enteric fermentation. Additionally, waste from abattoirs poses a major hazard to the environment and water supplies. Purification and a revolting odor are caused by leftover animal tissues and abattoir blood. The FAO predicts that a severe food scarcity would soon affect the entire planet as the demand for meat will likely rise by more than two-thirds. Alternatives to meat should be created as a strategy, such as cultured meat, proteins derived from plants, and analogues of insect proteins.

The idea of meat substitution is still somewhat hazy, which may have something to do with how it first became necessary to provide proteins and then move on to meat replacement. It is vital to define the vocabulary used to describe meat alternatives. "Meat alternative" is a catch-all phrase that refers to any protein source that can be utilized in place of the meal's meat. The phrase, which is closely connected to "alternative protein," primarily refers to the need to provide proteins and excludes the demands for precisely simulating all the nutritional and textural characteristics. A more accurate name for goods that replicate the functioning of meat in terms of processing, nutritional

content, and sensory qualities is "meat analogue" or "meat substitute". Texturized vegetable protein (TVP) technologies and plant biomass are frequently cited as the only structural underpinnings of meat analogues, leading to the idea that these products have advantageous ratios of essential amino acids, little saturated fat, and no cholesterol. Such attribution, however, does not apply to a number of commercially available meat substitutes (insect, microalgae, and other meat-based). As a result, "meat analogue" is defined as a quite broad category of goods that should be further classified into a) insect protein analogues, b) plant-based meat analogues, c) mycoprotein-based substitutes, and d) cultured meat. Typically, the terms "meat analogues" and "meat substitute" are used synonymously to describe physiologically, enzymatically, or physically structured meat imitates made of proteins, lipids, carbohydrates, and other ingredients derived from non-animal sources and uncommon animal species.

### **History Of Meat Analogues**

The use of meat analogue, also known as imitation meat and mimic meat, as a substitute for real meat in food products dates back to the early 1960s. In the past, tofu and tempeh (fermented soybean cake) were made with same ingredient: soy protein. Since 965CE, either these ingredients have been prepared through straightforward processing or fermentation methods, and many Southeast Asian nations have used them for millennia in their traditional cuisine. Along with these traditional Asian goods, dry texturized vegetable protein (TVP), made from extruded defatted soy meal, soy protein concentrates, or wheat gluten, was the first commercialized meat analogue. Early in the twenty-first century, meat substitutes became more popular due to consumer desire for nutritious foods and growing concerns about the environmental impact of consumers' diets. Meat analogue products, which may imitate the flavor, texture, appearance, and functionality of traditional meat-based goods, have been produced in the last ten years thanks to modern advancements in food science and manufacturing technology. Currently, greater attention is being paid to the direct creation of non-traditional protein sources in meat analogues, such as insect protein-based "meat" and cultured meat.

#### **a) Insect Protein Analogues**

With 5.5 million different species, insects are one of the most abundant living things on the planet. Nearly 2,000 species of insects are consumed worldwide, primarily in Southeast Asia, South America, and Africa. Eating insects is a long-standing tradition (known as entomophagy) in these areas that dates back at least 3,000 years. As a result of their high protein content and provision of



important amino acids for our daily needs, insects have long been valued as a source of protein. Coleoptera (beetles), Lepidoptera (caterpillars), Hymenoptera (ants, wasps, and bees), Orthoptera (locusts, grasshoppers, and crickets), Hemiptera (leafhoppers, planthoppers, and cicadas), Isoptera (termites), Odonata (dragonflies), and Diptera (flies) are the orders of insects that are most frequently consumed. However, eating insects is not widely accepted by western consumers, largely due to a poor perception of insects in general and their use in food in particular. Nevertheless, the value of edible insects has become apparent due to the growing demand for proteins other than meat. Insect sales have been rising significantly in recent years, and by 2023, they are anticipated to surpass \$522 million in US dollars. Several insects such as grasshoppers, cicadas, leafhoppers, and bugs are rich sources of proteins (20 to 76% of DM), fat (2-20% of DM), low in carbohydrates, and have reasonable amounts of minerals (Na, K and Ca), and trace elements such as Cu, P, Fe, Mn, Se and Zn, water-soluble or lipophilic vitamins such as pantothenic acid and folic acid. Insects are high in proteins containing more protein per 100 g compared to meat, fish, and egg. The wasps, bees, ants have highest protein contents.

The advantages of edible insects extend beyond just their high nutritional value to include their high feed/meat conversion rate as well as their reduced needs for land, water, and feed. Additionally, they require little space and have a high fecundity rate with year-round breeding. Some species (like palm weevil larvae) have a high capacity for recycling since the by-products can be fed to humans or other livestock.

#### **b) Cultured Meat**

The newest emerging meat substitute is cultured meat, also known as synthetic meat, lab-grown meat, bio artificial muscle, and Frankenstein meat. It can be characterized as synthetic meat made with stem cell technology. In 1932, former British prime minister Winston Churchill first broached the subject of cultured beef. Techniques for engineering cells and tissues have been created for use in medicine. However, they have recently been used in the realm of food technology for large-scale culturing due to advanced technological inputs. Based on these advancements, the first beef patty made from cow muscle cells was introduced to the general public in 2013. Beet juice and saffron were added to the muscle cells to create the patty, which had the consistency and color of meat.



In order to introduce cultured meat to the market, its production costs must be reduced and its quality standards must be raised. Since cultured meat will be the only true meat that contains animal protein and has a quality comparable to that of traditional meat, it could be crucial in boosting the supply of meat. Major businesses that produce cultured meat include Mosameat, Memphis Meats, Super Meat, and Integriculture. The market size is anticipated to be US \$4.3 million for meatballs, US \$3.7 million for burgers, and US \$3.3 million for sausages, among other varieties of cultured meat that may be introduced.

Benefits as meat substitutes: Since cultured meat is produced from farm animals, it resembles traditional meat the most and may even be more environmentally friendly. Because this product has a superior taste and texture to other meat alternatives, it can satisfy consumers' nutritional and sensory needs. In this regard, cultured beef may appeal to customers who are unwilling to alter their typical diet or meat-eating habits. In addition, a single cell can multiply multiple times during the creation of cultured meat, necessitating the use of fewer animals than in livestock farming.

### **c) Plant-Based Meat Analogues**

They can be produced using plant protein that has been extracted. In fact, one of our species' oldest dietary sources is plant protein. The use of tofu dates back to 965 CE, while other items like wheat gluten, yuba, and tempeh have been around for a long time in various nations and areas. However, compared to typical meat, the majority of them possessed quite distinct characteristics, particularly in terms of flavour and texture. Therefore, it took until the 1900s for these products to become successful on the market. The primary sources of plant-based meat substitutes are known to be wheat, soybeans, legumes, oil seeds, and fungus. Raw materials including soybeans, wheat, and peas (*Pisum sativum*) are processed to produce extracts and isolates that constitute the primary flavourings. These compounds are then put through processes to make items that resemble meat. Many goods, like wheat gluten, yuba, and tempeh, have been utilised for many years in various nations and areas. However, compared to typical meat, the majority of them possessed quite distinct characteristics, particularly in terms of flavour and texture. In terms of flavour and texture, it didn't make for a really enjoyable meal. With rising social expectations, the market for plant-based meat substitutes has recently been growing, and ongoing attempts are being made to enhance their sensory attributes. It currently holds the largest market share among the many meat substitutes, and it is anticipated that the industry would grow to more than \$21.2 billion US dollars by 2025.



#### d) Fungal Meat Substitutes

The hunt for acceptable, high-protein microbial alternatives has been prompted by worries that animal protein sources won't be enough to satisfy man's protein requirements. They found that filamentous fungi provided a suitably textured product and *Fusarium venenatum* A3/5 was selected as the best fungus for further product development. The continuous-flow fermentation of *F. venenatum* on a glucose substrate yields mycoprotein for commercial use. Cultures are kept at a pH of 6.0 at a temperature of 28 to 30 °C. Normally, the continuous fermentation process lasts for around 6 weeks. Mycotoxins are monitored throughout the production process and tested every 6 hours. In a separate tank, the fungal biomass is heated for 30 to 45 minutes at temperatures over 68 °C (ideally, 72 °C to 74 °C). After centrifuging the heat-treated culture broth, the mycoprotein is extracted as a paste. This paste serves as the base for a variety of foods that can be prepared for human consumption. In order to align the mycelia into a fibrous network with a texture like that of meat, the myco-protein paste and a binding agent (egg albumin) are mixed together during the production of the final goods. Then, using typical food processing technologies, product is formed. Chunks and mince are among the final products, along with sausages, burgers, fillets, and steaks.

*F. venenatum* myco-protein contains approximately 44% (w/w) protein, on a dry weight basis, and the net protein utilization value is comparable to that of milk. There are all nine necessary amino acids. The amounts of essential amino acids in mycoprotein are roughly equivalent to those found in eggs, despite the fact that the concentration of sulphur-containing amino acids is quite low. Additionally, mycoprotein offers a source of dietary fibre in the form of chitin and glucans from the mycelial walls, is free of cholesterol, and has a low saturated fat content.

#### Market Prospects

Without a question, as more customers look for sustainable food options and protein substitutes, the popularity of meat alternatives is exploding. Germany, France, the Netherlands, the United Kingdom, Italy, and Sweden are notable leaders in the research and production of meat replacements, with Europe controlling the world market. By 2029, it is expected that plant-based substitutes would increase at a compound annual growth rate (CAGR) of 10%. The market for meat replacements is very competitive since there are so many tiny businesses. Western nations account for the bulk of the industry for plant-based meat substitutes. Food neophobia is the cause of a lesser acceptance of meat alternatives in several Asian nations. The acceptance of novel meals will rise



over time as customers become more accustomed to the items, since this appears to be the case across all cultures. A poll has been carried out in three well-known nations—the USA, China, and India—to determine whether customers accept beef replacements. Surprisingly, China and India had the highest acceptance rates compared to the United States (74.7%). Healthy diets and concerns about animal welfare and the environment are not the only factors driving the shift away from meat consumption.

## Conclusion

It may be said that there is a significant market need for meat analogues in both the present and the future. The consumers interested about nutritious foods and a sustainable environment were the ones that showed the most significant interest in this product, not the rise in vegan consumers. However, there are still few meat alternatives on the market today, and most of what is offered is plant-based. The research and development needed to guarantee meat of a standard of quality required several years. Additionally, due to the availability of resources, alternative meat producers can always manufacture goods based on insects. But the biggest obstacle to be overcome, aside from safety concerns, is acceptability of meat made from insects.

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## Pathogenesis, diagnosis and treatment of copper toxicity in animals

Gunjan and Jay K. Desai

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### Occurrence

Accidental administration of a copper sulphate solution that is overly powerful or in excess for treating parasite disorders. consuming food that contains copper salts. The consumption of copper-rich foods and salt licks can both lead to chronic poisoning. grazing on pasture that had been treated with a copper salts-containing fungicide. Copper salts used in snail eradication campaigns have contaminated drinking water. In regions with copper-rich soil, chronic poisoning is possible. Consuming some plants, such as *Heliotropum europaeum* and *Echium plantagineum*, causes aberrant copper metabolism. Acute poisoning may develop from copper intakes of 20–100 mg/kg in sheep and young calves, and 200–800 mg/kg in mature cattle. Sheep grazing on pastures containing 15-20 ppm (dry matter) of copper and low levels of molybdenum may experience chronic poisoning with daily copper intakes of 3.5 mg/kg.

### Pathogenesis

Protein coagulants are copper salts that are soluble in water. As a result, they severely irritate the alimentary mucosa when consumed. If the initial intake is too high, gastrointestinal distress could result in shock and death. In other situations, where the irritability is not extreme enough to result in shock, copper enters the bloodstream and causes severe intravascular hemolysis. Hepatic insufficiency and hemoglobinuric nephrosis are present together. Hemolytic

crisis, which includes methemoglobinemia and deteriorating lesions in the brain's white matter, is the name given to the entire occurrence. It takes a certain amount of copper to trigger a hemolytic crisis, and if less than that, as in chronic copper poisoning in sheep, it tends to be stored in the liver. After six months, for example, when the maximum liver levels are reached, copper is rapidly released into the bloodstream, causing an acute, deadly hemolytic crisis. Thus, these sheep seem to be in good health before the hemolysis starts. Because the release of hepatic copper is influenced by stress, situations like fasting, travel, and lactation frequently trigger the illness. The liver and kidneys' copper levels rise during the prehemolytic and hemolytic stages of the illness. Additionally, during hemolysis, iron levels in the kidneys significantly rose, notably in the apical lysosomes of the proximal tubule cells. Digestive system absorption of copper results in a cumulative effect. Copper absorption is inhibited by high molybdenum levels. Copper is eliminated through the gastrointestinal tract, urine, and bile.

### **Symptoms**

There will be severe thirst and vomiting in the acute stage. vomiting that is green and has mucous. Abdominal discomfort that is severe will be felt. Diarrhea of a vivid green color. Death usually occurs within 24 hours of convulsions and coma. Loss of appetite and severe depression in chronic form. sluggishness and emaciation. Diarrhea followed by constipation. Jaundice with hemoglobinuria. loss of hair and thickening of the skin.

### **Post-mortem appearance**

In its acute form, gastro-enteritis is clearly present, and the mucous membranes thicken. Abdominal abrasion. spleen, liver, and renal enlargement. heart with hemorrhagic spots. Gastroenteritis in its chronic phase. hepatic fatty degeneration and widespread icterus. The liver and spleen accumulate hemosiderin. Friable and enlarged spleen. myocarditis, cystitis, and nephritis. The tubules in the kidneys may be clogged with bloody casts and enlarged. Greenish-brown bile that is viscous and bloated fills the gall bladder.

### **Diagnosis**

On the basis of a sudden death preceded by a hemolytic crisis and severe liver damage, a presumptive diagnosis is made. Measure copper concentrations in samples of the liver and kidney at the time of death if there is an elevated serum copper concentration, albeit the increase may be



temporary. Acute copper poisoning is characterized by blue-green ingesta, elevated faecal (8,000–10,000 ppm), and renal (>15 ppm, wet wt.) copper levels. Copper concentrations in the blood and liver rise during the hemolytic phase in chronic poisoning.

### **Treatment**

Symptomatic treatment for shock. Calcium versenate and penicillamine may be worth trying. Intervention should emphasize prevention because once liver damage and the hemolytic crisis have occurred, treatment is rarely effective. All animals have dismal prognoses. In animals with acute poisoning, supportive care for shock and gastrointestinal symptoms may be beneficial. If given in the early stages of the condition, penicillamine (50 mg/kg per day, PO, for 6 days) or calcium versenate may also be helpful to increase copper excretion. During a hemolytic crisis, vitamin C (500 mg/day per sheep, SC) may be able to lessen oxidative damage to RBCs. Both the treatment and prevention of copper poisoning work well with ammonium tetra thiomolybdate (1.7 mg/kg, IV, every other day for 6 days). Use this medication with caution as it improves copper excretion while decreasing copper absorption. This drug requires 10 days detox period. For three weeks, daily oral dosing of sodium thiosulfate (1 g) and ammonium molybdate (100 mg) may minimize the number of deaths in affected lambs. To lessen the absorption of copper, dietary supplementation with zinc acetate (250 ppm) may be helpful. Eliminate or restrict access to plants that cause phyto-genous or hepatogenous copper toxicity.

In sheep flocks at high risk, dietary sodium thiosulfate supplementation may be used to prevent or treat chronic copper toxicosis. Additionally, when molybdenum or thiosulfate is supplied, periodic assessment of the copper and molybdenum concentrations in feed as well as refraining from feeding cattle feeds to sensitive species like sheep may help prevent chronic copper poisoning. Additionally, it is preferable to refrain from overdosing animals with chelated minerals.



## Post-mortem appearance, diagnosis and treatment of molybdenum toxicity in animals

Gunjan and Jay K. Desai

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Molybdenum and copper are antagonistic to one another. Intake of Sulphur and molybdenum in excess leads to copper insufficiency. A compound made of copper sulphate and sodium molybdenum precipitates from a neutral solution. Because copper is biologically inaccessible when bound in a Cu-Mo complex, an animal may also contain such a complex.

### Occurrence

Sheep, goats, and cattle are vulnerable. Consumption of plants from soils high in molybdenum. emissions from several molybdenum-using companies contaminating grasslands. Cows that were feeding on pasture with 16 ppm copper in fresh grass (dry matter) and were close to an industrial facility that produced molybdenum oxide developed hypocuprosis. In dry matter, the fresh grass had 29–80 ppm molybdenum. Soon after receiving copper sulphate treatment, symptoms vanished.

### Pathogenesis

Consuming molybdenum interferes with the liver's ability to store copper, which results in an indirect copper deficit. Mo poisoning is aggravated when low intakes of copper and high intakes of Sulphur happen at the same time. With the exception of diarrhea, exostosis, and hemorrhages along the long bones, the indications are primarily those of copper insufficiency. Secondary abnormalities in the metabolism of minerals, in particular copper, iron, calcium, and phosphorus, are brought on by molybdenum toxicity.

### **Symptoms**

Diarrhea that is severe and persistent. Emaciation, weight loss, and a dry, glaring coat. Depigmentation of the hairs around the eyes, creating the impression of glasses. decrease in milk production. Lameness, a stiff walk, and aching joints. Overgrowth of the long bones' ends and osteoporosis. Calves' stunted growth and wastefulness. Young bulls lose their libido. Leucopenia and anemia.

### **Post-mortem Appearance**

No distinctive lesions. Dehydration, anemia, and emaciation. The loss of melanin pigment from the hair cortex and medulla, hemosiderosis of the lymph nodes, spleen, liver, lungs, and gut, degenerative changes in the liver, skeletal muscles, and testes, colloidal goitre, and stunted bone growth in goats with molybdenosis were only a few examples of the microscopic abnormalities.

### **Treatment**

Copper sulphate 1g for calves and 2 g for cows, daily in feed or 5 g weekly. Recovery within 2-3 days confirms the diagnosis. One gram of copper sulphate and 1 mg of cobalt per 100 lb body weight in drinking water at weekly intervals.



## Tips to Increase Profit Margins in Sheep Farming

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### *Abstract*

Sheep farming requires a lot of labor, so keeping production costs in check is essential if a person wants to make a healthy financial profit. Sheep are mostly valued for the production of their wool, milk, skin and excrement. Its meat is delicious, extremely nutritious, and well-liked by people all over the world.

### **Introduction**

India is leading in both the number of cattle and milk production. According to the 20<sup>th</sup> livestock census, which was carried out in 2019, there are currently 535.78 million animals in the country, an increase of 4.6% from the previous livestock census in 2012. India ranks third in the world with 6.4% of the global sheep population. According to the 20<sup>th</sup> livestock census, the country's total sheep population is 74.26 million, increased by 14.13% over the previous census (2012). Sheep, with their numerous uses for wool, meat, milk, skins, and manure, are a significant part of the agricultural economy, particularly in the country's arid, semi-arid, and mountainous regions. Sheep farming makes up around 8.5 percent of the overall value of animal agriculture's production, particularly in mountainous, desert, and semi-arid regions where cereals and dairy farming are unprofitable (Gupta *et al.* 2011). Through the selling of wool and livestock, it offers the farmers a steady source of income. They are crucial to the livelihood of a significant portion of small and marginal farmers as well as landless labors that breed sheep. Five million households in India



raise sheep, goats, and rabbits, among other small ruminants, as well as engage in related activities (Singh *et al.* 2020).

### **Why sheep farming?**

- ✓ Sheep can be reared on farms alongside other animals in smaller spaces and do not need expensive homes to live.
- ✓ Starting a business does not involve high costs associated with sheep husbandry.
- ✓ The sheep farming business uses less labor and generates a considerable amount of profit.
- ✓ Low-quality grass is sufficient for sheep to survive. They produce milk, meat, and wool, all of which have diverse uses.
- ✓ By using competent management, this business can be a reliable source of income from sheep farming and employment.
- ✓ In India, those who are unemployed can still make a solid living and obtain financing for sheep farming.

### **How to Begin a Sheep Farming Enterprise**

- ✓ Create a sheep and goat farming business plan that fits budget. Additionally, a financial analysis, a marketing plan, and income revenue are required in sheep farming business plan.
- ✓ To start sheep farming, pick a suitable place. Sheep need access to clean, freshwater sources, plenty of green food, competent medical care, and transportation. For the purpose of launching a sheep farming enterprise in India, one must take sufficient care and offer the necessary amenities.
- ✓ One of the most important factors for the sheep farming industry is the quality of breeds, which can increase output. There are numerous sheep breeds in the globe, however not every breed is ideal for every farming region. As a result, you will need to prepare the breeds in accordance with the local agricultural climate.
- ✓ Choose the sheep breed that is appropriate for your business plan e.g. Chokla, Magra, Sonadi, Marwari, Hassan etc.
- ✓ Construct a shelter for sheep that will shield them from harmful weather conditions and protect them from various diseases. Their home should be tidy, spotless, dry, and have enough light and



air. Additionally, the house should have a suitable drainage system for them so as to avoid dirtiness and odor.

### **Marketing strategies before starting sheep farming business**

Before beginning a business plan for a sheep farm, determine the marketing plans and methods.

- ✓ For better and more effective marketing, a person should focus on local markets and neighbourhoods. If a person has the necessary infrastructure and sheep farming expertise, selling sheep farming or sheep goods on the global market would be the best option.
- ✓ Small-scale households or individuals can benefit greatly from the sheep farming industry, with a few creative ideas and can even turn a profit from sheep farming on its own.

### **Management practices for reducing production cost**

1. When compared to cattle, buffalo, and pigs, sheep are simpler to handle.
2. Perfect pasture land is not necessary for sheep. They are content to eat shrubs, grass, and bushes that grow in unfavorable soil.
3. The term "**golden footed animal**" refers to sheep. The soil will receive fertilisation from sheep excrement. A former sheep pasture is a good place to grow crops. Its manure is of higher quality than that of other animals due to higher percentages of N, P, and K.
4. Purchase the sheep directly from the person who raised them. Look at the herd the lambs come from and talk to the person about animal and its parentage history.
5. Sheep are ruminant animals that primarily consume hay and new grass. However, if given nothing but good pasture, salts, vitamins, and mineral supplements, along with fresh water, they can survive and even thrive. Combine grass, bushes, and trees. A good pasture can typically support 5–6 sheep per acre.
6. Decide how many sheep you'll buy; the number of sheep you can raise will depend on your location and the productivity of your land. Additionally, market prices and potential returns must be taken into account if you want to make money from sheep.





7. Simple wooden logs are the best type of sheep fence because they are inexpensive and readily available. The temporary paddocks can be enclosed with electric mesh fencing. To keep the sheep on new pasture, rotate them among different paddocks.
8. Provide enough food (hay, silage or pasture). During the final trimester of pregnancy and until weaning, give adequate nutrition (pasture, high-quality grain).
9. Give growing lambs enough pasture and forage.
10. Start a low-investment, high-yielding semi-intensive sheep farming system in which a sheep spends half of each day in the field and the other half in the sheep shed.

### **Key rules for increasing sheep production**

1. Do not underfeed sheep.
2. Maximize the number of extra lambs produced
3. Think about selling first
4. Think about supplemental bedding options
5. Prepare your flock for the winter.
6. Keep accurate records.

### **Conclusion**

Many small and marginal farmers as well as landless laborers depend heavily on sheep farming for their living. Wool and sheep skins are used as the primary raw materials in a variety of agricultural businesses. Three more streams of income are generated each year through the production of manure, wool, and meat. Modern technology has been steadily introduced into sheep farming. The cost-benefit analysis of sheep farming has gained significance in light of this and the requirement for the establishment of policies for the development of sheep.

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## Japanese encephalitis: an epidemiological paradigm

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### *Abstract*

A Major havoc, flavivirus in the Asia-Pacific region is the Japanese encephalitis virus (JEV). Over two billion individuals are susceptible to infection because they reside in endemic and epidemic regions. The first native human case was recently reported in the African continent, while birds with the infection have been discovered in Europe. JEV might spread to different continents. The worldwide distribution of JEV is covered in the first half of this review along with some recent findings. The effects of JEV on people, including their natural course and immunity, are the main topics of the following sections.

**Keywords:** Epidemiological studies, blood-brain barrier, transmission, cellular events, Japanese encephalitis virus (JEV), flavivirus, JEV

### **Introduction**

The Japanese encephalitis virus (JEV), a recently identified flavivirus transmitted by mosquitoes, has connections to the virus that causes dengue (DENV), the Western Nile virus (WNV), the Zika virus (ZIKV), or the encephalitis brought on through the tick-borne viral (TBEV). JEV is an encapsulated virus with the virion size of approximately fifty nm, an RNA single-stranded genome that has a positive sense of about 11 kB, and minimal length changes between genotypes, as reported by Lu *et al.* (2017) & Desingu *et al.* in (2017). JEV is the primary cause of encephalitis in humans in the Asia-Pacific region. JEV originated from an African ancestor virus that had long since



relocated into the Indonesia-Malaysia area, from which it subsequently spread through Asia and all of the way to Australia, consistent with genetic studies (Solomon *et al.*,2003).One serotype and several genotypes have been mapped by serological and genetic investigations across Australia and New Zealand and also in Africa and Europe, demonstrating ongoing changes in the geographic distribution of JEV and JEV adaptation to novel circumstances (Oliveira *et al.*, 2018; Samy *et al.*,2018; Fang *et al.*,2019; Gao *et al.*,2019; Garjito *et al.*,2019;Karna *et al.*, 2019).While epidemic episodes were reported in temperate and cooler-climate regions, only tropical places have been considered to be endemic. Recently, Angola, Africa, has reported the first indigenous human case outside of Australasia [10], while Italy, Europe, has reported finding JEV in birds. But other transmission cycles and chains have been identified. Wild water and wading birds, especially migratory species including herons, egrets, and ducks, create a natural JEV reservoir. With the possible exception with the Middle East and certain regions of Australia, when the presence with the birds has been established but where no JEV cases have been recorded by Samy *et al.* (2018) have predicted the geographic spread of egrets throughout the Australasian region, this corresponds well to the observed presence of JEV cases (Gao *et al.*,2019).Therefore, one must take into account that eagles and egrets travel in the summer to the northwestern temperate parts of Eurasia for breeding.

They may also live year-round in the subtropical and less chilly parts of Europe, Africa, and Asia, but they move to the tropics for the winter. Therefore, JEV may move across the ocean out of endemic tropical locations into countries with temperate and tropical climates where JEV may reemerge due to long-distance migratory birds (herons and egrets). Ducks have been found to transmit JEV across seas as well as shorter distances, as was the case in Japan as well as Korea (Simon *et al.*,2017). JEV has been surprisingly discovered in the lymph nodes the birds in Italy, likely as a result of long-distance bird migration between Asia to Europe. However, nothing is known about these species' migratory paths.Future research estimating the propagation of JEV by migrating birds may benefit from technological advances, such as satellite-based tracking, as it has been demonstrated in recent research for the influenza H5N1 virus (Samy *et al.*,2018).

The fact that the infected birds are asymptomatic JEV carriers is crucial (Karna *et al.*, 2019). However, more recent research suggests that specific JEV strains can cause illness in newly hatched birds (Oliveira *et al.*, 2018). To comprehend the immune chemistry of JEV in various vulnerable



birds, more study is required. Bats, in addition to other birds, may serve as natural JEV reservoirs and contribute significantly to JEV transmission. Two scenarios are theoretically possible: first, migratory JEV carrying birds may have entered Angola, and second, JEV could have been spread by comparable indigenous mosquitoes. Second, one must take into account the fact that Angola has grown to be China's principal African trading partner, or vice versa. However, only additional cases in the future and related research will shed light on potential infection paths for African patients.

## Conclusion

The pathophysiology concentrating on humans and biological pathways that foster JEV infection were covered in the following sections. For the focused development of novel effective anti-viral medicines, a deeper knowledge of infection with JEV at the cellular or system level is necessary.

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# The influence of transposable elements on plant pathogen evolution

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## Introduction

Transposable elements (TEs) are the DNA sequences that have ability to change their position within a genome. These are also called as jumping genes, junk DNA, selfish DNA *etc.* TEs are present in every prokaryotic and eukaryotic genome and make up a large fraction of the genome and are responsible for much of the mass of DNA in a eukaryotic cell. Although TEs are selfish genetic elements, many are important in genome function and evolution. Transposons are also very useful to researchers as a means to alter DNA inside a living organism. As a result of their deep evolutionary origins and continuous diversification, TEs come in a bewildering variety of forms and shapes. Transposition often results in duplication of the same genetic material. Transposable elements were discovered by Barbara McClintock in 1965 through an analysis of genetic instability in maize and earned a Nobel Prize in 1983.

## Characteristics of transposable elements

- i. They code for enzymes which result in self-duplication and insertion into a new DNA site
- ii. Because transposons carry the genes for initiation of RNA synthesis, some previously dormant genes might be activated
- iii. It doesn't have a site for the origin of replication. As a result, it cannot replicate without the host chromosome as plasmids or phages
- iv. There is no homology between the transposon and its target site for insertion
- v. These elements can insert at almost any position in the host chromosome or a plasmid

- vi. Some transposons might seem likely to enter at some specific positions (hot spots), they barely insert at base-specific target sites

### Classification of transposable elements

TEs can be divided into two major classes based on their mechanism of transposition and each class can be subdivided into subclasses based on the mechanism of chromosomal integration (Table 1). Class 1 elements, also known as retrotransposons, mobilize through a ‘copy-and-paste’ mechanism whereby a RNA intermediate is reverse-transcribed into a cDNA copy that is integrated elsewhere in the genome (Boeke *et al.*, 1985). For long terminal repeat (LTR) retrotransposons, integration occurs by means of a cleavage and strand-transfer reaction catalyzed by an integrase much like retroviruses (Brown *et al.*, 1987). For non-LTR retrotransposons, which include both long and short interspersed nuclear elements (LINEs and SINEs), chromosomal integration is coupled to the reverse transcription through a process referred to as target-primed reverse transcription (Luan *et al.*, 1993). Class 2 elements, also known as DNA transposons, are mobilized *via* DNA intermediate, either directly through a ‘cut-and-paste’ mechanism or in the case of Helitrons, a ‘peel-and-paste’ replicative mechanism involving a circular DNA intermediate.

**Table 1: The hierarchical classification of transposable elements**

Level	Description
Class	It divides transposable elements (TEs) into two classes based on their transposition intermediate: RNA (class I or retrotransposons) or DNA (class II or DNA transposons).
Subclass	It separates TEs that transpose via “copy-and-paste” mechanism from those <i>via</i> “cut-and-paste” mechanism.
Order	It distinguishes TEs with different insertion mechanisms due to dissimilar encoded enzymes
Superfamily	Superfamilies within an order share the same insertion mechanism but are different in terms of enzyme organization, non-coding domains and/or TSD
Family	It is defined by DNA sequence conservation.
Subfamily	It is defined on the basis of phylogenetic data and might serve to differentiate autonomous and non-autonomous derivatives.



Each TE subclass is further divided into subgroups (or superfamilies) that are typically found across a wide range of organisms, but share a common genetic organization and a monophyletic origin. For example, Ty3/*gypsy* and Ty1/*copia* elements are the two major superfamilies of LTR retrotransposons that occur in virtually all major groups of eukaryotes (Malik and Eickbush, 2001). Similarly, Tc1/*mariner*, *hAT* (hobo-Ac-Tam3) and MULEs (Mutator-like elements) are three superfamilies of DNA transposons that are widespread across the eukaryotic tree (Feschotte and Pritham, 2007). At the most detailed level of TE classification, elements are grouped into families or subfamilies, which can be defined as a closely related group of elements that can be traced as descendants of a single ancestral unit. This ancestral copy can be inferred as a consensus sequence, which is representative of the entire (sub) family (Britten and Kohne, 1968). Thus, in principle, every TE sequence in a genome can be affiliated to a (sub) family, superfamily, subclass and class (Fig. 1). However, much like the taxonomy of species, the classification of TEs is in constant flux, perpetually subject to revision due to the discovery of completely novel TE types, the introduction of new levels of granularity in the classification, and ongoing development of methods and criteria to detect and classify TEs.

Transposons further can be classified as either "autonomous" or "non-autonomous" in both Class I and Class II TEs. Autonomous TEs can move by themselves, whereas non-autonomous TEs require the presence of another TE to move. This is often because dependent TEs lack transposase (for Class II) or reverse transcriptase (for Class I).

### **Functions of transposable elements**

#### **TEs: Adaptive drivers of evolution**

Genome plasticity enables organisms to adapt to environmental changes and occupy novel niches. Adaptive evolution mediated by TEs is facilitated by recombination events resulting in genomic diversification. This is achieved through genomic changes, which persist under positive selection in fungal pathogens. TEs contribute towards adaptive genetic variation through:

- (1) TE insertion into coding genes (Jangam *et al.*, 2017)
- (2) TE insertion into introns
- (3) TE transposition in proximity to genes
- (4) Generation of retro copies *via* reverse transcription and
- (5) Aberrant transposition and ectopic recombination through paralogous TEs.





**TEs: Structural transformers of the genome**

Genome plasticity can be described as alterations observed in a genome structure, which can be characterized by changes in genome organization, chromosome number and genome size. Genome plasticity is mainly influenced by environmental stresses faced by individual genes in host species that confer genetic adaptability traits for survivability where TEs may play an assistive role (Belyayev, 2014). Eukaryotic genome plasticity can be caused by TE-mediated chromosomal rearrangements through ectopic homologous recombination or alternative transposition. Furthermore, the mutation caused by TE insertion may also lead to generation of new proteins promoted by exon shuffling and TE domestication (Castanera *et al.*, 2016). TE insertions within functional genes may bring about alternative splicing resulting in altered protein synthesis. All of these TE insertion-promoted processes, *viz.*, exon shuffling, TE domestication and exonization, can generate novel genes with possible specific functions in the host (Fig. 1).

**TEs: Mediators of pathogenicity and host range**

Effector proteins that are secreted by fungal pathogens to promote colonization interfere with host defense and result in necrosis. By the same token, effector proteins can be recognized by their complementary plant resistance genes, leading to the activation of defense responses in plants (Venner *et al.*, 2009). Pathogenicity genes that are often clustered within a specific region of the genome codes for these molecules that promote infection of host plants. TEs' recurring association with pathogenicity can be seen by looking at the positional aspect. TEs often sit in proximity with pathogenicity factors as seen in *M. oryzae* where genes that encode secreted proteins are found within 1 kb flanking distance from TEs (Bao *et al.*, 2017).

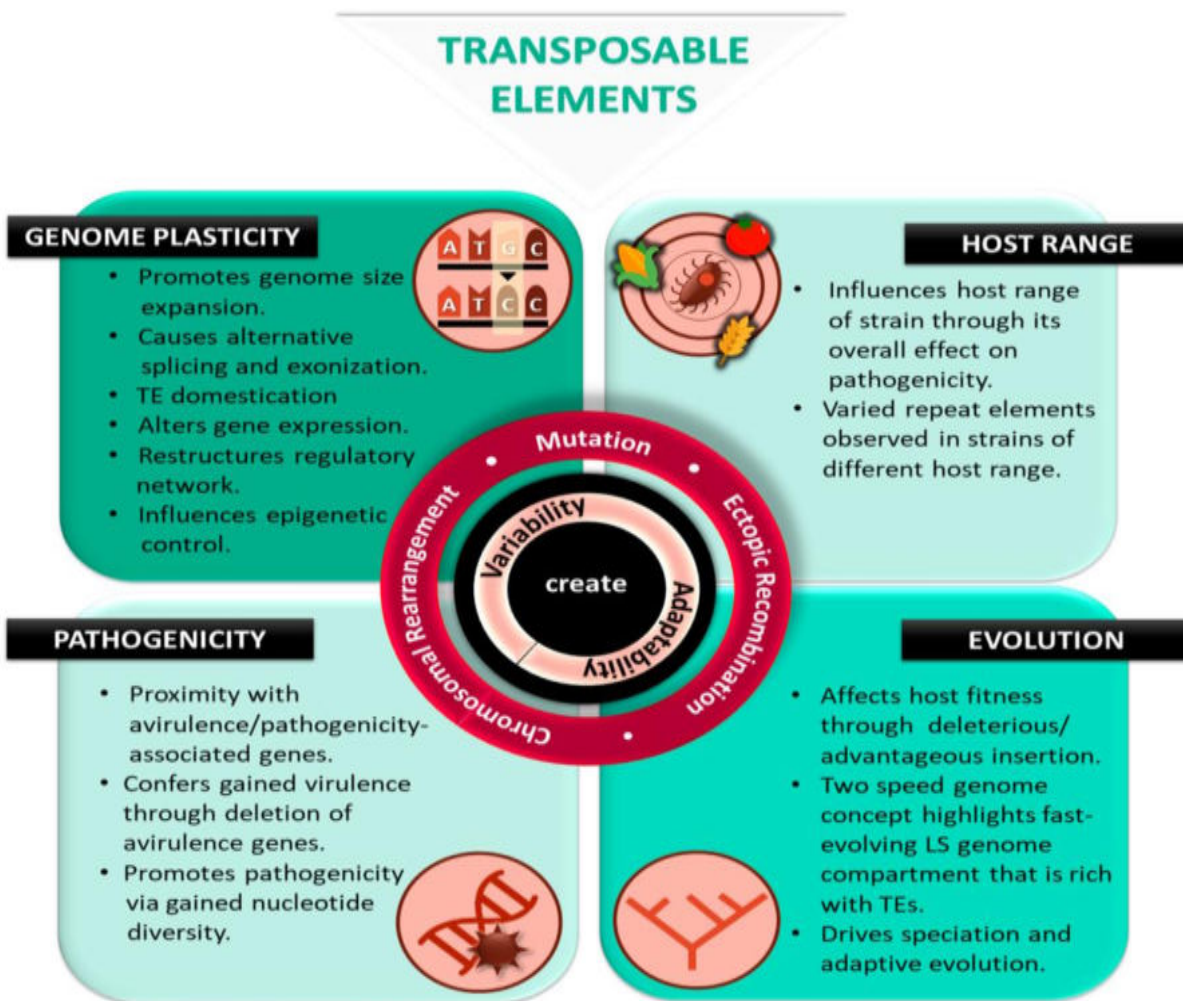
TE insertion mediates pathogenicity through mutational effects on pathogenicity-associated genes. Mutations from TE insertions can lead to genetic variability that generates many new pathogenic variants with conferred ability to invade previously resistant host plants (overcome host plant resistance) and thence expand on its host range. TE mediated inactivation or deletion of PAMP-encoding genes or effector genes important for host recognition results in gain of virulence (Fig. 1) by evading the plant's immune system (Dean *et al.*, 2005). A genomic region occupying *Avr4/6* in *Phytophthora sojae*, which is responsible for virulence in soybean, revealed a Ty1/*Copia*-like



element in proximity to this locus. This TE insertion caused point mutations conferring virulence to this locus (Basnayake *et al.*, 2009).

### Transposable elements in plant pathogenic fungi

In fungi they were first identified in the yeast *Saccharomyces cerevisiae* (Boeke, 1989) but only very recently in filamentous fungi. Despite extensive investigation of molecular genetics of some species used as models for fungal genetics, exemplified by the well-studied ascomycetes *Neurospora crassa* and *Aspergillus nidulans*, no evidence for the activity of transposable elements has been revealed that might be the consequence of continuous selection for phenotypic stability.



**Fig. 1. The role of transposable elements in affecting genome plasticity, influencing host range and pathogenicity and shaping evolution of phytopathogens**



Paradoxically, most of our knowledge of TEs in fungi comes from studies on undomesticated species: plant pathogens, industrial and field strains. Most of these species lack the sexual stage and generally exhibit a high level of genetic variation, which attracts speculation that they contain active transposons.

Fungal TEs have been identified by a variety of strategies, mainly by the characterization of dispersed repetitive sequence or by trapping them in a target gene. The nitrate reductase gene has been particularly useful for this purpose because chlorate resistance can select loss-of-function mutants (Daboussi, 1997). Other elements were found by heterologous hybridization or polymerase chain reaction (PCR) amplification with degenerated primers deduced from conserved domains (Daboussi, 1997). Finally, as genome segments began to be cloned and sequenced, the discovery of new TEs accelerated (Cambareri *et al.*, 1998). The TEs presented in Fig. 3 are found in three orders of fungi, Ascomycota, Basidiomycota and Zygomycota. However, most were identified in Ascomycota species. This bias is probably due to the number of researchers working on ascomycetes, using *A. nidulans*, *N. crassa*, *A. immersus* and *Podospora anserina* as models, or their involvement in plant interactions and biotechnology processes. The 60 TE sequences have been assigned to the major groups previously described in the different kingdoms (Capy *et al.*, 1997).

### **Transposable elements in plant pathogenic bacteria**

There are two main types of transposable elements in bacteria having different size and structure.

1. Insertion sequences (IS elements)
2. Prokaryotic Transposons (Tn): Composite and non-composite transposons

### **Insertion sequences:**

These are simplest type of bacterial transposable sequences that can insert at different location of bacterial chromosome and plasmid through illegitimate recombination. They are typically short sequences and contain only one gene that encodes the enzyme for transposition. A bacterial chromosome may contain several copies of a particular type of IS element

### **Characteristics of Insertion sequences**

1. IS elements are compactly organized and containing about 1000 nucleotide pairs and contain only genes (open reading frame) which encode for enzyme for regulating transposition.



2. Many distinct types of IS elements have been identified. The smallest IS element is IS<sub>I</sub> which is 768 nucleotide pairs long.
3. Each type of IS element contains inverted terminal repeats at both end and a transposon sequence in between those inverted repeats. Transposon is the only gene that code for transposition of IS element.
4. The inverted terminal repeats is 9-40 base pair long and is the characteristics of most IS element
5. IS element have the capacity to duplicate the inserted sequence at the site of insertion; known as target site duplication.

### **Transposition of insertion sequence in bacteria**

IS element containing single open reading frame (ORF) which encodes for the enzyme transposase, catalyzing its own transposition. The enzyme transposase is like restriction endonuclease which binds to terminal inverted repeats (IR) of IS element which is the restriction site. Then the enzyme cut and excise IS elements from chromosome or plasmid. The excised IS element is mobile in nature and moves along the length of chromosome to recognize the target site for insertion on same or different chromosome or plasmid. Once recognizing the target site, it generates staggered cleavage (cut the single strand of DNA) generating sticky and itself gets inserted. As IS element get inserted, the proofreading mechanism of DNA results in duplication of the DNA sequence at the target site of the insertion such that one copy of target DNA is located on each side of IS element. Thus, IS elements helps in target site duplication.

### **Prokaryotic Transposons (Tn)**

Prokaryotic Transposons are similar to IS element but they are larger and also contain other genes (mostly antibiotic resistance gene) in addition to gene that encode transposase. Transposons are several thousand base pairs long and contain inverted terminal repeats. There are two types of prokaryotic transposons- composite and non-composite transposons. The composite transposons and Tn<sub>3</sub>-like elements are more complex than IS elements, containing some genes that encode products unrelated to the transposition process.

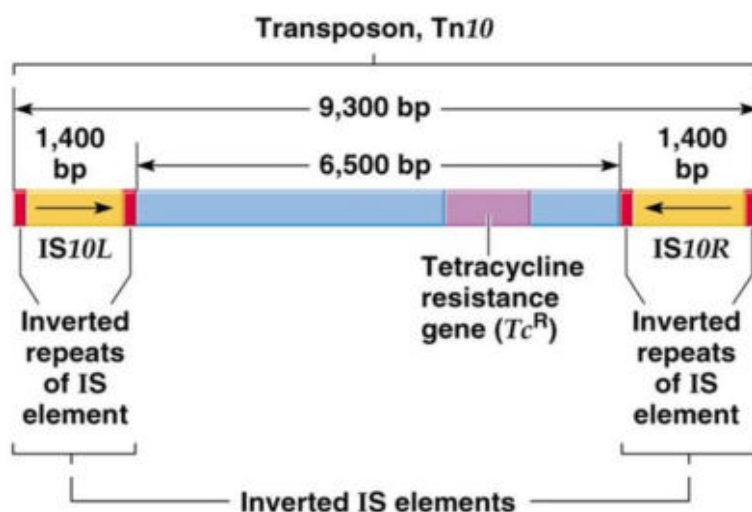
**Composite transposons:** Composite transposons are created when two IS elements insert near each other and the region between the two IS elements can then be transposed when the elements act jointly. For example, Tn<sub>10</sub> is composite transposons of 9.3kbp which contains 1.4 kbp terminal



inverted repeats and in between them is gene for transposase and gene for antibiotic resistance (Fig. 2).

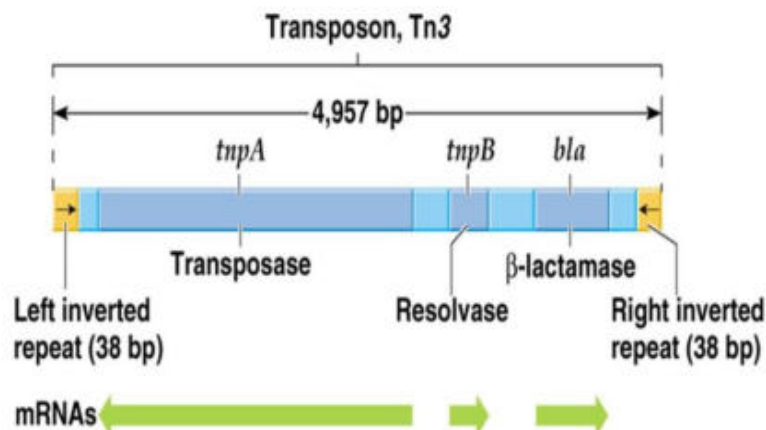
**Non-composite transposons:** The non-composite transposons are a sequence of DNA containing gene for transposase and multiple other genes in between terminal inverted repeats. Unlike composite transposons, it does not contain IS elements at each end but instead it contains simple inverted repeats of 38-40 nucleotide pairs at each end. For example, Tn3 is non-composite transposons of 5kbp which contains three gene for beta-lactamase (*bla*), transposase (*tnpA*) and resolvase (*tnpB*). The beta lactamase provide resistance to the antibiotic ampicillin, and the other two enzymes play important roles in transposition and recombination (Fig. 3).

ISs can generate significant variability in bacteria and contribute to their evolution (Jackson *et al.*, 2011) in part because they are usually present in more than one copy per genome and thus represent mobile regions of recombination. Their mobility, together with their capacity to mobilize unrelated DNA in their proximity, can lead to panoply of mutations and rearrangements in the host bacteria, which include insertions, deletions, duplications, translocations, co integrations, inversions and gene activation (Craig *et al.*, 2002). From these activities, it easily follows that they have an enormous potential to alter the genome and influence bacterial evolution. They can also shuffle DNA among different genetic replicons such as chromosomes and plasmids sustaining a gene trading activity that widely contributes to the horizontal spread of genetic information.



**Fig 2. Composite transposons in bacteria**





**Fig. 3. Non-composite transposons in bacteria**

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## Role of antimicrobial peptide in fish immunity

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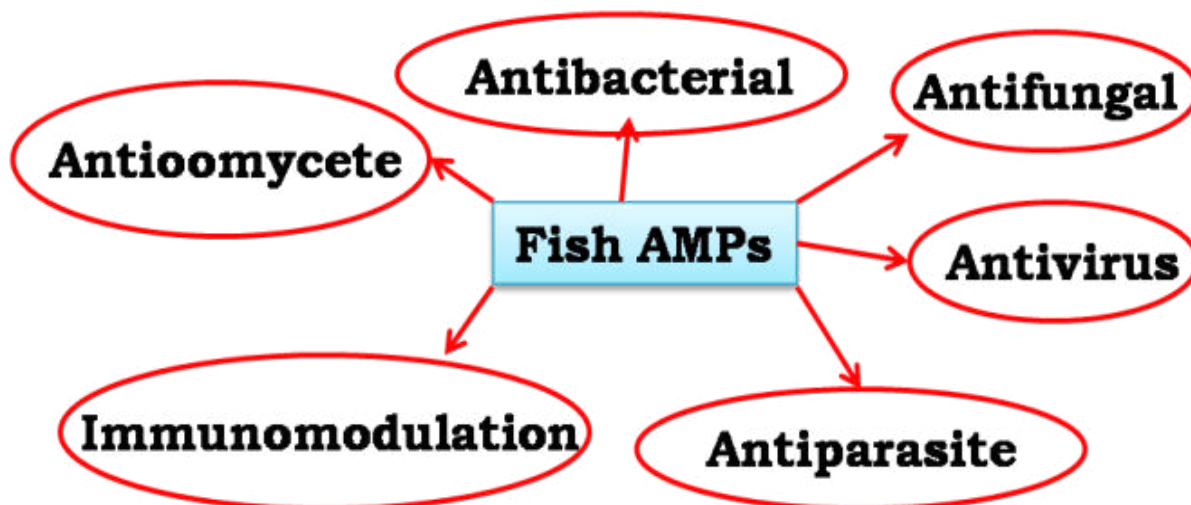
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### Introduction

Antimicrobial peptides (AMPs) are an important component of innate immunity across all classes of life ranging from prokaryotes to higher animals. Being a key component of innate immunity, AMPs are produced in the sites which are at high risk of pathogen invasion. The main advantage of AMPs possesses broad spectrum antimicrobial activity and can function without memory and specificity, against bacteria, fungi, virus and even parasites. They also exhibit diverse biological functions such as immunomodulation, neutralization of endotoxin and induction of angiogenesis, wound healing, antitumor in addition to its antimicrobial activity. AMPs generally consist of 10 to 50 amino acids and most of them are cationic in nature with a net charge of +2 to +9, although some anionic (negatively charged) AMPs have also been discovered. They are divided into several subgroups such as anionic, cationic  $\alpha$ -helical, cationic  $\beta$ -sheet and extended cationic AMPs based on the amino acid sequences, net charge and structure. An attractive feature of AMPs is the attack on microbial membranes and rapid killing action reducing the chances of developing resistance by the microbes. Further, AMPs have also been recognized as one of the promising alternatives to antibiotics as they are effective against multi-drug resistant bacteria. Till date, many AMPs have been identified from different sources including fish. Being an aquatic animal, fish survive in an environment loaded with saprophytic and pathogenic microbes. Since the adaptive immune system in fish is less developed, they rely more on their innate immune system to shield themselves from the attack of pathogenic microbes. This indicates that fish must produce numerous non-specific antimicrobial molecules which play critical roles in maintaining its health.





**Different functions of fish antimicrobial peptide**

### AMPs of fish

Fish produces all major classes of AMPs similar to mammals in addition to a specific class called piscidins. Fish AMPs exhibit immunomodulatory functions and broad-spectrum antimicrobial activity against both fish and animal pathogens. AMPs derived from fish and their functions are discussed below;

- i) Piscidins: They are a family of linear amphipathic AMPs with potent antimicrobial activity against a variety of microorganisms. They are active against bacteria, fungi, water molds, virus and parasites. They are mainly produced in gill, skin, and intestine and also found in head-kidney and spleen. Expression of piscidin gene can be induced by stimuli like presence of bacterial or bacterial cell component such as LPS. Piscidins have immunomodulatory functions and are able to modulate the expression of pro-inflammatory and other immune-related genes.
- ii) Defensins: These are the cysteine rich cationic AMPs which are active against both Gram-negative and -positive bacteria and fish specific viruses. They exhibit chemotactic activity and can stimulate antimicrobial activity in phagocytes. It is constitutively expressed in early developmental stages as a part of the defense system. Its expression can also be induced by stimuli like LPS,  $\beta$ -glucans and peptidoglycan.
- iii) Hecpudin: These are cysteine-rich peptides with antimicrobial activity against a wide variety of fish pathogens at the low micromolar range. It has the ability to modulate the expression of different immune-related genes in fish. Its expression can be detected as early as in the



fertilized egg and can also be induced by exposure to both Gram-positive and Gram-negative bacteria, fungi and viruses. It also acts as iron regulator and may also serve as pleiotropic sensor for other divalent metals.

- iv) Cathelicidin: Fish cathelicidins have variable antimicrobial activity depending on its source. Some cathelicidin has activity against only Gram-negative bacteria while others are active against both Gram-negative and positive bacteria and even fungus. Its expression is observed as early as in embryonic stage suggesting its role in immunity. Expression can be induced by bacteria, bacterial DNA and oomycetes. The immunomodulatory activity has been demonstrated in Atlantic salmon cathelicidins.
- v) Histone-derived proteins: These are the fragments of proteins derived from histones with broad-spectrum activity against fish pathogens including water molds and parasitic dinoflagellate. They are expressed in fish skin, and found in other tissues, such as gill, spleen and the gut and can be induced under stress conditions.

## Conclusion

Production of different classes of AMPs in fish right from early developmental stage to adult is indicative of its importance in defense mechanism. Presence of these peptides in the sites such as skin and gills which forms the physical barrier also indicates that AMPs play a role to prevent the entry of the pathogens. As fish rely heavily on innate immunity, the non specific bioactive molecules including AMPs are crucial to keep its body guarded from the pathogenic microorganisms and maintain its health. Further, fish has been recognized as a good source of AMPs having broad spectrum activity against not only fish pathogens but also human pathogens. AMPs are also considered as a unique candidate to fight against antibiotic resistance due to its membrane disruption ability and rapid killing action. Therefore, fish AMPs can be explored to discover new AMPs for development of novel therapeutic agents.

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## Applications of nanoparticles in aquaculture

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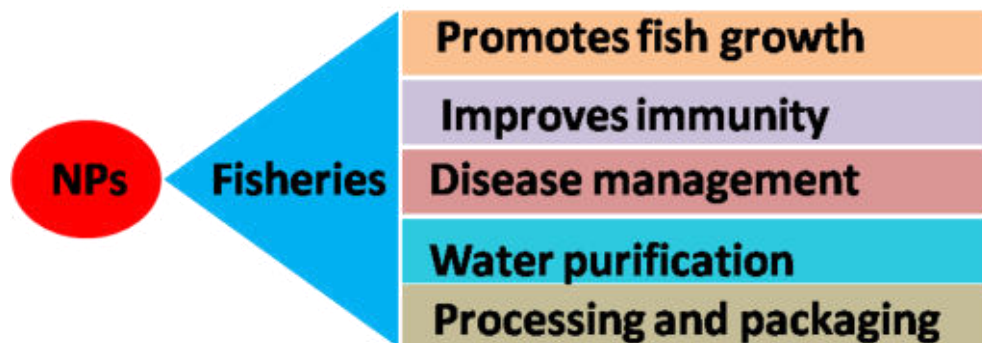
### Introduction

Nanoparticles (NPs) are small particles made of single or multiple materials having at least one dimension in nanoscale ranging from 1 to 100 nm. The outstanding features of NPs, such as larger surface area and high particle number per mass unit as compared to their bulk material, superior mechanical performance, possibility of surface functionalization makes them perfect candidate for wide applications. The main factors that make the NPs significantly different from the larger size material are the surface effects and quantum effects. These effects provide them many enhanced features in terms of mechanical, thermal, magnetic, electronic, optical, and catalytic properties. NPs can be of different shapes such as spherical, conical, cylindrical, tubular, and spiral. They are generally classified into three groups, organic, carbon-based, and inorganic NPs based on their composition. Organic NPs are made of proteins, carbohydrates, lipids, polymers, or any other organic compounds. Carbon-based NPs are made exclusively from carbon atoms while the inorganic NPs (for example metal NPs) are prepared differently from the other two classes.

### Synthesis of nanoparticles

The processes for synthesis of NPs are categorized into bottom-up (arranging atom by atom) or top-down (converting large materials to nanoscale) method. The most commonly used bottom-up methods are sol-gel, spinning, chemical vapour deposition (CVD), pyrolysis and biosynthesis. Among these, sol-gel is the most preferred method due to its simplicity and suitability to synthesize most of the NPs. For large scale production of NPs in industries, pyrolysis method is mainly followed. While the other methods use conventional chemicals, biosynthesis, a environmental

friendly approach is a method for the synthesis of nontoxic and biodegradable NPs using bacteria, plant extracts, fungi, etc. The top-down method involves the reduction of a bulk material to particles of nanoscale size. Some of the most widely used top-down processes are thermal decomposition, mechanical milling, nanolithography, laser ablation, and sputtering.



**Schematic diagram showing effects and uses of nanoparticles in fisheries sector**

### Applications in aquaculture

Aquaculture is considered as a fast-growing industry playing an important role to meet the demands of protein by the increasing population. Global consumption of food fish has increased and side by side the production has also escalated. However, there are concerns about the sustainability of aquaculture due to many challenges like environmental pollution, prevalence of diseases causing unwanted impact on productivity and production. So, various technical innovations or strategies need to be applied to increase the productivity, for successful breeding, for management of water quality and diseases in fish. Among these, nanotechnology has number of potential applications such as in production of effective fish feed, in aquariums and management of water qualities. Some of the applications of NPs in aquaculture are listed below.

**1. Feed supplement:** Fish feed incorporated with nano-selenium and NPs of iron can enhance weight gain and their antioxidant status. Fish fed with nano-selenium has more selenium content in the muscle. Also, addition of selenium, zinc and manganese NPs in feed of early stage improved stress resistance and mineralization of bone. Manganese NPs promoted growth and antioxidant system in freshwater prawn. Copper NPs improved the digestive enzyme activities, antioxidant system and non-specific immune response in freshwater prawn and red sea bream. *Azolla microphylla* based



gold NPs increases hepatoprotective activity and antioxidant effects against chemical induced toxicity in common carp. Aloe vera NPs supplemented feed promote the growth of Siberian sturgeon.

**2. Disease control:** Nano-delivery system is an alternative strategy for delivery of vaccines in fish which enhance the efficacy and is also safer. For example, alginate particles are used for oral delivery of vaccines to aquatic animals. Chitosan, a biopolymer found in the exoskeleton of crustaceans and insects are used for preparation of formulations for delivering different kinds of vaccines in fish. Another biodegradable polymer, Poly (D,L-lactic-co-glycolic acid) is used for encapsulation and delivery of different compounds in fish. This compound when used for encapsulating DNA vaccine showed improved immunological parameters in Japanese flounder. Ginger NPs can protect common carp fingerlings from motile *Aeromonas septicaemia*. Neem based silver NPs can improve immunomodulatory and antibacterial activity in mrigal (*Cirrhinus mrigala*).

**3. Water quality management:** Nano-device has the ability to reduce the rate of water exchange and increase the quality of water. Nanoscale iron powder can also be used for the cleaning of less toxic, simpler carbon compounds from water. Silver, copper, zinc and chitosan nanoparticles in different forms are used for removal of microbial contaminants such as *Escherichia coli*, *Enterococcus faecalis*, *Cryptosporidium parvum*, *Staphylococcus aureus*. Other nanoparticle systems of titanium dioxide, silicon dioxide, nanocrystalline cellulose, graphene oxide, iron–aluminium oxide are used to remove contaminants like aureomycine hydrochloride, fluoride, lead, mercury etc.

**4. Fish harvest and processing:** Fishing lures nano-coated with a polyimide film increases the probability of catching fish two to three times. NPs can be used in processing and packaging to extend the shelf life and also to preserve the freshness and quality. For example, silver NPs integrated nano-ice reduced the microbial load and also inhibit the growth of *Acinetobacter* on flathead grey mullet. Chitosan NPs are effective antibacterial agent that can form an edible coating material for sea foods to reduce microbial load and extend the shelf life.

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# Macrophage Differentiation Under Metabolic Stress Conditions

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## *Abstract*

Macrophages are essential players in the immune system, performing diverse functions ranging from pathogen clearance to tissue repair. Emerging evidence suggests that the metabolic status of macrophages plays a crucial role in determining their functional phenotypes. This article explores the impact of metabolic stress conditions on macrophage differentiation and its implications for immune responses. We discuss the influence of nutrient availability, such as glucose and fatty acids, as well as altered mitochondrial function on macrophage polarization and function. Furthermore, we highlight the potential therapeutic targets and strategies to modulate macrophage metabolism, aiming to enhance immune responses in various disease contexts.

## **Introduction**

Macrophages are versatile immune cells that exist in different tissues throughout the body. They possess a remarkable plasticity, allowing them to adapt their phenotypes and functions based on the surrounding microenvironment. Macrophage polarization refers to their ability to differentiate into distinct functional states, broadly classified as pro-inflammatory M1 or anti-inflammatory M2 phenotypes. The balance between these polarized states is critical for maintaining tissue homeostasis and mounting appropriate immune responses. Macrophages, as key players of the immune system, play a critical role in maintaining tissue homeostasis, resolving infections, and promoting tissue repair. They possess remarkable plasticity and can undergo distinct functional polarization states in response to various microenvironmental cues. One such crucial determinant of macrophage behavior is metabolic stress, which can profoundly influence their differentiation, activation, and effector functions. Metabolic stress conditions, such as nutrient deprivation, hypoxia, and altered metabolite





availability, are encountered by macrophages in diverse physiological and pathological settings, including tumors, ischemic tissues, and inflamed microenvironments.

Recent studies have unveiled the impact of metabolic stress on macrophage differentiation, shedding light on the intricate interplay between cellular metabolism and immune function. Understanding how metabolic stress shapes macrophage differentiation is of utmost importance, as it not only contributes to the maintenance of tissue homeostasis but also holds implications for the pathogenesis of several diseases. Dysregulated macrophage responses under metabolic stress conditions can lead to aberrant immune reactions, impaired tissue repair, and exacerbated inflammation. Therefore, unraveling the intricate interplay between metabolic stress and macrophage differentiation has garnered increasing attention in recent years.

### **Nutrient Availability and Macrophage Polarization**

Macrophages require an adequate supply of nutrients to sustain their energy demands and carry out their immune functions. The availability of specific nutrients, such as glucose and fatty acids, has been found to shape macrophage differentiation. Altered nutrient availability under metabolic stress conditions can promote the development of pro-inflammatory or anti-inflammatory macrophage phenotypes, depending on the context.

### **Glucose Metabolism in Macrophage Differentiation**

Glucose metabolism is a crucial determinant of macrophage polarization. Enhanced glycolysis, driven by the activation of glycolytic enzymes, promotes the M1 pro-inflammatory phenotype. On the other hand, oxidative phosphorylation, which occurs in the mitochondria, supports the M2 anti-inflammatory phenotype. Dysregulated glucose metabolism during metabolic stress can disrupt this balance, leading to aberrant macrophage activation and impaired immune responses.

### **Lipid Metabolism and Macrophage Polarization**

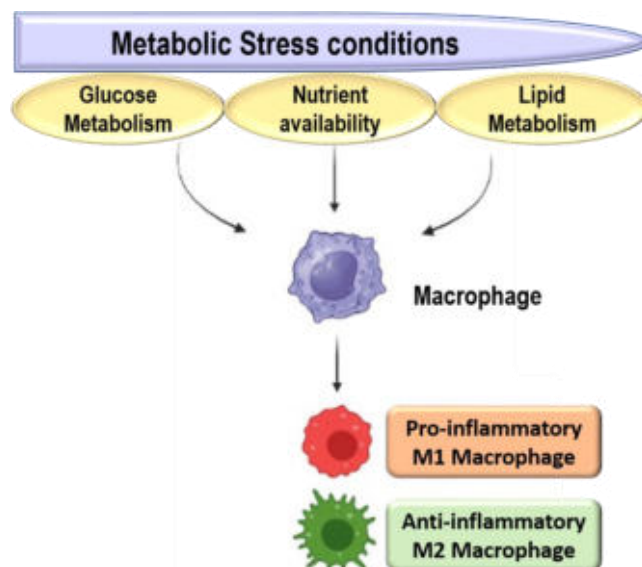
Lipid metabolism plays a significant role in macrophage polarization. Fatty acids act as signaling molecules and can modulate macrophage phenotype. Lipid accumulation and altered lipid metabolism can skew macrophages towards a pro-inflammatory phenotype, contributing to chronic inflammation and metabolic disorders.

### **Mitochondrial Dysfunction and Macrophage Function**

Mitochondria play a central role in energy metabolism and cellular homeostasis. Dysfunctional mitochondria can impair macrophage functions and alter their polarization states.



Mitochondrial dysfunction under metabolic stress conditions can skew macrophages towards a pro-inflammatory phenotype and compromise their ability to respond effectively to pathogens or promote tissue repair.



**Figure 1. Macrophage Differentiation Under Metabolic Stress Conditions**

### **Therapeutic Strategies for Modulating Macrophage Metabolism**

Understanding the interplay between metabolism and macrophage differentiation opens up new avenues for therapeutic interventions. Targeting metabolic pathways and metabolic regulators could help modulate macrophage polarization and enhance immune responses in diseases such as cancer, infectious diseases, and chronic inflammatory conditions.

### **Implications of Macrophage Metabolic Stress in Disease Pathogenesis**

Metabolic stress conditions, such as obesity, diabetes, and atherosclerosis, profoundly influence macrophage metabolism and function. Altered macrophage phenotypes in these diseases contribute to disease progression and tissue damage. Unraveling the mechanisms underlying macrophage metabolic stress could offer insights into disease pathogenesis and guide the development of novel therapeutic approaches.

### **Future Perspectives: Unraveling the Complexities of Macrophage Metabolism**

Despite significant advances, our understanding of the intricate interplay between metabolism and macrophage differentiation remains incomplete. Further research is needed to decipher the precise mechanisms by which metabolic stress conditions shape macrophage



phenotypes. Integrating omics approaches, advanced imaging techniques, and computational modeling will help unravel the complexities of macrophage metabolism and pave the way for personalized therapeutic strategies.

### **Conclusion**

Macrophages play critical roles in immune responses and tissue homeostasis. Their differentiation and functional polarization are intricately linked to metabolic stress conditions. Nutrient availability, including glucose and fatty acids, as well as mitochondrial function, significantly impact macrophage differentiation and function. Understanding the metabolic regulation of macrophages under stress conditions holds great promise for developing targeted therapies to enhance immune responses and combat various diseases. Further investigations in this field will undoubtedly contribute to our knowledge of immunometabolism and open new avenues for precision medicine.

### **Acknowledgement**

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## Nanosilver: Sword to Combat Mastitis

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### Introduction

Mastitis is a prevalent infectious disease affecting the dairy industry worldwide. It is primarily caused by bacterial pathogens, including *Staphylococcus aureus*, *Streptococcus spp.*, *Escherichia coli*, and others. It can be classified into clinical and subclinical mastitis. Clinical mastitis presents with visible changes in milk appearance, udder swelling, and systemic signs of illness. Subclinical mastitis, on the other hand, lacks apparent clinical signs but is characterized by increased somatic cell count in milk, indicating underlying infection. Its impact on economy is very vast because if untreated then the utility of that animal for that lactation period is doomed. For the countries like India, where cattle slaughter is banned in several states, the situation of economic losses is worse. Understanding the epidemiology and the range of pathogens involved is crucial for effective treatment strategies to cope up with these conditions.

One of the strategies to combat with this, is the use of nanoparticles having antimicrobial properties e.g., silver, zinc and iron nanoparticles and the promising one is silver nanoparticles. Silver nanoparticles are nano-sized particles composed of silver atoms, typically ranging from 1 to 100 nanometers in diameter. They exhibit unique physico-chemical properties, such as a high surface-to-volume ratio, which contribute to their enhanced antimicrobial activity. Various synthesis methods, including chemical and physical approaches, are employed to produce silver nanoparticles with controlled characteristics. Silver nanoparticles exert their antimicrobial effects through multiple mechanisms. They can disrupt bacterial cell membranes, leading to increased permeability and leakage of cellular components. Moreover, silver ions released from the nanoparticles can interfere

with bacterial enzymes and biomolecules, disrupting vital cellular processes and ultimately causing bacterial death.

The role of silver nanoparticles in the treatment of bovine mastitis holds great promise for the dairy industry. Their antimicrobial properties, broad-spectrum activity, and various forms of application provide valuable tools in combating mastitis-causing pathogens. By harnessing the potential of silver nanoparticles, we can strive towards improved udder health, enhanced milk production, and sustainable management of bovine mastitis in the dairy industry. Topical applications, intramammary infusions using nanoparticle-loaded matrices or nano-emulsions, and silver nanoparticle-coated implants offer different approaches to deliver effective treatment.

### **1. Topical Applications**

**a) Silver Nanoparticle Gels/Creams:** Silver nanoparticles incorporated into gels or creams provide extended contact with the affected area, improving antimicrobial efficacy. These formulations enhance tissue adherence and minimize nanoparticle dispersion, ensuring sustained antimicrobial activity.

**b) Silver Nanoparticle Solutions:** Topical application of silver nanoparticle solutions directly to the udder has shown promise in treating bovine mastitis. The nanoparticles penetrate the bacterial cell walls, impair cellular functions, and inhibit bacterial growth. Formulations such as sprays, dips, or washes may be used for efficient delivery.

### **2. Intramammary Infusions**

**a) Nanoemulsions:** Nanoemulsions containing silver nanoparticles can be used as intramammary infusions. These emulsions enhance nanoparticle stability, facilitate deep penetration into infected tissues, and provide effective antimicrobial action.

**b) Silver Nanoparticle-Loaded Biodegradable Matrices:** Incorporating silver nanoparticles into biodegradable matrices, such as hydrogels or nanoparticles encapsulated within polymers, allows for controlled and sustained release of silver ions. This approach prolongs the antimicrobial effect and reduces the frequency of treatment administrations.

### **3. Silver Nanoparticle-Coated Implants:**

**a) Catheters and Tubes:** Silver nanoparticle-coated catheters and tubes used in milk collection systems can help prevent bacterial growth and biofilm formation. This reduces the risk of bacterial contamination during milking, contributing to improved udder health.



**b) Teat Implants:** Silver nanoparticle-coated teat implants, composed of biocompatible materials, can prevent bacterial colonization on the teat surface. These implants act as a physical barrier and inhibit biofilm formation, reducing the risk of infection.

#### **4. Combination Therapies:**

Combining silver nanoparticles with traditional antibiotics or antimicrobial agents has shown synergistic effects in treating bovine mastitis. These combination therapies enhance treatment efficacy, reduce the development of antibiotic resistance, and potentially shorten the duration of treatment.

To ensure their safe and effective use in veterinary practice. It is essential to conduct rigorous clinical trials and studies to evaluate the optimal dosage, treatment duration, and potential side effects of silver nanoparticles in bovine mastitis treatment. Moreover, it is crucial to consider the long-term implications of silver nanoparticle use, including the potential for environmental contamination. Researchers and regulatory bodies must assess the impact of silver nanoparticles on ecosystems and develop guidelines to mitigate any adverse effects. It is important to emphasize the importance of proper hygiene practices, including regular udder cleaning, maintaining a clean milking environment, and appropriate teat disinfection, as a preventive measure against bovine mastitis. Silver nanoparticle-based treatments should be considered as adjunct therapies to complement these preventive measures and conventional mastitis management strategies.

In conclusion, the role of silver nanoparticles in the treatment of bovine mastitis shows significant promise. Their antimicrobial properties, when harnessed through various forms of application, offer potential avenues for targeted and effective treatment. However, further research, collaboration between scientists, veterinarians, and industry stakeholders, and adherence to regulatory guidelines are crucial to realizing the full potential of silver nanoparticles in bovine mastitis treatment. By combining advancements in nanotechnology with established management practices, we can strive towards healthier udders, improved milk production, and enhanced animal welfare in the dairy industry. However, further research is necessary to establish optimal dosages, safety profiles, and long-term efficacy. Additionally, considerations such as potential environmental impacts and bacterial resistance must be thoroughly addressed.



## Antibiotic Residues in Milk

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### *Abstract*

Antibiotic residues in milk pose a significant risk to public health, contributing to the global concern of antibiotic resistance. The indiscriminate use of antibiotics in food-producing animals and the use of certain antibiotics as feed additives without proper regulation are major factors leading to the presence of residues in milk. International organizations play a crucial role in regulating the use of antibiotics in animal production, setting maximum residue limits (MRLs) to minimize risks. Detecting and regulating antibiotic residues in milk requires the development of highly sensitive detection tools and appropriate confirmation and quantification methods. Public awareness, adherence to withdrawal periods, regular monitoring, and surveillance policies are essential in controlling antibiotic residues.

**Keywords:** Antibiotic residue, maximum residue limit, health risks, detection

### **Introduction**

Antibiotics are substances that occur naturally or are created through semi-synthetic or synthetic processes, which possess antimicrobial properties. These compounds can be administered in animals via various routes, including injection, oral ingestion, or topical application. For over six decades, antibiotics have been employed in the healthcare of livestock to prevent and treat common ailments such as mastitis, respiratory infections, and foot diseases.

The global rise of antibiotic resistance has become a significant concern in the field of public health, with an estimated 0.7 million deaths attributed to this issue each year. Alarmingly, this number is projected to increase to 10 million deaths annually by 2050.

Antibiotic use in food-producing animals contributes to the presence of drug residues in products like milk, meat, and eggs, leading to the development of antimicrobial-resistant bacteria. These residues can be released into the environment and contaminate animal-derived food items. This practice not only promotes the spread of antimicrobial resistance among animals but also poses a substantial risk to human health. Additionally, the contamination of the environment with antibiotic residues presents an ecological hazard, potentially giving rise to resistant bacteria that could endanger public health.

### **Causes of Antibiotic Residues in Milk.**

The presence of antibiotic residues in milk can be attributed to various factors related to the therapeutic use and management of antibiotics. Indiscriminate usage of antibiotics in the treatment of infectious diseases, failure to follow instructions on antibiotic labels, concentrations, or conditions, and inadequate maintenance of withdrawal times can lead to higher concentrations of antibiotic residues in milk. Additionally, improper cleaning of equipment contaminated with antibiotics, improper disposal of antibiotic containers, and the impaired metabolic process of antibiotics in diseased animals can also lead to higher risks of antibiotic residues.

### **Impact of antibiotic residues on dairy products formulation**

Antibiotic residues in milk used for fermented dairy products can affect technological methods, leading to lower product quality and potential economic implications for the dairy industry. The presence of antibiotic residues in milk inhibits the development of lactic acid bacteria, thereby delaying acid production. Similarly, in the cheese-making process, acid development is very important as it accelerates the activity of enzyme rennet and speeds up the coagulation process. Additionally, the presence of antibiotic residues in milk and inadequate pH levels can promote the growth of spoilage organisms such as Clostridia and yeasts, further affecting the quality of the final products.

### **Health Risks and Concerns**

The existence of antibiotic residues in milk can present a significant risk to human health. Regulatory authorities establish Maximum Residue Limit (MRL) values for antibiotics, which are determined based on the Acceptable Daily Intake (ADI). The presence of antibiotic residues can potentially lead to various toxic effects in humans including carcinogenicity, allergies, mutagenicity, nephropathy, anaphylactic shock, reproductive disorders, bone marrow toxicity, and even





hepatotoxicity. The hazards associated with antibiotic residues can be classified into two categories based on the duration of exposure and the time it takes for health effects to manifest: direct short-term hazards and indirect long-term hazards.

Several international organizations, including the World Health Organization (WHO), Codex Alimentarius Commission (CAC), and Food and Agricultural Organization (FAO) play a crucial role in regulating the use of drugs in animal production, specifically to addressing the issue of antimicrobial residues in animal-derived foods such as milk and meat. These organizations have developed maximum residue Limits (MRLs) for livestock products, and regular monitoring, control, and surveillance programs are in place to minimize the risk to human health. Monitoring and preventing illegal antibiotic residues in milk is essential for public health. Adhering to withdrawal periods, maintaining hygiene, and implementing effective management practices can reduce residue levels. Regulatory standards and strong management are crucial for ensuring milk safety.

### **Detection and Regulation of Antibiotic Residues**

Various analytical techniques have been developed to detect drug residues in milk, categorized as screening tests and confirmatory tests. Screening methods, such as thin-layer chromatography and microbial inhibition tests, are qualitative in nature and primarily used for the initial detection of residues. On the other hand, confirmatory methods are more expensive, time-consuming, and require trained personnel. Examples of confirmatory methods include Liquid Chromatography (LC) coupled with detectors like mass spectrometry (MS) and UV, as well as High-Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE).

In order to address the issue of antibiotic residues in milk, it is crucial to raise public awareness through effective activities organized by experts and organizations in the field. To effectively control antibiotic residues in milk, it is crucial to develop highly sensitive detection tools to minimize false negatives and employ appropriate confirmation and quantification methods to reduce the risk of false positives. Any milk samples that exceed the maximum residue limit (MRL) should be promptly discarded. Regular monitoring and surveillance policies at a national level should be implemented to ensure that the levels or concentrations of antibiotic residues in milk are regularly monitored. Additionally, exploring herbal sources of medicines as an alternative option for treating diseases could be considered.



## Conclusion

The presence of antibiotic residues in milk is a significant concern due to the indiscriminate use of antibiotics in animals and the potential health risks associated with consuming such residues. These residues can lead to allergic reactions, toxic effects, and the development of antibiotic-resistant bacteria in humans. Raising public awareness, developing sensitive detection tools, and implementing effective monitoring and surveillance programs are crucial for addressing this issue and ensuring the safety of milk for human consumption.

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## Loose flower cultivation in Tamil Nadu

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### Introduction

Tamil Nadu continues to take the first place in the production of loose flowers in the Country, the production being 5.15 lakh MT during the year 2021 – 2022. Tamil Nadu takes the third place in regard to area, by cultivating the flowers in an area of 42,920 Ha (NHB, 2022) and Dharmapuri, Salem, Dindigul, Krishnagiri, and Tiruvannamalai districts play a major role in achieving the target. Assistance is extended for cultivation of loose flowers, bulbous flowers and cut flowers. Quality planting materials are being produced in State Horticulture Farms and distributed to farmers in subsidized cost to encourage flower cultivation.

In this study, we collected data of the present status of farmer's technique and their production technology for loose flower cultivation in Tamil Nadu. These data has been collected from various parts of Tamil Nadu. The flower crops namely *Jasminum sambac*, *Jasminum auriculatum*, *Jasminum grandiflorum*, tuberose, chrysanthemum, nerium, rose, marigold and ixora are taken into the study. The locations taken for the study are Madurai, Karamadai, Nilakottai, Salem, Vadalur, Mettupalayam and Trichy. The crop wise analysis of the loose flowers gives us the clear view of the different cultivation practices among farmers all over Tamil Nadu.

The other important aspect is the cost economics. The profit of the farmers determines the growth of the flower area and production. It also paves the way for the new farmers to take flower cultivation in Tamil Nadu.

### *Jasminum sambac* (Gundumalli)

Suresh, a farmer from Paraipathy, Madurai cultivates *Jasminum sambac* in 2.5 acres. He

follows organic farming for the past 15 years. He uses 2–3-month-old cuttings for propagation with spacing of 6ft x 6ft and 5ft x 3ft (**1.25 m x 1.25 m**) and plants all around the year. The plants last up to 5 – 10 years. His cultivation practices are quite different from other farmers. From ploughing and making irrigation channels then digging holes and filling it with vermicompost +FYM + seaweed application. Dipping with VAM + *T.viride* + *B.subtilus* and planting. Irrigation is given at 2 days interval and weeding monthly once. Pruning is done Yearly once and Bio fertilizers weekly twice and Micronutrients monthly once. Major problem is budworm and thrips.

<b>Particulars</b>	<b>Amount (rupees)</b>
Gross income	9,60,000
Expenses	3,00,000
Profit	6,60,000
Benefit Cost Ratio	3.2
<b>Break-even point</b>	<b>1.5 yrs</b>

Rajan from Eliyarpatti, Madurai is a conventional farmer who cultivates J. sambac over 2 acres. He uses 6 - 12-month-old cuttings for propagation with spacing of 2ft x 2ft and 3ft x 3ft and plants all around the year. The plants last up to 7– 10 years. 12000 cuttings are required for an acre. The cultivation practices are Ploughing with plant leaf mould (7-10T). Irrigation’s weekly once upto 2 months then application of Complex + urea + Sulphate. After 2 months application of monochrotopas or rogor and then weekly once weeding. Pruning is yearly thrice and often the field is allowed for the goat gazing for better yield and also to reduce labor cost. Average yield is 1.8 T/acre.

<b>Particulars</b>	<b>Amount (rupees)</b>
Gross income	2,60,000
Expenses	1,00,000
Profit	1,60,000
Benefit Cost Ratio	2.6
<b>Break-even point</b>	<b>1 yr</b>

### ***Jasminum auriculatum* (Mullai)**

Prabhakaran, a farmer from Chinnathottipalayam, Karamadai grows J. auriculatum in 2 acres over a period of 10 years. He uses 1.5 – 2-month-old cuttings for propagation with spacing of 6ft x



6ft and plants all around the year. The plants last up to 10 - 12 years. 1000 cuttings are required for an acre. The cultivation practice starts with Ploughing with FYM (10T/acre) + DAP (100kg/kg). Then 1 ft x 1 ft digs with 1 or 2 cuttings in it and irrigate 2-5 interval. After 1.5 months urea application and weeding after 2-3 months. The different operation which he uses is Pinching after 3 months and complex application along with it. Application of 17:17:17 (10-15 kg/plant) is done and FYM (2 kg). After 4 months, application of monochrotophas (15ml / tank). The **Pinching is done upto 5 months. The average yield** starts with 100g and goes upto 50-60 kg after 1.5 years. Pruning's done yearly once (**last week of Jan**). Major problem is mites and thrips.

Particulars	Amount (rupees)
Gross income	22,40,000
Expenses	7,50,000
Profit	14,90,000
Benefit Cost Ratio	2.98
<b>Break-even point</b>	<b>1.5 yrs</b>

### ***Jasminum grandiflorum* (Jathimalli)**

Subbaiyan, a farmer from T.G.Pudhur, Karamadai grows *J. grandiflorum* in 10.5 acres over a period of 25 years. He uses 3-month-old cuttings for propagation with spacing of 9ft x 4.5ft and plants all around the year. The plants last up to 7 - 10 years. 600 cuttings are required for an acre. Ploughing is done without FYM application. After that DAP + Urea – 50g/plant is given. Weeding is done along with application of FYM (cow or chicken). Irrigation is given with 3-4 days interval. Pruning yearly once and takes 4 months to set flowers then continuous flowering for 8 months. (**Pruning time: last week of dec**). The average Fresh yield is 1kg / acre and 2nd year yield is 30kg / acre (after pruning).

Particulars	Amount (rupees)
Gross income	3,00,000
Expenses	1,00,000
Profit	2,00,000
Benefit Cost Ratio	3
<b>Break-even point</b>	<b>.5 - 1 yr</b>



## Nerium

Shanmuga Sundaram, a farmer from S. Pungampalayam, karamadai has been growing nerium for the past 7 years around an area of 2 acres. He uses 1 year old cuttings with spacing of 10ft x 8ft and plants them during May – June period. It last up to 6 to 10 years. Application of Complex + FYM +DAP. After 5 months pruning is done. 10 days once irrigation is given for 3hrs/day. Bending is done whenever is necessary. Harvesting time is usually 6-8 am. Major problem is the harvesting time.

Particulars	Amount (rupees)
Gross income	4,00,000
Expenses	110,000
Profit	2,90,000
Benefit Cost Ratio	3.63
<b>Break-even point</b>	<b>1.5 yrs</b>

## Chrysanthemum

G. Chinnathambi from Kuthiraikuthipallam, Salem is a chrysanthemum farmer and has a nursery for the flower crop. He cultivates chrysanthemum in 2.5 acres. He uses 2-month-old cuttings (rooted) for propagation with spacing of 1ft x 1ft and plants from july to december. The plants last upto 4 – 5 months. Irrigation is given from 3<sup>rd</sup> day with 5 days interval. Then 15<sup>th</sup> day, humic acid @ 10ml / 10 lit is given. 30<sup>th</sup> Redomil gold +SAAF applied. For pest, proclain is sprayed. Finally, 40<sup>th</sup> day - DAP-50kg/acre is given. Harvesting starts from the third month it is done with an interval of 7days once (90-110 days), 2days once (110-130 days) and daily (5<sup>th</sup> month). Major problem is thrips and uneven climate.

Particulars	Amount (rupees)
Gross income	2,25,000
Expenses	1,44,000
Profit	81,000
Benefit Cost Ratio	1.56
<b>Break-even point</b>	<b>1.5 yrs</b>

## Tuberose

Ramasamy from malliyampatti, Nilakottai is tuberose farmer for over 15 years in an area of 3 acres He uses 4-year-old corms for propagation with spacing of 15cm x 15cm and plants from



April – May. The plants last up to 4 – 7 years. 500kg corms/1 acre is required. Irrigation is given in 5days interval. After 1.5 to 2 months DAP -250kg/acre + groundnut cake is applied. It takes 3 months to flower and Bud to flower-10 to 20 days. Commercial flowers come after 6 months. No pesticides and fungicides are used. Sprinklers are installed for Pests (**mealy bug**) and it is used in between 2days interval. The average yield is 100-150kg/acre of flowers. During (Apr-Jun) Season, flower price is ₹100-150/kg and normal - ₹50-70/kg. During (Dec-Mar) is the Lowest - ₹10-30/kg.

Particulars	Amount (rupees)
Gross income	14,00,000
Expenses	6,65,000
Profit	7,35,000
Benefit Cost Ratio	2.1
<b>Break-even point</b>	<b>2.1 yrs</b>

## Rose

Krishna Kumar from Vellipalayam, Karamadai grows rose in an area of 1.5 acres over a period of 8 years. He uses 4 months old (clonal) cuttings for propagation and plants with a spacing of 7ft×7ft. the crop duration is 5years. For 1 acre, 800 cutting are required. Ploughing is done with FYM 10 T and application of Feuradon (2g/dig). Irrigation is given with 3-4 days interval. DAP + Potash + Urea is given when required. Groundnut cake + Sesame cake is given after weeding after 6 months. Harvesting yields over 7,20,000 flowers/year/acre. Major problem is budworm, mites, powdery mildew and crown gall.

Particulars	Amount (rupees)
Gross income	2,88,000
Expenses	2,00,000
Profit	88,000
Benefit Cost Ratio	1.44
<b>Break-even point</b>	<b>2 yrs</b>

## Ixora

Kumar from Neithalur, Trichy grows ixora in an area of 1.5 acres over a period of 10 years.



He uses 3 month old cuttings (rooted) for propagation with spacing of 7ft x 6ft and plants from June - July . The plants last up to 2 – 6 years. 1000 cuttings are required for an acre. Ploughing with FYM until powdery form comes. Digging holes and drying it out. Planting is done with 2 cuttings per hole. Irrigate in 3 days interval and accordingly apply DAP (10g) in the field. After those 15 days once pesticide spraying is done (Dithane + propanopas)) and 10 days once in winter. 20-30 days once weeding and soil loosening is done regularly and checking for earthworms. Roping and pruning is done after 6 months then flowers arrive.

Particulars	Amount (rupees)
Gross income	7,00,000
Expenses	3,00,000
Profit	4,00,000
Benefit Cost Ratio	2.33
<b>Break-even point</b>	<b>1.5- 2 yrs</b>

## Marigold

Ethiraj, a farmer in Vadalur, Cuddalore cultivates marigold in 2 acres. He uses 18 – 20 old plants for propagation with spacing of 120cm x 30cm and plants all around year. The plants lasts upto 3 months. 700 seedlings are required for an acre. Ploughing along with application of FYM (4-5T/ acre) and DAP (50kg/acre) during winter and rainy season. Transplanting and after 20-25 days earthening up is done. After 45 days staking is done. After 50 days harvesting stage arrives. Complex + micronutrient is applied during earthening up and 19: 19: 19 during flowering stage (10 – 12kg/acre). Major problem is rot and powdery mildew.

Particulars	Amount (rupees)
Gross income	1,50,000
Expenses	60,000
Profit	90,000
Benefit Cost Ratio	2.5
<b>Break-even point</b>	<b>2 months</b>

## SWOT Analysis of loose flower cultivation in Tamil Nadu

### Strength:

- High returns
- Regular market flow
- Year around cultivation





- Family labors
- Connection with various markets and industries

### **Weakness:**

- Unavailability of labors during harvest
- Pest and disease management
- Lack of good quality planting material
- Lack of knowledge

### **Opportunities:**

- Growing flower markets
- Increased customer preferences
- More seasonal Price of flowers
- Regular demand for traditional flowers
- Festival demand

### **Threats:**

- No awareness over pest control
- Huge decrease in Agricultural labor
- Flower quality maintenance
- Taxes for goods

## **Conclusion**

As the farmers use very traditional and different method for cultivation of loose flowers, many advantages and disadvantages are there. More communication between farmers and scientists will lead to a prosperous future for the flower production not only in Tamil Nadu and also all over India and the world. Hence the farmers can get better yield and more income. Knowledge over markets and their fluctuations will help the farmers be more aware of the demand and supply of the flowers. Even with all the differences our farmers are doing an excellent job in the loose flower production. Innovative adaptation of modern technologies will also help the farmers. Therefore, farmers are going in the right path in flower business to increase their income and also nations GDP. From the above data it proves that the farmers are earning in profit in the flower with a good return with traditional and conventional production technology.



## Retailing: Ubiquitous Experience

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### *Abstract*

The fashion industry forms part of a larger social and cultural phenomenon known as the “fashion system,” a concept that embraces not only the business of fashion but also the art and craft of fashion, and not only production but also consumption. As consumers have access to multiple distribution channels such as online retailing, telemarketing and more recently, social media retailing. However, the final point to consider concerning omni-channel retailing is that whilst it is seamless and integrated, it is able to provide a ubiquitous experience, as channels can be accessed anywhere and at any time using a variety of devices.

**Keywords:** Fashion, Consumer, Retailing, Business and Product.

### **Introduction**

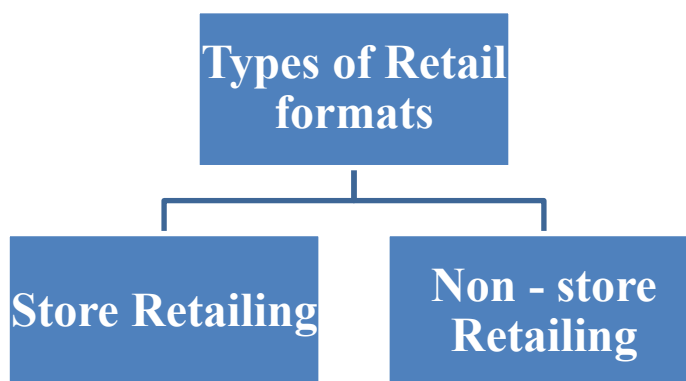
The Indian retailing industry, which was traditionally dominated by small and family-run stores, has come of age. The retail sector is the second largest employer after agriculture in the country and also the second largest untapped market after China. There are some 12 million retail outlets in India. Besides, the country is also dotted with low-cost kiosks and pushcarts. Organized retailing is only 2% of the total retail industry.

Over the past couple of years there have been sweeping changes in the general retailing business, mainly in apparel retailing which was once strictly a made-to-order market for clothing, has changed to a ready-to-wear market. Flipping through a catalogue, picking the color, size and type of clothing a person wanted to purchase and then waiting to have it sewn and shipped was standard practice.

Recently, India is increasingly being looked upon as a major supplier of high-quality fashion apparels and Indian apparels have come to be appreciated in major markets internationally.

Retail is any business that directs its marketing efforts towards satisfying the final consumer based upon the organisation of selling goods and services as a means of distribution. Retailing includes all activities involved in selling, renting, and providing goods and services to ultimate customers for personal, family or household use. In the channel of distribution, retailing is where the customer meets the product. It is through retailing that exchange occurs.

With the advent of modern format retailers and the growth of plastic cards, affluent urban Indian women are shopping like never before. They spend mornings browsing in stores looking for deals or latest styles. Upper income urban women are adopting ethnic chic. These are designer clothes that incorporate Indian motifs, ethnic fabrics and are a fusion of western and Indian styles.





**I. Forms of Store retailing:** Fashion retailers selling apparel, footwear, bags and accessories exist in various retail formats. However, all formats of stores may not offer all fashion items. Let's take a look at the most famous ones prevalent at present:

1. **Mom-and-Pop** (Kirana Stores as they call them in India): is a retail outlet that is owned and operated by individuals. The range of products are very selective and few in numbers. These stores are seen in local community often are family-run businesses.
2. **General store** - a store which sells most goods needed, typically in a rural area. It carries a broad selection of merchandise, sometimes in a small space, where people from the town and surrounding rural areas come to purchase all their general goods.
3. **Department stores** - Department stores usually sell products including apparel, furniture, home appliances, electronics and additionally select other lines of products such as cosmetics, photographic equipment, jewelry and sporting goods.



4. **Discount stores** - A discount store is a type of department store, which sells products at prices lower than those asked by traditional retail outlets. It tends to offer a wide array of products and services, but they compete mainly on price offers extensive assortment of merchandise at affordable and cut-rate prices.
5. **Specialty Stores:** Specialty stores are small stores which specialize in a specific range of merchandise and related items. The pricing policy is generally in the medium to high range, depending on factors like the type and exclusivity of merchandise and ownership.
6. **Hypermarkets:** provides variety and huge volumes of exclusive merchandise at low margins. In commerce, a hypermarket is a superstore combining a supermarket and a department store. Hypermarkets allow customers to satisfy all their routine shopping needs in one trip.
7. **Supermarkets:** is a self-service store consisting of grocery, apparel, home furnishing and limited products on nonfood items. (Wal-Mart, Big Bazaar)
8. **Malls:** It has a range of retail shops at a single outlet. They endow with different products, food and entertainment under a roof. Example: Life style and Sahara mall in Gurgaon.

			
Mom-and-Pop	General store	Department stores	Discount stores
			
Specialty Stores	Hypermarkets	Supermarket	Malls

**II. Forms of Non - store retailing-** this type of retailing is mainly use new and advance method of retailing.

**1. Automatic Vending Machines** - A vending machine is a machine which dispenses items such as sanitary napkin to customers automatically, after the customer inserts currency or credit into the



machine. This is an automated piece of equipment wherein customers can drop in the money in machine and acquire the products.

**2. Direct Mail & Catalogs** - Direct mail is a way of advertising in which advertisers mail printed ads, letters or other solicitations to large groups of consumers. A form of direct marketing in which customers order from catalogues which are sent to them by mail; the ordered goods are shipped directly to them.

**3. Television Home Shopping-** Home shopping commonly refers to the electronic retailing/home shopping channels, which includes such billion-dollar television-based and e-commerce companies as HSN, and Amazon.com. It allows consumers to shop for goods from the privacy of their own home, as opposed to traditional shopping.

**4. Online Retailing & Telemarketing** - The customer can shop and order through internet and the merchandise are dropped at the customer's doorstep. Example: Home shop 18, e-bay, amazon.com). Telemarketing is a method of direct marketing in which a salesperson solicits prospective customers to buy products or services, either over the phone or through a subsequent face to face or Web conferencing appointment scheduled during the call.

			
Automatic Vending Machines	Direct Mail & Catalogs	Television Home Shopping	Online Retailing

### Conclusion

As apparel retail is led by fashion, a player needs to keep a close watch on fashion amongst teenagers as they are the trend setters. Role of Bollywood in spreading fashion needs to be understood. Seasonal variations on stocking pattern and need to clear inventory at the end of season should be understood by apparel retailer. Typically, once an item is sold from the outlet, retailer ensures that there is no repetition of same. It gets replaced by different design, style and colour.



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## Immunomodulatory effects of lactoferrin

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### Introduction

Lactoferrin (LF) is an 80 kDa iron-binding protein of the transferrin family that is abundantly expressed in most biological fluids. It is now recognized that this glycoprotein is a key element in the mammalian immune system, playing an important role in host defence against infection and excessive inflammation. Lf can bind and sequester lipopolysaccharides (LPS), thus preventing proinflammatory pathway activation, sepsis and tissue damage. Lf is considered to be a cell-secreted mediator that bridges the innate and adaptive immune response. It is released from neutrophils (PMNs) in the blood and inflamed tissues. Lf has a direct antimicrobial role, as it limits the proliferation and adhesion of microbes (e.g. bacteria, viruses and parasites) and/or kills them. This effect of Lf is the result of its ability to sequester iron in biological fluids and destabilize the membranes of microorganisms. The metals that it binds are the Fe<sup>2+</sup> or Fe<sup>3+</sup> ions, also can exist free of Fe<sup>3+</sup> (apo-Lf). It is a simple polypeptide chain folded into two symmetrical lobes (N and C lobes), which are highly. These two lobes are  $\alpha$ -helix between in human LF $\alpha$  connected by a hinge region containing parts of an (hLF). Lf also acts as a first-line of defence by significantly impacting the development of adaptive immune responses. Iron sequestration by Lf reduces oxidative stress, thus altering the extension and specific production of cytokines. Lactoferrin has a strong modulatory effect on the adaptive immune system by accelerating the maturation of T-cell precursors into competent helper cells and by the differentiation of immature B-cells into antigen-presenting cells.

Lf can also modulate both innate and adaptive immunities by acting as a Toll-like receptor (TLR4) or by interacting with lymphocytes and antigen-presenting cells (APCs).

### **Antimicrobial activities of lactoferrin**

Several functions have been attributed to Lf. It is considered to be a key component of the innate host defence system because it can respond to a variety of physiological and environmental changes. The structural features of Lf provide additional functionalities beyond the Fe<sup>+3</sup> homeostasis function common to all transferrins. Specifically, Lf exhibits strong antimicrobial activity against a broad spectrum of bacteria (Gram<sup>+</sup> and Gram<sup>-</sup>), fungi, yeasts, viruses and parasites, although it seems to promote the growth of beneficial bacteria like *Lactobacillus* and *Bifidobacteria*. It also exhibits anti-inflammatory and anticarcinogenic activities and has several enzymatic functions. Lf plays a key role in maintaining cellular iron levels in the body. One of the first antimicrobial properties discovered for Lf was its role in sequestering iron from bacterial pathogens. This was believed to be the sole antimicrobial action of lactoferrin because apo-lactoferrin possessed antibacterial activity. It was later demonstrated that lactoferrin can also kill microorganisms through an iron-independent mechanism in which lactoferrin directly interacts with the bacterial cell surface.

### **Immunomodulatory and anti-inflammatory activity**

Lf displays immunological properties that influence both innate and acquired immunities. Its relationship with the immune system is evident from the fact that people with congenital or acquired Lf deficiency have recurring infections. Oral administration of bLf seems to influence mucosal and systemic immune responses in mice. Lf can modulate both specific and non-specific expression of antimicrobial proteins, pattern recognition receptors and lymphocyte movement related proteins. The role that Lf plays in regulating innate immune responses confirms its importance as a first line host defence mechanism against invading pathogens, modulating both acute and chronic inflammation. Most intriguing is the ability of Lf to induce mediators from innate immune cells that subsequently impact adaptive immune cell function. Lf's positive charge allows it to bind to negatively charged molecules on the surface of various cells of the immune system, and it has been suggested that this association can trigger signalling pathways that lead to cellular responses such as activation, differentiation and proliferation. Lf can be transported into the nucleus, where it can bind DNA and activate different signalling pathways. In addition to inducing systemic immunity, Lf can promote skin immunity and inhibit allergic responses. It activates the immune system against skin allergens, causing dose-dependent inhibition of Langerhans cell migration and the accumulation of dendritic





cells in lymph nodes. Leukocytes exposed to Lf modulate their cytokine production; proinflammatory cytokines, TNF- $\alpha$ , IL-6, and IL-1 $\beta$  can be modulated by Lf to increase or decrease. It is well documented that IL-12 plays an important role in driving development of helper T-cell type 1 immunity. Therefore, the role of Lf in the regulation of proinflammatory cytokines and IL-12 clearly demonstrates communication between innate and adaptive immune responses.

### **Lactoferrin from other species**

The different benefits of Lf have led us to be interested in using molecular strategies to develop recombinant Lf from different species to increase its availability. Because of its broad antimicrobial capacity, Lf could be used as a nutraceutical protein or adjuvant drug. Although colostrum contains high Lf levels, industrial companies will require the production or purification of Lf without affecting the alimentary industry uses of milk. Currently, highly purified bovine lactoferrin (bLf) and human lactoferrin (hLf) can be produced. In addition, lactoferrin from other species (*eg*, mouse, rat, chimpanzee, boar, sheep, goat, buffalo, camel and dog) was sequenced and found to vary by 2112 to 2530 bp. It has been possible to produce recombinant Lf specific to human, bovine, equine, porcine, caprine, yak and Kunming by using various expression systems (*e.g*, bacteria, fungi, yeast, cell lines, insects, mammals and plants). While Lf is produced in quantities ranging from 0.756 mg/L to 10.6 g/L, human Lf remains the most expressed among all of the different expression systems.

### **Future applications of Lactoferrin**

Lf has multiple activities, it can bind a significant number of compounds and substances, such as lipopolysaccharides, heparin, glycosaminoglycans, DNA and metal ions (*eg*, Fe, Al, Mn, Co, Cu, Zn) is involved in iron homeostasis; has a wide range of antimicrobial activity against bacteria, virus, fungi and parasites; and has anti-inflammatory, immunomodulatory, anticarcinogenic and enzymatic activities. Antibiotic-resistant microorganisms are extremely dangerous to humans, and extensive scientific research has resulted in the development of new antibiotics with different effects in an effort to solve the issue. The scientific community has targeted Lf as a promising candidate to help break the vicious cycle of antibiotic resistances. Oral Lf supplementation in human newborns can prevent infection or decrease the severity of an existing infection.

### **Conclusions**

Lf is a versatile molecule that was molded by natural selection to be a first-line defence in mammals. Its ability to exert multiple regulatory effects due to its cationic nature allows it to bind a



large number of surface molecules or metal ions during the development of microorganisms and induce host immune-modulatory activation, which influences the adaptive and innate immunities. The development of Lf expression systems for food and pharmaceutical applications are required, due to its plethora of abilities as a multifunctional, nutraceutical protein.



## Chhurpi – The Himalayan Cheese

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### Introduction

The Himalayan region in India is home to one of the most bio-diverse ecosystem ranging from Jammu and Kashmir to Arunachal Pradesh. In these regions preparation and consumption of traditional fermented milk products are very common. The ethnic population of Himalayan region prepare various traditional naturally fermented artisan dairy products which are both nutritious as well as shelf-stable. One of such product is chhurpi, which is a protein-rich cheese product that has a smoky flavor and hard consistency. It is a very popular traditional cheese in some pockets of eastern India, Nepal, Tibet and Bhutan. It is also known as Himalayan chewing gum as it becomes gradually softer the longer we chew it. People living in Himalayan regions have the habit of chewing it during winter mornings to keep their mouths busy so that they can keep themselves warm and bear the extreme cold climate. Chhurpi is traditionally made from yak milk, chauri (a cross between male yak and female cow) or from cow milk.



**Fig. Chhurpi Churpi cheese making process**

In general, two types of churrpi are prepared – Hard and soft chhurpi. Hard chhurpi is mainly prepared from yak milk and has a harder and chewier consistency and can be used as a snack, whereas, soft chhurpi is prepared fermented cow milk and has a slightly sour taste and softer texture.

Soft chhurpi is prepared by making curds from whey. These white and soft curds are left to ferment by wrapping them in soft clothes and hanging them in room temperature to drain out water. Soft chhurpi is used as a substitute to vegetables in the mountainous regions where the availability of fresh vegetables is scarce. It is also used in curries or pickles and is an excellent source of protein. In case of hard chhurpi or dudh chhurpi, the cooked curds are cured for 2-3 days at room temperature by pressing them and then cutting them into rectangular pieces. These pieces are then sun dried or under wood fire in a low temperature for 5-7 days. Hard chhurpi can be kept for many years without spoilage and can be kept up to 20 years if stored properly in yak skin. This higher keeping quality of hard chhurpi is attributed to its low moisture content. This property is mainly beneficial to yak herders of remote Himalayan highlands while they travel long distances to transport or market these products. These cheeses are sold as rectangular pieces having a hard and compact body. It is known as one of the world's hardest cheese. There is a wide variation among the chemical composition and sensory qualities of chhurpi prepared in different parts of the Indian subcontinent as preparation of chhurpi is an age-old family tradition and its preparation varies according to the culture of that region.

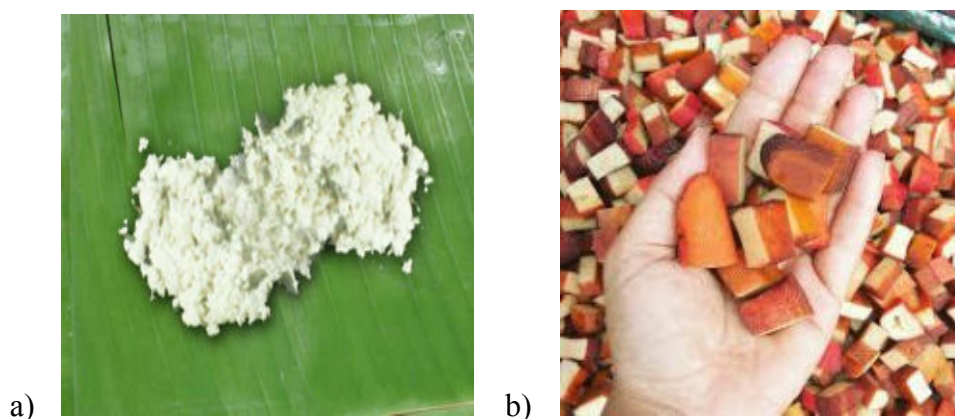


Fig. a) Soft chhurpi b) Hard chhurpi

### Health benefits of chhurpi

According to several researches, fermented milk products have different health promoting properties such as antimicrobial, immunomodulating, anticarcinogenic and cholesterol-lowering effects. Traditional fermented milk products such as chhurpi has a huge influence on the health and economy of the rural regions where they are prepared and consumed. Chhurpi is very healthy as well as nutritious milk product due to its low fat and high protein level, which makes it perfect for people trying to control diabetes or build muscle tissues. It also has a very high Vitamin A content as well



as around 15% higher amino acid content than cheese made from cow milk. It is also a good source of calcium, phosphorus, magnesium, zinc, omega 3 fatty acids and antioxidants.

The production of chhurpi is also organic as the yak or chauri from which these products are prepared grazed exclusively on herbs and grasses in the high alpine regions. Also, the traditional preparation methods do not use any preservatives or additives. The microbes mostly found during fermentation process are the LABs (Lactic acid bacteria), which have several health benefits such as ability to produce bioactive peptides during fermentation process. They also induce antimicrobial effects and produce distinct sensory qualities. Hard chhurpi is beneficial in removing plaque and tartar and keeping the gums strong.

### **Culinary uses of chhurpi**

*Churrpi* is one of the most popular traditional fermented milk products consumed by the people of Himalayan region of Darjeeling, Sikkim, Nepal, Bhutan, and Arunachal Pradesh. Average per capita consumption of Chhurpi is 6.9 g/day in Darjeeling hills, and 9.9 g/day in Sikkim. Soft churrpi is immediately consumed after processing. It is used as a filling for momos or as soups. It is also used in preparation of various vegetables, curries, pickles and other regional delicacies. It has a white, soft, mild to strong flavor and consumed with boiled rice meals. Hard churrpi is usually used a snack or chewing gum to provide energy. It can also be used as a dog treat to provide them healthy fats and essential nutrients.

### **Conclusion**

Ethnic fermented foods are produced by the ethnic people using their native knowledge of food fermentation from locally available raw materials of plant or animal sources either naturally/spontaneously or back-slopping or by adding starter culture(s) containing functional microorganisms which modify the substrates biochemically and organoleptically into edible products that are culturally and socially acceptable to the consumers. The preparation processes of these traditional fermented foods such as chhurpi come from earlier generations and have been passed down from generation to generation. Therefore, registration of different information about traditional food is part of the preservation of a nation's culinary heritage and culture. Traditional fermented foods have achieved an important place in human society as fermentation enhances the shelf life, texture, taste, aroma, and nutritional value of food. Churrpi is a traditional milk fermented cheese product having high nutritional quality. Local people consume this product by cooking with vegetables, meat, or by adding it into soups to get its health benefits as well as to enjoy its



characteristic flavor. Therefore, *chhurpi* is not only a food product but also an integral part of their life. However, a very few scientific research have been done on these products. The production process is also not standardized as every ethnic community of the Himalayan highlands has their own traditional way of preparing *chhurpi*. So, efforts should be made to popularize this ethnic product. With its rich history dating back centuries, *chhurpi* offers a captivating story of traditional cheese-making techniques, unique microbial cultures and the preservation of food in challenging environments. Along with its growing popularity in international markets, there is a potential for further research and innovation in the field of *chhurpi* cheese making.

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## Effect Of Pesticides in Aquatic Environment

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### Introduction

Pesticides are chemical compounds which are toxic in nature to destroy insects in aquatic environment. Pesticides are widely used in modern aquaculture to enhance the quantity and quality of yield in efficient manner. Organochlorines organophosphates, carbamates, pyrethroids and neonicotinoids are four major group of pesticides used. Pesticides tend to bio accumulate in food chain. Aquatic animal's aquatic sources are precious herbal belongings. Micro-organisms also contribute significantly to primary production, nutrient cycling and decomposition in estuarine eco system. Both structure and function of microbial communities may be impaired by pesticide toxicity. Herbicides are generally more toxic to phototrophic organisms Atrazine is most widely used herbicide.

- It can also use as fungicide, rodenticide, and insecticide.
- Annually 40% of the food produced worldwide is destroyed due to pests, therefore it is necessary to implement effective pest management using wide range of pesticide.
- Pesticides effectively increase the quality and quantity of yield in agriculture as well as aquaculture with less economic cost.
- After 1950's gradual increase in the manufacture and utilization of pesticides in agriculture and aquaculture
- Pesticides are basically classified into four major groups they are, Organochlorines, organophosphates, carbamates, and pyrethroids among all these pesticides **organochlorines** are the oldest group of pesticides
- Most of the pesticides are Broad spectrum insecticides for control of agriculture, aquaculture and domestic insects.
- These pesticides show long term persistence atmosphere.
- The mode of action of organochlorines is to disrupt the nervous system and paralysis of insects

- Most common examples for organochlorines are DDT(Dichlorodiphenyltrichloroethane), lindane Endosulfan, Aldrin, dieldrin.
- even DDT has banned in many developed countries still it is used in many developing countries
- In India it is used for malaria vector control
- Organochlorines are widely used till 1995's after that increased use of organophosphates
- Organophosphates are also broad-spectrum pesticide which acts as stomach poison and contact poison
- Malathion, diazinon and glyphosate are similar to organophosphates
- Carbofuran, category, synthetic pesticides like pyrethroids, neonicotinoids are highly toxic to insects and fishes but less toxic to mammals and aquatic bird's
- Cypermethrin and permethrin are most widely used synthetic pyrethroid pesticide
- Pesticides can be carried to aquatic ecosystem through agriculture run off, spray drift, soil erosion etc.
- It is also seen that farmers do not follow proper safety protocols during application of pesticides.
- Thus, safety use of pesticides is important with respect to health and protection environment and ecosystem the infant government has recommended maximum residual limit (MRL's) under food safety and standards.

Table 1: Permissible limits of major pesticides in drinking water

Pesticides	ISI Limit ( $\mu\text{g/L}$ )
<b>DDT</b>	<b>42</b>
<b>Aldrin</b>	<b>17</b>
<b>Lindane</b>	<b>56</b>
<b>Organic phosphate</b>	<b>100</b>
<b>Carbamate</b>	<b>100</b>
<b>Heptachlor</b>	<b>18</b>
<b>Dieldrin</b>	<b>17</b>

### Aquatic toxicology

- Aquatic toxicology is the study of the effects of environmental contaminants on aquatic organisms, such as the effect of pesticides on the health of fish and other aquatic organisms.
- The pesticide's capacity to accelerate the harmful effect of fish and aquatic animals are large.
- toxicity always depend upon exposure time, dose rate, persistence time in the environment.
- Brief exposure to some chemicals may have little effect on fish, whereas longer exposure may cause harm Bio-concentration is the accumulation of pesticides.
- Soil are ecologically important for aquatic habitat, which plays a significant role in nutrients holding capacity.
- Highly polluted sediments or accumulation of nutrients are adversely affecting the ecological functioning of rivers

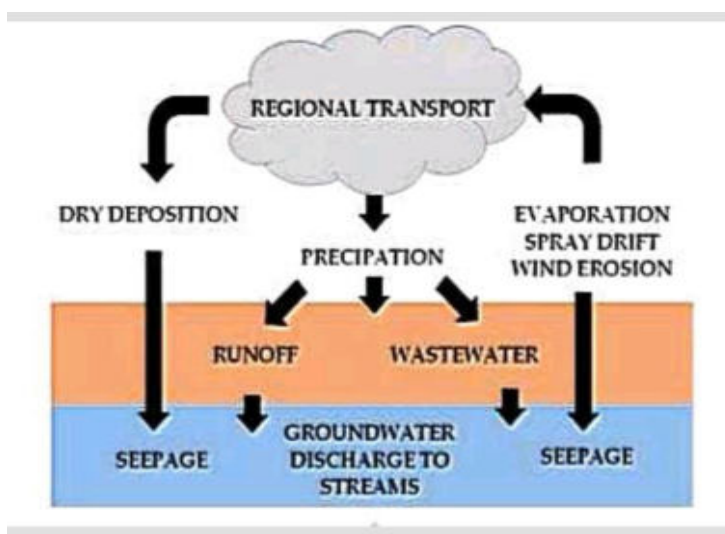




- Due to persistence in the environment and long-range transport. Repeated exposure to certain insecticides occurs frequently
- In decreased fish egg production and hatching, nest and brood abandonment, decrease resistance to disease.
- reduced body weight, hormonal modifications, and reduced avoidance of predators.

### Effect on Aquatic Ecosystem

- Individual pesticides are harmful to the ecosystem, but combining effect together results in enhanced toxicity
- Knowledge about interaction of specific pesticides belonging to these group in aquatic organisms is lacking
- The extent of bioaccumulation of different pesticides in fish is influenced by the polarity and water solubility of the pesticides
- There is an inverse relation between water solubility of pesticide chemicals and bioaccumulation of that chemicals in fish.
- If the solubility of the pesticides in water increases extent of bioaccumulation decreases.
- Hence water solubility is an important parameter in decreasing the dynamics of pesticides in aquatic environment
- The rate of elimination of pesticide is to be species specific.
- The level of pesticides in given species is determined by rate of absorption and rate of elimination reaction.
- Many toxic effects associated with organophosphates, carbamate and organochlorines have been discovered by residue analysis program (RAP)



### Effect On Micro Organism

- Microorganisms are important inhabitants of aquatic ecosystem system they play critical role in primary productivity, nutrient cycling, decomposition.
- Aquatic environment receives direct and indirect pesticides input exposing microorganisms and aquatic animals to pesticides.

**Pesticidal effects on humans:** Pesticides and their residues cause harm full effect on humans Pesticides can enter in to human body and food chain through direct contact, food and polluted air. Many symptoms like skin rashes, cramps, impaired vision.

**Bioaccumulation of pesticides:** Pesticides could accrue into aquatic animals via several means



directly from water through skin (or) gill, ingestion of polluted food (bio magnification). The effect of pesticide in fish result in behavioral change sluggish movement of fish and alteration of swimming ability makes more susceptible to predators

**Immune system:** Low concentration of pesticide disturbs the fish immune system, also work as impersonator of sex hormones function of immune system is modified by pesticides leading to immune depression

**Geno toxicity:** The Geno toxicity effect has become the key biomarker for assessing contamination related to damage.

**Behavioral variation:** Fish behavior is mostly affected by the uptake of contaminants; fish eat and accumulate various pollutants including pesticides. Imbalanced swimming, darting, and erratic movement etc.

#### **Microbial Sensitivity to Pesticides**

- It is not surprising that there is a considerable diversity in the sensitivity of micro-organisms to pesticides
- the majority of available pesticide data regarding aquatic micro-organisms is for algae.

#### **Chlorinated hydrocarbons**

- Various responses were observed among marine and freshwater algae to the effect of chlorinated hydrocarbons
- Aldrin, dieldrin, and endrin had no significant effect on respiration of green and blue green algae.
- At higher concentration lowered ATP levels but not population density

#### **Endosulfan**

- Endosulfan is a synthetic, chlorinated cyclodiene insecticide it is applied to grains, fruits, vegetables Etc.
- Endosulfan inhibit blue green algae Anabaena.

#### **Atrazine:**

- Atrazine is an s- triazine herbicide used to control broad leaf plants and greasy weeds.
- Inhibiting photosynthesis by blocking electron transport.

#### **Legislation in India to tackle pesticide pollution:**

- The government of India has taken significant measures to protect environmental resources
- The first law in 1972, wild life protection act, by national committee on environment planning and coordination.
- In 1974 water act (prevention and control of pollution) ,1986 Environment protection act
- Environment protection act 1986 encompassing legislation providing a single regulatory body for protection of environment.
- Directorate of plant protection, Quarantine and storage (DPPQS) have passed insecticide act in 1968, these regulate the import, registration process, manufacture, sale, transport distribution and use of pesticides.
- All pesticides are sold in India have to mandatory undergo approval process with central insecticide board and Regulation committee



### **Conclusion**

The popular article on the existing literature on pesticides pollution reveals that it is widespread in aquatic ecosystem in India. As aquatic organisms have a tendency to bio accumulate these pesticides along with food chain. It is necessary to have a strict implementation of existing policies and development of mitigation strategies in India.

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Available on request



# Human Babesiosis: An Emerging Human Zoonosis

Rashmi Singh, Akanksha Chaudhary & Ashok Baidha

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## Introduction

Babesiosis, caused by infection with intraerythrocytic parasites of the genus *Babesia*, is one of the most common infections of free-living animals worldwide, and it is gaining attention as an emerging human zoonosis. It is also a vector-borne disease with major public health implications. Three *Babesia* species, *B. divergens*, *B. microti*, and *B. venatorum*, are primarily responsible for zoonotic babesiosis in many parts of the world. *Babesia divergens* originated from bovines. Ticks of the genus *Ixodes* are the primary vectors of babesiosis, and their geographical distribution determines the prevalence of the pathogens involved. Worryingly, the geographical range of *Ixodes* ticks is expanding as a result of climate change. *Ixodes* ticks are also vector of *Borrelia burgdorferi*. the main vector species of *Ixodes* are *I. ricinus*, *I. scapularis*, and *I. persulcatus*. Babesiosis is transmitted to humans through infected tick bites, but it can also be transmitted through blood transfusion and transplacental transfer from mothers. Human babesiosis symptoms include headache, fatigue, loss of appetite, fever, chills, nausea, and shortness of breath, with the elderly at a higher risk of severe symptoms like hepatomegaly, kidney failure, hemolytic anaemia, splenomegaly, and splenic complications, which can lead to death. Blood smear examination is commonly practiced for diagnosis.

Asia as a region is currently undergoing extensive human modification of natural habitats, threatening its biodiversity. The destruction of Asia's habitats increases the risk of zoonotic disease spread, benefiting known reservoirs of zoonotic diseases, particularly rodents, which are more adaptable and resistant to habitat change. The impact of zoonotic transmission of babesiosis in Asia



is being worsened by a lack of molecular diagnostic techniques and clinical diagnostic expertise, insufficient medical awareness, and low capacity to detect the pathogens responsible. Human babesiosis is an underrepresented disease in Asia due to its low incidence, and its occurrence has not been thoroughly investigated in this region. Nonetheless, it is known that specific *Babesia* species are to blame for the spread of babesiosis throughout Asia. It has also been reported that in Asia, rodents and their associated ticks serve as major reservoirs of *Babesia* spp., including *B. microti*. *Babesia microti* was also reported in wild animal populations in Thailand and Japan, with evidence of human case reports also found in Singapore, Mongolia and India. There is a lack of information on the current status of zoonotic babesiosis in Asia in terms of species distribution, reservoir diversity, intensity, and prevalence.

Human babesiosis has also been reported in India, though only in one case of a man in the Baroda (Gujarat) region, where the genus *Babesia* was identified through microscopy. A 51-year-old man presented to a private hospital with fever, loss of appetite, and vomiting, and *Babesia* was identified morphologically from smears taken after antibiotics failed to treat his symptoms. Plasmodia antigen testing was negative, indicating that the patient was not infected with malaria. Malaria is prevalent in many parts of India, and the intraerythrocytic parasites are commonly referred to as plasmodia. Only a careful examination of peripheral blood smears can distinguish *Babesia* from malarial parasites. To determine the true prevalence of human babesiosis in India, extensive peripheral smear examination and serological surveys may be required. People may get sick from the *Babesia* infection and may show flu-like symptoms, such as fever, chills, headache or body aches, a low red blood cell count (anaemia). Some people are more likely to develop severe illness, including: elderly people, people with other illnesses, individuals without a spleen

Preventive measures should be taken by the person who involve in animal rearing and taking animals to feed on dense pasture where ticks might be found. Preventive measures are especially important for people who are at high risk of developing severe babesiosis like, people who do not have a spleen. The best defense is to limit your exposure to tick habitats. Other things should be taken care for prevention from Babesiosis are like walk on cleared trails, avoid areas with overgrown grasses or brush. Wear long pants, long-sleeved shirts, and socks when outdoors. Tuck the pant legs into the socks. Apply tick repellents to skin and clothing etc.



## Foot and Mouth Disease in Pigs: An overview in Indian Perspective

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### Introduction

Foot-and-mouth disease (FMD) is a viral disease of all cloven-footed animals caused by the *Aphthovirus* of the *Picornaviridae* family. In India, where FMD is prevalent, the cases have been documented throughout the year from different regions of the nation. The multiplicity of viral strains in circulation with serotypes O, A, and Asia-1, unrestricted movement of the animals from diseased areas, and apparent infection in small ruminants have compounded the disease situation in India. Type O is the most common serotype and is responsible for 83-93% of outbreaks, followed by Asia 1 (3-10%) and A (3-6.5%). However, the disease occurrence, clinical disease severity, and outbreak frequency have all gradually and significantly decreased in areas receiving routine vaccinations, primarily under the FMD control program and partially under the ASCAD (Assistance to States for Control of Animal Diseases) and RKVY (Rashtriya Krishi Vikas Yojana) of the Government of India.

India's livestock wealth (534.54 million) includes 9.06 million pigs as per the latest census which has been reduced in comparison to the previous census owing to the increased piglet mortality and diseases. FMD is a major contributor of economic losses to the swine industry. Respiratory aerosols and direct or indirect contact with sick animals are the two main ways of viral spread. Under specific meteorological conditions, it is thought that the FMD virus can be transmitted via aerosols over lengths of up to 30 miles. The infected pigs are very effective virus spreaders and can produce aerosols with much higher virus concentrations than other species. Hence, pigs have been referred as the amplifier hosts for FMD virus. Even after recovering from FMD pigs may carry the virus for

weeks, months, or even years.

The affected pigs exhibit mild to severe clinical signs depending upon the type of virus involved and immune status of the animals. The common clinical signs include high fever (104-106°C), dullness, excessive salivation, vesicles in the mouth, particularly on the tongue and gums, ulcers in the inter-digital space of the hoof, blisters on the teats, infertility and retarded growth, thimbling (totally missing hooves). In some cases, abortions and death may occur during extreme circumstances.

The FMD is amongst the most serious diseases of pigs in terms of economic impact and is globally recognized as a priority disease for control and eradication. The economic losses suffered by farmers due to this disease are enormous and continue during the life cycle of the animal.

To reduce the danger of disease transmission and eventually eradicate it preventive measures must be adopted. Basic biosecurity measures are important in minimizing the spread of FMD. Standardize pig movements, restricted people and vehicles movements, placement of foot dips at all entrances, service and feed delivery points, proper cleaning and disinfection procedures, proper health monitoring, isolation of sick animals, treatment of diseased animals, proper sanitization of feed and water are few crucial steps to be taken for control of the disease and minimize the associated losses.

Routine immunization may be used in endemic and high-risk areas primarily to protect breeding stock. Pig vaccination presents challenges due to the limited duration of protection, which is only around six months. Additionally, it's partly because there are three FMD serotypes prevalent in India, and immunity to one makes animals vulnerable to the others. In most endemic areas, vaccines must be multivalent (several serotypes) for effective management of the disease.



## Chemical applications improve cotton yield by alleviating water stress in cotton belt of Punjab (south-

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Cotton is an important cash crop during *Kharif* season that is growing for textile industries. In Punjab, cotton is grown on an area of 249 thousand hectares with a total production of 639 thousand bales. In the state, cotton is a second major *Kharif* season crop after rice which is mostly grown under south-western districts of Punjab where irrigation water is a major problem during *Kharif* season because of scanty rainfall and extended canal closures as well as brackish underground water. Consequently, the delayed monsoons, non-availability of canal water or failure of irrigation facilities often create moisture deficit conditions in the cotton growing areas resulting declined seed cotton yield up to 42.9 %. The foliar application of Osmo protectants such as Glycine betaine and salicylic acid at appropriate concentration and time may be an effective approach for sustaining the high cotton productivity under water deficit scenarios.

### Causes of water stress

In the south-western districts of Punjab, crops depend more on canal water and rainfall than underground water because of brackish underground water. So, meager rainfall and canal closure causes irrigation water deficit conditions.

### Effect of water stress on cotton

Cotton is warm season crop required 4-6 irrigations depending upon the seasonal rainfall but peak vegetative and early reproductive stage is sensitive to water deficit conditions, causing a significant reduction in yield of up to 42.9 % as well as the fibre quality and biomass yield of the crop up to 55 %. Timely availability of irrigation water to cotton remains the main production hurdle to sustain cotton productivity in cotton belt of Punjab.

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### Mitigation strategies to overcome water stress on cotton

Farmers need to understand the appropriate time and quantity of irrigation water to the cotton crop. A heavy pre-sowing is required and first irrigation applies after 30-35 DAS. During peak vegetative stage and early reproductive stage, proper application of water may reduce the adverse effects of water scarcity during rest of the season. Proper use of nitrogenous fertilizer (30-42 kg/ha depends on the varieties and hybrids) also improve water absorption capacity of the plant roots.

### Chemical management to overcome effect of water stress

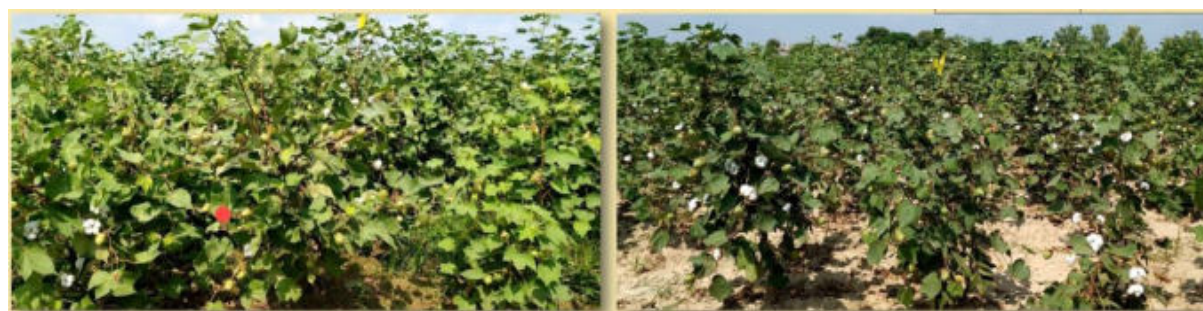
In water shortage conditions, plants are unable to retain water from their roots due to low osmotic pressure in the root cells, so certain chemicals found by researchers increase osmotic pressure, which eventually increases the ability of roots to absorb water. With a certain concentration, some chemicals such as Salicylic acid and Glycine betaine can effectively counteract the harmful effects of water stress on cotton crops. Some important aspects are described below: -

#### Preparation of solution

Prepare solution for Salicylic acid and Glycine betaine @ 100 ppm. In simple language, to prepare a 100-ppm solution, take 10 ml of Salicylic acid and Glycine betaine in 100 liters of water. Dissolve 12.5 g Salicylic acid or Glycine betaine in 375 ml of Ethyl alcohol and then add it to 125 liters of water for one acre area of cotton crop.

**Table 1: Concentration of different chemicals used to overcome effect of water stress**

	Parts per million (ppm)	Percent solution (%)	In 125 L of water
Salicylic acid	100 ppm	0.01	12.5 g
Glycine betaine	100 ppm	0.01	12.5 g



a) Chemical applied on water stressed crop

b) Water stressed cotton crop

**Fig.1: Effect of Salicylic acid and Glycine betaine spray on the water deficit cotton crop.**



### **Mode of application**

The water stress can be effectively overcome by foliar application with sufficient solution concentration.

### **Selection of spray pump and nozzle**

Foliar spraying can be done with knapsack sprayer (manually or battery operated) or tractor operated sprayer with cone type nozzle. Mostly 125-150 liter of water is required per acre, so prepare solution according to quantity of water / area to be sprayed.

### **Method of foliar spray**

Nozzle height at the time of spraying is very important. Maintain nozzle height around 1.5 feet from the crop canopy during spraying.

### **Time of spray**

Water stress at the peak vegetative and early reproductive stage affects a significant loss of cotton seed production. So, during these stages of the crop, these chemicals should be sprayed. Cotton is an indeterminate crop, so its particular growth stage is complex to describe. Although, time gap between two consecutive stages also depends on the factors like date of sowing, soil texture, rainfall, air temperature and stresses., but 70-80 days after sowing, researchers considered a good time for these chemicals to be applied. Only one foliar spray application is appropriate.

**Table 2: Time of application**

<b>Chemical</b>	<b>Time of application (DAS)</b>	<b>Number of sprays</b>
Salicylic acid	70-80	1
Glycine betaine	70-80	1



## Management of Heifers Turning to Adult Cows

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Heifers are the animals that are not given birth to calf yet, when they experience calving the stress level elevate to higher level as compared to other adult cattle that had given birth previously. So, heifers experience this stress most. Everything is novel for them like, a newborn calf, new calving pens, and milking operation. Farmers need that their heifer should swiftly adjust after calving and begin milk production. And after that, will be healthy enough to become pregnant and begin the process all over again.

To cope up with this stress we have to adopt some strategies to improve heifer's immune system at the time of transition. Also, we have to ensure that heifers are prepared for success when they join the milking herd. There are a few important management techniques we may use in the months before calving to ease the transition to milking cattle.

### Selection of Heifer

Selection should be made for the largest heifers because they reach puberty earliest as compared to smaller heifers. Heifers should be structurally large, physically sound, in good health, and have good growth potential. For selection records could be very helpful, we can refer to performance records to determine growth potential and to identify heifers from high production cows. Heifers those are obese at weaning have a tendency for reduced milk production ability.

### Heifer Nutrition

- To ensure birth to a healthy calf from first time calvers, it is important to ensure that their nutritional needs are being properly met. So, while formulating their diet, keep care that feed contain high-quality ingredients. Heifers can be fed a variety of feed ingredients if the rations are properly formulated to meet their nutritional needs in-terms of energy, protein, minerals, and vitamins.
- Proper nutrition is necessary to reach the goals of early puberty, early breeding, and proper body weight and size at calving. Keep in mind that formulated ration should be sufficient for adequate growth, but do not make animal obese. Heifer should have 60-65% of mature body weight at the time of breeding. If this body weight goal not achieved then it could cause problems during calving e.g., Dystocia for low milk production afterwards.

We can classify heifer based on their stages of growth as following:

- **Weaning to nine months of age.** Goal should be lean growth of muscles and skeletal tissues, achieving 30% of mature weight at six months. This will result in increased body frame size while maintaining a consistent body condition score. Improved skeletal development results fewer calving difficulties.
- **From 9 months to mating:** heifers should achieve 43-47% of mature body weight, one to two months before mating and shall continue to grow till mating (60% of mature body weight) for improved conception rates.
- **Mating to calving:** Live weight should be gained targeting 90% of mature body weight at pre-calving stage. Body condition tells how well an animal has been fed.

#### **Managing of the housing environment:**

In order to prepare a heifer for calving, reduce environmental stress so that we can maintain better growth rates and improve general health. For this purpose, following techniques can be incorporated:

- Provide sufficient of bedding and ensure that the heifer's bedding should be dry, absorbent, non-irritant and soft.
- Make housing environment of heifer dry, well-ventilated, and draft-free, this could be achieved with well-planned housing system.
- Avoid overcrowding and give animals enough space to eat to lessen stress and competition. Recommended space allocation is very important to make animal feel comfortable during confinement.
- Housing facilities should be such that animals remain protected from the environmental elements and temperature fluctuations. The immune system of a heifer might deteriorate when exposed to extreme climatic changes, and this will lead to diseases or decreased production.



**Minimize potential health problems:** Heifers that are healthy from birth through pregnancy are often less likely to have health issues after giving birth and are better able to adjust to life in the milking herd. Pregnancy tests, diet, and vaccinations are essential for sustaining health.

▪ **Vaccination:**

Replacement heifers need to be on a good health program. They should be vaccinated prior to breeding for brucellosis, vibrio, leptospirosis, IBR. (red nose), and other diseases prevalent in the area. A good vaccination program is a good insurance policy. There are a lot of vaccines out there, and not every option is right for every operation. It's important to work with a veterinarian to understand what diseases are on the farm that requires vaccination.

▪ **Pregnancy check:**

Regular heifer pregnancy checks may seem unimportant, but not knowing a heifer's reproductive status can be expensive. The cost of lost milk production and additional raising expenses per heifer for every month the first calving is postponed beyond 22 months can be very high. Pregnancy tests should ideally be performed around 30 days after breeding and once more a month later to catch any pregnancy losses.

▪ **Calving Difficulty**

Dystocia is a great concern for farmers with first-calf heifers, as it is the primary cause of calf losses at birth. The major causes of dystocia in first calf heifers are either oversized calf or an undersized heifer. A large calf and/or a heifer with a smaller pelvic girdle cause difficulty during parturition. To reduce calving difficulties farmers should focus on to developing heifers that have larger body size at calving. If heifers weigh around 80% to 85% of their mature body weight at calving, they should have less dystocia. Also measure pelvic area of heifers and those with small areas should be culled.

▪ **Culling**

Making decisions about culling can also be aided by monitoring disease incidence. Any animal that has received repeated medical attention for a disease should be eliminated before breeding starts. Also eliminate poor producers or animals those have repeated breeding problems. Every heifer ought to have a report card that includes crucial details like growth rates, disease incidence, and vaccination records. These records can give us more insight into the possible causes of heifers' subpar performance prior to or following calving. Records should assist us in determining out what's wrong and what could be the remedial actions.



## How packaging becomes a marketing strategy for poultry products

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With an output of 3.8 million tons of chicken meat and 66.45 billion egg per annum in 2013, India has emerged as the 4<sup>th</sup> largest world poultry meat producer and 3<sup>rd</sup> largest egg producers. The broiler production has been growing at an incredible rate of 12-15 % per annum and egg production at the rate of 6-8% over the last decade. Despite spectacular increase in egg and broiler meat and production, both processed poultry product and value-added product sectors in India are in a nascent stage. Even today the bulk (80%) of chicken are sold alive or as skinned carcasses in wet market and the rest are sold as dressed whole carcass, cut-ups or further processed value-added products. Establishment of about a dozen modern, mechanized poultry processing plants and vertically integrated poultry companies with their own retail outlets/ restaurants, proliferating fast food outlets and entry of a few multinational fast-food chains have given some momentum to the growth of this sector over the last decade. Hence efforts are needed to evolve cost-effective and efficient technology for the production of shelf-stable, consumer-oriented convenient products to penetrate the vast until now untapped market.

In order to gain a competitive advantage in the dynamic market, multinational companies in the food processing sector give more focus on the quality of the product, functional aspect of the product, specifications of the product, aesthetic design of products etc. But in competitive market almost all the products of the same category have more or less same quality, functionality and features. So the companies target on providing uniqueness amongst themselves on these grounds.

And this makes them to give more emphasis on the aesthetic designing of packaging of their product to grab the attention of the customers.

Many multinational food processing firms are spending approximately 10% of their retail pricing on the packaging, even in some scenario it becomes the most expensive part for the company. Often packaging is considered as a key element for the promotion of the product. Reaction to the packaging determines the success and failures to the product. As in a dynamic marketing environment, adopting new designs are unavoidable for the lively presence of the firms in the market.

The basic purpose of packaging is to protect meat and egg products from undesirable impacts on quality including microbiological and physio-chemical alterations. Packaging protects foodstuffs during processing, storage and distribution from:

- **contamination by dirt** (by *contact with surfaces and hands*)
- **contamination by micro-organisms** (*bacteria, moulds, yeasts*)
- **contamination by parasites** (*mainly insects*)
- **contamination by toxic substances** (*chemicals*)
- **influences affecting colour, smell and taste** (*off-odours, light, oxygen*)
- **loss or uptake of moisture** (*evaporation or water absorption*)

Above all packaging should ensure the protection of the product from various breakages, spills through shipment, storage etc. Practically all films used for meat packaging derive from **synthetic “plastic” materials**. The most common synthetic materials used for meat packaging are:

Polyethylene (PE)	(oxygen + , water vapour -)
Polypropylene (PP)	(oxygen + , water vapour -)
Polyvinylchloride (PVC) (soft)	(oxygen + , water vapour -)
Polyester (PET)	(oxygen ± , water vapour -)
Polyamide (PA)	(oxygen - , water vapour +)
+ = relatively permeable & - = relatively impermeable	

Moreover, packaging is one of the crucial elements which decide the purchasing decision of people. Since food products were available in wide range and varieties, unique packing design is one of the strategies that can adopt to get the customer attention. Customers have certain expectation regarding the packaging of a product, viz; the packaging material, complete and simple labeling listing the ingredients, instructions to use, nutritive quality, storage conditions etc.



Packaging should be able to meet the customers' need, so as to meet the customers from various market segments. Since Indian traditional joint families are converting into nuclear ones and globalization makes people to travel and stay away, food packaging development should cater the changing consumption patterns and smaller households. Single and smaller serve products will reduce waste by meeting the needs of single and two person households. Increased use of retail ready packaging also reduces double handling and damage and improve stock turnover. Recently the manufacturers as well as the retailers adopted a new strategy in the packaging that is 'eco-friendly' packaging technique which attracts consumers mainly because of easiness in disposal.

People are very sensitive to the quality aspect, especially when it comes to food items. That is the reason why 70% of customers prefer transparent packaging than a closed packaging for food items. Also, transparent packaging films normally used for meat products allow attractive product presentation as the packaged product is visible. However, such films provide no protection against possible light impact on meat products which may cause unattractive colour changes, oxidation and rancidity.

Packaging has a vital role to play in containing and protecting food as it moves through the supply chain to the consumer. Innovations in packaging materials, design and labeling provide new opportunities to improve efficiencies. Recognition of the benefits of packaging technologies, development of economically viable packaging systems and increased consumer acceptance for packaged products opens new frontiers for entrepreneurs in marketing the poultry products.

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## Unraveling the causes of summer anestrus in buffaloes: effective strategies for mitigation

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### *Abstract*

Summer anestrus refers to a temporary cessation of reproductive activity and the absence of estrous cycles in female buffaloes during the hot summer months. It is a common phenomenon that affects buffalo herds worldwide, leading to reduced fertility rates and economic losses in the dairy and livestock industry. This article explores the causes underlying summer anestrus in buffaloes and provides potential strategies to ameliorate its impact.

### **Introduction**

Buffaloes in the Indian sub-continent is highly valued for their productive potential and are often referred to as the "Black Gold." They are primarily raised in India for their milk, which has a higher fat content, as well as for their meat. However, they face certain reproductive issues such as delayed puberty and sexual maturity, seasonal reproduction patterns, lack of observable estrus signs, and extended periods of non-breeding after giving birth. This phenomenon, commonly known as summer anestrus, is prevalent in buffalo populations worldwide. Anestrus is characterized by the absence of regular estrus behavior, lack of discernible follicular or luteal structures in the ovaries. An inactive or non-functional ovary is a significant factor contributing to anestrus in buffaloes. Anestrus is a frequently occurring reproductive problem in buffalo populations. Buffaloes experiencing summer anestrus do not display estrus due to disruptions in their endocrine profile, leading to inactive ovaries. The combination of longer daylight hours and high ambient temperatures results in elevated prolactin levels, which suppress the secretion of gonadotropins and disrupt ovarian steroid production. Heat stress also has detrimental effects on follicle development, the microenvironment of the follicular fluid, and the quality of oocytes.

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## **Seasonality in the reproductive pattern**

Buffaloes are a polyestrous species, meaning they exhibit estrous throughout the year. However, different countries, including India, Pakistan, and various regions worldwide, have reported a distinct seasonal pattern in buffalo reproductive behavior. Environmental factors, particularly ambient temperature, photoperiod (day length), and feed availability, have been identified as key influences on this seasonal variation, rather than genetic factors. Estrus expression is limited during the colder winter months, while sexual activity decreases during the hot summer. The indirect impact of climate on vegetation patterns appears to be the primary cause of buffalo's natural reproductive patterns during the summer season. Photoperiod, through its influence on pineal gland secretion, plays a significant role in controlling these reproductive shifts. In some regions of the world, buffaloes breed consistently throughout the year, with a slightly higher frequency in spring and a slightly lower frequency in autumn.

### **Environmental factors**

Ambient temperature, humidity, and photoperiod significantly contribute to the occurrence of anestrus in buffaloes during the summer. Research has shown that ambient temperature and relative humidity directly impact breeding efficiency. Unfavorable environmental conditions, such as high temperatures and humidity, disrupt the length of the estrous cycle and reduce the duration of estrus. Buffaloes are particularly vulnerable to heat stress during the summer, especially when exposed to direct sunlight, as they have a limited capacity for cutaneous evaporative cooling due to their low density of sweat glands. Their black skin and sparse hair coat provide minimal protection. High levels of relative humidity further exacerbate these conditions. Additionally, the duration and intensity of light exposure have an influence on the initiation of the estrous cycle in buffaloes.

### **Endocrine factors**

Hormones play a crucial role in the development of summer anestrus in buffaloes, primarily due to the suboptimal functioning of the hypothalamo-hypophyseal-gonadal axis. The low reproductive efficiency of buffaloes during the summer has also been attributed to decreased luteal activity, characterized by lower average progesterone levels and reduced peak progesterone levels, which may explain the poor conception rates. Anestrus buffaloes during the summer exhibit low estradiol concentrations. Anestrus buffaloes exposed to thermal stress during the summer show higher levels of serum corticoids, which in turn lead to alterations in gonadotropin secretion and ultimately trigger the state of anestrus.

The role of melatonin in regulating reproductive seasonality has been extensively studied in seasonal breeders like sheep and mares, but its role in buffalo reproduction has received less



attention. Melatonin is produced by the pineal gland and its secretion follows a circadian rhythm, with significant levels observed only during the dark period. Research has shown a clear seasonal variation in melatonin production in Italian Mediterranean buffaloes, with the highest concentrations occurring during periods of shorter day length.

Prolactin (PRL), a hormone associated with ambient temperature, is believed to mediate the seasonal effects on buffalo reproduction. During the hot summer months, buffaloes exhibit hyperprolactinemia, which is thought to be influenced by seasonal changes in pineal metabolism and considered a potential cause of summer anestrus in the species. Prolactin may interfere with the hypothalamic mechanism responsible for the episodic release of luteinizing hormone (LH) or inhibit the positive feedback of estrogen on LH secretion, thereby affecting the estrous cycle and fertility.

Buffalo heifers exhibit seasonal fluctuations in circulating follicle-stimulating hormone (FSH) levels that align with the breeding pattern. The lowest FSH values are observed during the non-breeding season (March to June), which coincides with the hot months. Generally, the FSH levels in anestrus buffaloes remain lower compared to the baseline levels seen in normally cycling buffaloes. Furthermore, the pre-ovulatory FSH peaks that typically coincide with LH peaks are reported to be absent in non-cycling buffaloes during the hot months. The ratio of FSH to LH is also lower during the hot summer months compared to the peak breeding season.

Luteinizing hormone plays a significant role in contributing to ovarian inactivity in buffaloes during the summer months. Compared to the winter season, LH secretion is lower during the summer. Additionally, optimal LH surges are often absent in anestrus buffaloes during the summer, although some animals may experience an increase in LH levels from 3.06 to 8.67 ng/ml on certain days. The decrease in LH levels is attributed to the inhibitory effects of progesterone and PRL.

Anestrus associated with low thyroid function is common in buffaloes during the summer season. It has been postulated that high ambient temperatures lead to hypothyroidism, resulting in reduced ovarian responsiveness to pituitary gonadotropins, which contributes to summer infertility.

### **Management factors**

Management practices play a crucial role in the rearing of buffaloes during the summer season. It is widely recognized that buffaloes exhibit weak estrus behaviour, which becomes more pronounced in hot weather conditions. A significant number of buffaloes experience silent estrus during the summer, characterized by less noticeable estrus signs with shorter durations. It has been observed that buffaloes tend to exhibit estrus mostly during the night-time or early morning hours, which often goes unnoticed by many farmers. Therefore, routine observations alone are not sufficient for detecting estrus in buffaloes, resulting in prolonged service periods during the warmer months.



## **Strategies to ameliorate summer anestrus**

To enhance the reproductive efficiency of buffaloes, a range of strategies can be implemented, including environmental modifications, enhanced nutrition, optimized breeding practices, improved suckling management, and the use of hormonal therapy.

### **Managerial practices**

To improve the reproductive efficiency of buffaloes during the summer breeding period, altering farm management practices has been found to be a more favorable option than relying solely on hormonal treatments, particularly for rural buffaloes reared in field conditions. Implementing measures to protect buffaloes from direct solar radiation is considered a fundamental aspect of effective management during the hot summer months. Additionally, a modern management approach has been recommended to optimize reproductive efficiency and production in buffaloes during the summer, focusing on stress reduction and improving the nutritional status of the animals. Implementing measures to mitigate heat stress, such as providing shade, adopting a loose housing system, and utilizing water-based cooling methods like sprinkling, washing, or wallowing facilities during the hottest part of the day in summer, can significantly reduce the negative impact on buffalo fertility. Research has shown that buffaloes exposed to showers in addition to wallowing facilities achieved higher conception rates, possibly due to the prevention of early embryonic mortality through body cooling. In hot and humid areas, practices like showering, splashing, or spraying water on the body, along with appropriate housing and a shift from daytime to night-time grazing, are recommended to enhance the reproductive and productive efficiency of the animals. The conventional heat detection methods traditionally used in buffaloes are insufficient for detecting estrus during the summer season. Since buffaloes exhibit estrus predominantly during the nocturnal hours, implementing a system of heat detection during the cooler parts of the day or night-time, using a teaser bull, may enhance the efficiency of estrus detection.

### **Hormonal treatments**

Various hormonal treatment regimens are employed to address anestrus, stimulate ovarian activity, induce or synchronize behavioral estrus, or control ovulation in buffaloes. Different hormone combinations or single hormone treatments have been utilized with varying degrees of success. Progesterone-based treatments such as PRID, CIDR, and progesterone injections, either alone or in combination with gonadotropins and PGF<sub>2</sub> $\alpha$ , have been reported to be highly effective in inducing ovarian activity during summer anestrus in buffaloes. Additionally, the combination of PRID and PMSG has shown superior results compared to PRID alone in terms of estrus induction, conception, and pregnancy rates. The ovsynch protocol, either alone or in combination with other

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hormones, has also proven to be beneficial in inducing estrus and improving conception rates in buffaloes. Attempts were made to utilize GnRH and its analogues for inducing the resumption of the estrous cycle in buffaloes during summer. However, these treatments did not yield the desired results due to their limited half-life and varying response depending on the size and stage of the follicles at the time of treatment. Additionally, it was observed that pre-treatment with insulin promotes follicular development, making the ovary more responsive. In cattle, insulin has been reported to increase the production of estradiol, leading to a feedback increase in LH release from the pituitary gland. Clomiphene citrate has been widely studied for the treatment of summer anestrus in buffaloes, with varying success rates in inducing estrus and conception. It has also been used in combination with progesterone, showing favourable outcomes. In addition to these conventional approaches, melatonin implants have been investigated as a treatment for seasonally anestrus buffaloes. A more recent advancement in this area is the utilization of antioxidants such as selenium, vitamin E, and zinc-methionine to improve fertility during the summer season.

### **Nutritional management**

Implementing an appropriate feeding strategy for buffaloes during hot climates is crucial to mitigate infertility issues, particularly summer anestrus. Strategies such as providing night feeding, allowing grazing only in the cooler morning and late afternoon periods, can help alleviate heat stress in buffaloes. This helps minimize heat stress and allows them to digest their food more efficiently. Additionally, offering green fodder, silage, or hay, along with ad-libitum water and mineral supplementation, can enhance reproductive efficiency during the summer. Well-fed buffaloes are more likely to exhibit estrus during the night, facilitating regular breeding. Provide access to salt blocks or mineral supplements formulated for buffaloes. This helps to replenish essential minerals lost through sweating and promotes overall health. Feeding buffaloes roughage during the night can help reduce the animal's heat load.

### **Conclusion**

Summer anestrus in buffaloes is a multifactorial issue influenced by heat stress, photoperiod, nutrition, and management practices. By implementing appropriate strategies to manage heat stress, optimize nutrition, manipulate photoperiod, and improve overall breeding and management practices, the adverse effects of summer anestrus can be minimized, leading to improved reproductive efficiency and productivity in buffalo herds.



# Revolutionizing Veterinary Science: The Advent of Nanotechnology

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## *Abstract*

The term "nanotechnology" describes the processing of materials at the atomic or molecular level, particularly for the creation of minuscule devices capable of calculation, function, and organization. The size range of 1–100 nm is commonly referred to as the microscopic level. Through the creation of a system for the delivery of smart medications, nanotechnology has a significant impact on the treatment of diseases in veterinary medicine and other facets of animal production. These days, nanotechnology has completely changed veterinary medicine and animal science fields by introducing novel, miniature tools and materials that are advantageous to living things. Quantum dots, magnetic nanoparticles, nanopores, polymeric nanoparticles, nanoshells, fullerenes, liposomes, and dendrimers are a few examples of the nanoparticles that are utilized for illness detection, therapy, drug administration, animal breeding, and reproduction. Although nanotechnology is recognized as one of the most important technologies that had previously been used in a variety of fields, veterinary science is only just beginning to use it.

## **Introduction**

The ability to calculate, operate, and organize matter at the nanoscale is known as nanotechnology. The scale typically refers to particles with at least one dimension between 1 and 100 nm, while it is frequently expanded to encompass particles smaller than 1 nm. It is an enabling collection of technology that spans all spheres of activity and scientific fields, rather than being restricted to a single industry. Nanotechnology makes use of the principles and methods of the nanoscale to comprehend and alter bio-systems, which make use of biological ideas and components to create new nanoscale systems and devices. Among the most astounding man-made materials, rationally created nanostructures display unique chemical, physical, and/or biological properties. These qualities allow for a remarkable range of applications for the nanostructures in industries as

diverse as electronics, agriculture, and health care.

Closing the gap between macroscopy and microscopy, where nanoparticles are the ideal medium to connect with biological systems, is one of the main benefits of nanotechnology. Large active surfaces, easily adjustable surface chemistry that enables binding to small molecular medicines, imaging labels, and ligands including antibodies, peptides, and nucleic acids are just a few of the characteristics that set nanoparticles apart from bulk materials. Additionally, because of their small size, they can interact only intracellularly and extracellularly, allowing for extravasation via endothelial cells as well as enhanced permeability and retention in tumor tissues. In the twenty-first century, it is anticipated that nanotechnology will lead to a significant number of innovations that will advance the practice of clinical veterinary medicine and have the potential to modernize veterinary care, animal welfare, and other sectors of animal production. Animal nutrition scenarios ranging from nutrient uptake and use, animal waste adjustment as expelled from livestock, pathogen detection, and much more will be made possible by veterinary nanotechnology. It will also improve systems of diagnosis and treatment delivery, offer new gears for molecular and cellular breeding, animal history from birth to consumer table, and much more.

### **Classification of nanoparticles**

Nanoparticles are minuscule particles that range in size from 1 nm to 100 nm. Over the past few decades, a wide range of newly created materials have been used to create particles such as nanocrystals, polymers, dendrimers, silica oxides, carbon, metal oxides, lipids, and quantum dots. Below are some of the often-utilized nanoparticles.

- **Fullerenes-** Fullerenes are nanoparticles made completely of carbon-based compounds. Their potential for use in nanomedicine has been thoroughly investigated, and their usage in the industrial industry is already well-established. High aspect ratio, thermal, electrical, and mechanical properties are only a few of the qualities that carbon nanoparticles possess that make them effective in therapy and regenerative medicine. Carbon Nanotubes (CNTs), which can have a single or multiple walls (SW, MW), are one of the fullerenes most frequently employed in nanotechnology. Due to their 'needle-like' capacity to penetrate cells and ability to obtain nuclear exposure by shortening their length, SWCNTs provide a novel method for drug delivery. Since CNTs can be charged or conjugated with medications to increase therapeutic efficiency, this phenomenon is very helpful.
- **Quantum dots-** When stimulated by light, quantum dots, which are nanocrystals that range in size from 2 to 10 nm, can fluoresce. They are made up of an inorganic center, whose size determines the color of the inorganic shell that is emitted, and an aqueous organic coating that is mixed with



biomolecules. It can be utilized for both clinical and diagnostic biomedical objectives. It can also be used to image sentinel nodes in cancer patients in order to stage tumors and plan therapy.

- **Liposomes-** Liposomes are spherical, artificial nanoscale vesicles made of cholesterol and phospholipids from natural sources. The first drug delivery system to be tested used liposomes. They are colloidal or micro-particular carriers, typically ranging in size from 80 to 300 nm. These can be utilized as efficient drug administration systems. Amphotericin and hamycin, two cancer chemotherapy drugs, exhibit higher efficacy and protection when administered as liposomal medications as opposed to conventional preparations.
- **Magnetic nanoparticles-** The capacity of antibodies to bind to the surfaces of magnetic nanoparticles, such as iron oxide paramagnetic compounds, and the potential for targeting using an external magnetic field make them interesting candidates for the therapy of disease. Super paramagnetic iron oxide nanoparticles with a diameter of less than 10 nm and outstanding magnetic characteristics are typically the most effective materials. They are tiny, thermally agitated magnets that are referred to as "ferromagnetic fluids" or "ferrofluids" in liquids. Super-paramagnetism can only exist in the absence of a magnetic field; if this field is taken away, magnetization will disappear, particles will stop interacting, and potential vascular embolization can be prevented.
- **Nanopores-** Desai and Ferrari first proposed the idea of nanopores in 1997. These consists of wafers with a high porosity density and a maximum diameter of 20 nm. The pores let the movement of insulin, glucose, oxygen, and other substances. However, the passage of cells and immunoglobulins through them is not necessary. Utilizing nanopores can help shield transplanted tissues from the host defense mechanism. The recipient's body may be implanted with beta pancreatic cells that have been folded inside the nanopore system. This tissue sample avoids rejection because it takes up nutrients from the neighboring tissues while evading detection by the immune system. It could be used as a more recent therapeutic method for diabetes mellitus that is insulin-dependent.
- **Nanoshells-** West and Halas have both produced nanoshells. A thin metal layer covers the silica nucleus nanoparticles that make up nanoshells. This can be applied utilizing immunological techniques to the right tissue. This method is being investigated for tumor therapy. Using nanoshells that exhibit the nanoshell's thermoablative feature, which is the ability to absorb infrared radiation when exposed from a source outside the body.

### **Nanotechnology applications in veterinary science**

Nanotechnology is utilized to create nanoscale pharmaceuticals, control delivery methods, identify contaminants, and create molecular and cellular biology nanodevices. In addition to playing





a crucial role in disease prevention through the adoption of a smart medication delivery system, it will play a significant role in the domains of animal welfare, veterinary medicine, and other animal production. One currently under development application of nanotechnology in medicine is the use of nanoparticles to deliver medications or other ingredients to particular cell types. Diseased cells are programmed to attract particles that make some cells handle directly.

- **Nano vaccines-** A novel approach to immunization is emerging: the nano vaccine. Nano vaccines are more efficient than conventional vaccines and can induce both a humoral and a cell-mediated immune response. They offer to direct the immune system of the body to fight pathogens and stop the spread of illnesses and disorders. The practice of using live, dead creatures for immunization has been replaced by a contender that is far safer: synthetics and recombinants. Such novel vaccine candidates require a designed adjuvant that improves immunogenicity because they are frequently weakly immunogenic and susceptible to deterioration. The development of nanotechnology has led to a number of innovative antigen-carrying techniques because conventional adjuvants are not adaptable. Such adjuvants based on nanoparticles can be created for a comfortable route of administration and a lower dose frequency to produce a specific target immune response, such as the intranasal route to enhance the target mucosal immunity. This makes them especially well-suited for veterinary care, when handling numerous animals at once is necessary or where standard immunization methods are unfeasible due to complex management systems or limited accessibility.
- **Nano-pharmaceutics-** In contrast to other areas of veterinary medicine, pharmacology and nano pharmaceuticals are at the forefront of what nanotechnology can develop. Reiterating that nanotechnology allows for the manufacturing of new pharmaceuticals and the possibility of reworking conventional compounds to obtain better results in efficacy is crucial when taking the field of pharmacology into account. In comparison to the free product equivalents, the pharmacokinetics and therapeutic indices of the drugs can be significantly improved by attaching them to nanoparticles via physical encapsulation, adsorption, or chemical conjugation. To treat bacterial intracellular infections, germs endocytose host cells and subsequently release medication payloads. Treatment for intracellular infections brought on by bacteria involves the use of drug-charged nanoparticles that enter host cells by endocytosis and subsequently release therapeutic payloads.

The use of nanoparticles for medication administration has various advantages, including improving the therapeutic effectiveness and pharmacological characteristics of the medicine. The efficiency of nanoparticles in increasing pharmacokinetics, minimizing undesirable side effects, and optimizing transfer to disease locations has been demonstrated by several nano-drug delivery



methods. As the active ingredient is concentrated just in the morbid area during this technique, the required amount of the medicine is employed, and side effects are significantly reduced. Costs and agony for patients will be decreased by this highly targeted strategy. In turn, the use of nanoparticulate drug carriers will solve a number of significant drug delivery issues, such as improving drug solubility and safety, lengthening drug half-lives in blood, minimizing side effects in organs other than the target, and concentrating drugs at the site of the disease. A drug may be dispersed in a hydrophobic nucleus, encased in a vesicle, disseminated across a gel, or attached to a nanoparticle sheet. Liposomes, polymeric nanoparticles, dendrimers, ceramic-containing capsules, micelles, and other nanoparticle-based drug delivery systems have all been employed to transport therapeutic agents for small molecules, peptides, and oligonucleotides.

Liposomes, which are spherical vesicles made of phospholipids and steroids, bilayers, or specific surfactants that shape spontaneously as other lipids are distributed in aqueous conditions where liposomes may form, were the first to be examined as drug carriers. Drug solubility and pharmacokinetic features, such as the therapeutic index of chemotherapeutic medications, faster synthesis and a decrease in negative side effects, as well as an improvement in in-vitro and in-vivo anticancer behaviour, have all been found to be improved by liposomes. During the encapsulation procedure, a medication is embedded in liposomes. Drug release from liposomes is influenced by the liposome's structure, the pH, the osmotic gradient, and the environment around it. It is possible to comprehend lipid connections with cells through adsorption, fusion, endocytosis, and transition of lipids. There are numerous drug sources for liposomal versions of drugs such as anticancer drugs, neurotransmitters, antibiotics, and anti-inflammatory drugs.

Dendrimers have a broad range of application in drug delivery due to the molecular variety's potential therapeutic value. Dendrimers have a number of adaptable divisions with voids where drug molecules can get physically trapped. Fantastic encapsulation results from this small design. Their structure had a significant impact on nanotechnology by supplying well-controlled useful building blocks. They serve many purposes, including the dispensing of medications and the treatment of cancer. Dendrimers are an effective drug delivery system because they deliver a drug at a precise rate through chemical modulation, either by adjusting the hydrolytic release conditions or by selectively releasing drug molecules based on their shape or size. High-load dendrimers have shown quick pharmacological effects and improved efficacy.

Alternatively, stronger and more stable carbon nanotubes can also be used as product transporters. Cell specificities can be achieved by fluorescent or radioactively labelling antibodies that bind to carbon nanotubes. Nanotubes can enter cells either by cell membrane penetration or



endocytosis. Carbon nanotubes can be made more soluble and used to carry drugs and other compounds by including carboxylic or ammonium groups into their arrangement. Cancer cell death with indium-111 carbon nanotube-labeled radionuclides is being researched.

- **Animal breeding and reproduction-** Reproduction and reproductive health have seen a recent boom in nanotechnology. These animal reproduction studies using nanotechnology have the following objectives: characterizing the nanoscale properties of gamete cells using atomic force microscopy and related scanning microscopy techniques; developing nano-bio sensors for physiological or altered detection of the reproductive status; developing chemical methods for the production of metal nanoparticles for fertility control applications; and developing nanodevices for secure cryopreservation of gametes and embryos. For dairy producers and pig farmers, managing the breeding process can be expensive and time-consuming. A nanotube implanted under the skin is one method now being tested to allow real-time monitoring of changes in blood estradiol levels. Because the nanotubes can bind and detect the estradiol antibody by near-infrared fluorescence during the time of estrus, they are utilized to track estrus in animals. A central breeding monitoring and control system will incorporate the signal from this sensor to actuate it. Additionally, nanotechnology tools like microfluidics, nanoparticles, and bioanalytic nano-sensors can help to find answers to other conundrums regarding animal health, development, and disease prevention and treatment. Modern techniques for enhancing traditional in-vitro fertilization operations and the growth of in vitro embryos include microfluidic and nanofluidic. Recent studies have demonstrated how effective microfluidics is at insulating motile sperm without the need for centrifugation.
- **Disease diagnostics-** In veterinary medicine, a disease's diagnosis may take a few days, a few weeks, or even several months, especially in the case of chronic disorders without any outward signs of illness. As a result, the need to wipe off the entire herd may have arisen at that point due to an infection. Nanotechnology has the potential to be identified and eliminated extremely early since it functions on the same scale as a virus or disease-infecting particle. As a result, nanotechnology can be an effective tool for delicate clinical diagnostics. The employment of nanotechnology tools for the investigation of animal diseases or as animal models for the diagnosis of human diseases is exceptional, according to one school of thinking on health. Recent studies suggest using quantum dots for in vivo imaging in tiny animal models. Individual Photon Emission Positron Emission Tomography (PET) and Computed Tomography (SPECT) are nuclear medicine imaging modalities that offer metabolic and functional information as opposed to CT and MRI, which only provide anatomical information. The combination of SPECT and PET with CT and MRI, however, offers comprehensive anatomical and metabolic data. Using non-invasive, targeted molecular imaging

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modalities that provide anatomical and physiological in-vivo information to perform functional molecular alterations in-vivo in order to control disease progression before it manifests with conventional morphological imaging techniques or laboratory tests. These molecular imaging techniques are provided by nuclear medicine, which monitors the body's absorption of radiopharmaceutical substances (gamma and positron emitters) that can be seen by SPECT or PET scanners.

- **Cancer treatment and diagnosis-** Cancer is a prevalent disease that has been the subject of much investigation. Because traditional chemotherapeutic agents do not target tumour cells specifically, people who receive them often experience toxicity problems. The objective is to develop a mechanism that can use medicines to destroy cancer cells while protecting healthy ones in order to find a solution to the issue. According to some, nanotechnology is a cutting-edge, intelligent technology that develops tools with the ability to distribute medications to various parts of the body. Submicron nanoparticles constructed of various materials or electronics are one example of such systems. Due to their unique ability to have a high surface-to-volume ratio, nanoparticles can attract various functional groups, which can then bind to specific tumour cells. Nanoparticles' modest size (10–100 nm) enables them to be gathered preferentially at tumour locations because tumours don't have a sufficient lymphatic drainage system. It is possible to create multifunctional nanoparticles that can recognize, diagnose, and then treat a tumour as a prospective cancer therapy. Cancer imaging is crucial for determining treatment strategies and gauging the efficacy of suggested regimens. Traditional imaging modalities like MRI and ultrasound may now better identify cancer, thanks to the use of nanoparticles for picture comparison and enhancement. New methods like optical-based cancer detection imaging have also been made possible. The medication's ability to target and kill cancer cells while leaving healthy cells unharmed is specifically linked to the treatment's efficacy. Thus, one of the most important properties of innovative anticancer medicines would be their high level of selectivity for cancer cells. In this area, combining nanotechnology and medicine offers a viable way to enhance cancer treatment.

## Conclusion

In summary, nanotechnology allows us to engage at the nuclear and molecular levels to investigate, manage, and apply nanometer-dimensional space. It is an exciting and rapidly developing area of engineering. It has made new potential uses in molecular biology and biotechnology possible. Almost all of the veterinary and animal scientific fields have seen a revolution thanks to nanotechnology, specifically in industrialised nations. This is because it can provide detailed information and reveal what is happening inside an organism's deeper tissues.

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Quantum dots, magnetic nanoparticles, nanopores, polymeric nanoparticles, nanoshells, fullerenes, liposomes, and dendrimers are only a few examples of the various nanoparticles that are employed in the diagnosis, treatment, drug administration, animal breeding, and reproduction processes. Compared to other sister disciplines, nanotechnology is one of the major achievements now used in a variety of fields, but it has only recently been applied to veterinary science. In addition, the technology's complexity and expensive cost made it difficult for underdeveloped nations, in particular, to utilize it in the field of animal science.

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## Role of Feed Enzymes in Livestock Production

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### *Abstract*

Nowadays, the demand for animal-based products has been increasing at a rapid rate which requires newer strategies for improving animal productivity. The main problem with the livestock sector in the developing countries is high feed cost and low available resources to meet their nutritional requirement. With the advancement in research, feed enzymes have been proven to improve the livestock feed efficiency. All the animals in nature require enzymes to digest the feed which are produced by animals themselves or by the gut microflora. However, the digestive system of animals may not be effective totally in utilizing the nutrients in the diet. In the animal production, the biggest portion of investment is in the feed. If the animal doesn't utilize the feed efficiently then production suffer huge losses. Therefore, supplementation of the specific enzymes improves the feed ingredient value thereby leading to improved feed utilization.

### **Introduction**

The supplementation of feed enzymes along with the animal diet has been in practice since late 1980s. The inclusion of these enzymes in the feed formulation have been predominant in poultry and pig. In the recent years the use of these enzymes in ruminant nutrition and aquaculture have been gaining potential interest. The dependency on this enzyme supplementation has been increased where the enzyme activity also plays role in breakdown of antinutritional components of the feed in addition to improvement in nutrient utilization. In order to be beneficial to the animal, the feed enzymes must target specific feed components which are either harmful or of little use or no use. This leads to wider utilization of ingredients required to improve the animal performance.

### **Mode of Action**

Enzymes used in the supplementation exhibit diverse modes of action. Recognized as feed

additives, several studies have been done to understand the mechanism of action of feed enzymes (Bedford and Partridge, 2011; Ravindran, 2013). The exogenous enzymes can break the bonds of those feed components which are not hydrolyzed by endogenous enzymes. This breakdown of the bonds helps in degrading of the unfavorable factors of feed which are responsible for increased gut viscosity and reduced digestion, thus achieving better nutrient utilization. They can interfere with endosperm integrity and also help in the release of nutrients bound to the cell wall. For example,  $\beta$ -glucanase can disrupt the endosperm wall of barley and cellulase and pentosanes can cause release of protein from the aleurone layer. Low digesta viscosity can be maintained by enzymes by reducing chain length of polysaccharides to improve nutrient absorption. Feed enzymes can manipulate the number of substrates present in the gastrointestinal tract to cause a change in bacterial population in the gut to support the increase in the beneficial bacteria and decrease in the harmful bacteria (Bedford and Cowieson, 2012).

### **Types of Feed Enzymes used in livestock**

#### **Carbohydrases**

Enzymes are divided based on the substrate on which they act on. Carbohydrases are enzymes which break carbohydrates into simpler sugars. They are divided into those that target non starch polysaccharides (fibre degrading enzymes) and starch degrading enzymes.

#### **Fibre degrading enzymes**

Fibre is made up of complex carbohydrates in plant cell walls. Nutrients such as starch and proteins are trapped in insoluble fibrous cell walls. Pigs and poultry lack enzymes to digest this fibre and therefore cannot utilize the nutrients. Soluble fibre forms viscous gels in digestive tract, trapping nutrients and slowing down the passage of feed in gut. Xylanase and  $\beta$ -glucanase are main fibre-degrading enzymes used in animal feed. Other fibre degrading enzymes currently used in animal nutrition, but to a lesser extent, include B-mannanase, pectinase and  $\alpha$ -galactosidase. The addition of enzymes in poultry feed have been linked to decreased digesta viscosity, decrease in the quantity of dirty eggs and improved yolk color.

#### **Starch-degrading enzymes**

Amylases causes breakdown of starch in grains, grain by-products and vegetable proteins. In pigs and poultry, starch digestion leads to more energy extraction in feed which is utilised in meat and egg production. In diets of piglets, amylases supplementation compensates for less amylase secretion due to low feed intake.



## Proteases

Proteases are the protein-digesting enzymes used in pig and poultry nutrition to break down storage proteins. Storage proteins can bind to starch. Proteases can help break down storage proteins, releasing bound energy-rich starch that can then be digested by the animal. Proteinaceous anti-nutrients such as trypsin inhibitors and lectins inhibit digestion by blocking the enzyme trypsin and reducing digestibility respectively. Proteases can be used to reduce the levels of trypsin inhibitors and lectins, thus improving protein digestibility.

## Phytases

Phosphorus is an important element for bone development and metabolic processes in pigs and poultry. In the plants, phytate forms complexes with minerals (such as phosphorus and calcium), proteins and starch, making them unavailable for absorption. Pigs and poultry do not produce the phytase enzyme that breaks down phytate. Phytate supplementation releases phytate bound minerals which can then be digested and absorbed by the animal to improve the efficiency of meat and egg production.

Feed enzymes are most widely adapted by broiler industry. As digestive system is simple in poultry the specific feed enzymes are useful in overcoming the defects in digestion. Broilers are sensitive to various antinutritional factors and show a more favorable response than layers.  $\beta$ -glucanase supplementation reduces sticky droppings in poultry which is helpful especially in layers where less dirty eggs can be obtained. Multienzyme supplementation is more effective in broilers. The young piglets rely mostly on enzymatic digestion for the utilization of nutrients. Hence supplementation of feed enzymes is necessary for digestion of high fibre cereals and protein meals thus improving the digestion in piglets (Prokopieva *et al.*, 2020). Plant feed sources are cheaper than the traditional feed meal in aquaculture species. Therefore, feed enzymes can play effective role in increasing utilization of plant protein sources. Phytase supplementation in fishes may decrease the inorganic phosphorus requirement in fish diets. Enzyme supplementation in ruminants is complex. However recent studies have shown that exogenous supplementation of fibrolytic enzymes helps in improving fibre digestibility and helps in efficient feed utilization in ruminants (Arriola *et al.*, 2017).

## Conclusion

The supplementation of enzymes in feed helps in efficient nutrient utilization and aids in the better growth and performance of the animals. However, effective feed strategies are required for efficient use of feed enzyme supplementation. The feed enzyme supplementation in the future can be improved by refining enzyme formulations measuring various specific enzyme activities, antinutritional compounds affecting the growth of the animal. Though there have been various



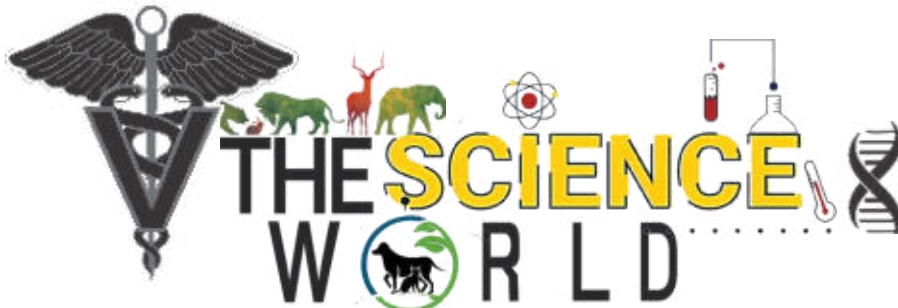


studies in the poultry and piglets regarding the improved nutrient utilization by addition of specific enzymes in diet, still research is required in the ruminants as well as aquaculture species. Feed enzyme is a promising field in the future research aspects where the enzymes can be modified to suit the needs of the animal based on their gastrointestinal tract environment. Hence improved nutrient utilization and growth performance can be observed.

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Popular Article

## Biofortification: A way to combat hidden hunger

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### Introduction

Food is a basic necessity of all living creatures and is the source of vital energy. Food has a great impact on the health of individuals and thereby building a healthy society. Availability of quality food is equally important along with quantity. The food crop has undergone significant improvements in productivity and production over the last few decades, under the moniker "Green Revolution". As a result, we are in a reasonably secure position to satisfy the demand for food. However, the major focus was emphasized on production and productivity to hasten the food supply rather than giving importance to its nutritional status. The micronutrient content of staple food crops is not at its optimal level, and processing further decreases this concentration. Regular consumption of calorie-rich food with low nutrient status will cause an invisible deficiency of nutrients in the body called hidden hunger. There are various methods to combat this problem. In this chapter, an overview of strategies to overcome hidden hunger is explained with a major emphasis on biofortification.

### Balanced diet

One of the natural, healthy, and sustainable ways to overcome nutrition deficiency is a balanced diet. A balanced diet contains an adequate amount of all the nutrients required by the body to grow, remain healthy and be disease-free. In addition, a healthy, balanced diet provides the necessary energy requirement, protects against vitamin, mineral, and other nutritional deficiencies, and builds up immunity. Even though it is one of the best methods of a healthy diet, the availability of various kinds of food sources is seasonal and location-specific. People living in the poverty-stricken area cannot afford healthy food and often depends on a few staple foods crop available in

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the locality. Hence, enhancing the nutrient content of staple food crops has become the need of the day.

### **Food supplements**

Food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect that are marketed in the form of pills, capsules, syrups, etc. They are mainly intended to correct some deficiencies in individuals for quick resort. Supplementation of iron, calcium, and vitamin supplements is commonly recommended for people whose daily diet has an inadequate quantity of these vitamins and minerals. Governments are running such supplementation programs for children and women for enhancing their health. However, this is purely an artificial method and requires government and institutional effort to supply doses to the people. Implementation and acceptance of this program are scanty in reality.

### **Food fortification**

Fortification is the practice of deliberately increasing the content of one or more micronutrients (i.e., vitamins and minerals) in food or condiment to improve the nutritional quality of the food supply and provide a public health benefit. Food fortification is done to prevention, reduction, and control of micronutrient deficiencies. It can be used to correct a demonstrated micronutrient deficiency in the general population (mass or large-scale fortification) or in specific population groups (targeted fortification) such as children, and pregnant women. Some of the compulsory fortification programs are done by the government such as table salt fortification with iodine, flour fortification in industrial mills, etc. even though it is a successful program but fortified elements will not be an integral part of the food. Hence, biofortification came as an alternative, sustainable, easy, and natural way of enhancing food quality by biological means.

### **Biofortification**

The term 'biofortification' was coined in the year 2001 by Steve Beebe. Enhancing the nutrition status of crop plants following biological or natural ways is called biofortification. It can be considered as a subclass of food fortification utilizing biological processes. Biofortification has the advantage of sustainable increment in the food's nutritional status and nutrients will be an integral part of tissues enhancing the bioavailability. Bio-fortification is the process of increasing vitamin and mineral density in a crop through conventional plant breeding, transgenic approach, or agronomic practices. The regular consumption of staple bio-fortified crops produces measurable improvement in human nutrition and health. Among the different micronutrients main targets of biofortification are iron, zinc, selenium, vitamins, and some important amino acids like thymine. Biofortification can be done through agronomic, genetic, or transgenic means.

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### **Agronomic biofortification**

sustainable agriculture, natural or organic farming with low-yielding landraces can maintain soil health. However, to meet food demand, large-scale cultivation of high-yielding varieties and monoculture lead to the exploitation of the available nutrient status of the soil. It has become necessary to feed crops before they feed us. The application of nutrients in the form of fertilizer or foliar spray to enhance the nutrient content of food grains is called agronomic bio-fortification. For example, enhanced zinc and iron content in wheat or rice can be achieved by either foliar spray or soil application of zinc and iron fertilizers. Agronomic biofortification (fertilizers) for selenium has been made mandatory in Finland to ensure sufficient selenium content in crops. Even though it is an easy method of biofortification, educating farmers and mandatory application of fertilizer with micronutrients is taken care of.

### **Genetic biofortification**

Using different breeding methods, the nutrient levels of staple crops can be increased to the target level without altering the agronomic traits and compromising yield levels. Available variations for nutrient content in the germplasm lines, pre-breeding lines, wild relatives, and landraces can be used for enhancing the nutrient status of food crops. Breeding targets for each micronutrient are based on the consumption pattern of the target population, nutrient bioavailability, and losses during processing and storage. Conventional breeding approaches following hybridization, segregation, and screening are one of the most commonly followed approaches to develop lines with higher nutrient content. However, the influence of the environment is very high in the expression of micronutrient content in plants, especially, soil nutrition status, irrigation, and abiotic stress. Advanced breeding tools such as marker-assisted selection, QTL mapping, and genome-wide association study were followed.

Some examples of, varieties with enhanced Zn, Fe, and selenium are available in staple crops like wheat, rice, maize, pearl millet, etc. WB2 and BHU1 in wheat, HHB229 and AHB 1200 in maize, Jalmagna in rice, Dhanshakti in pearl millet, Parbhani Shakti in sorghum, IPL220 in lentils are some examples of biofortified varieties for micronutrient released in India. Other important components of food such as vitamins and anti-oxidant contents were also enhanced by breeding approaches.  $\beta$  Carotene and anthocyanin-rich varieties in wheat, sweet potato, and cauliflower were developed. Quality protein maize (QPM) with high lysine, tryptophan, and pro-vitamin A content are also targets of breeders to tackle deficiency of these amino acids in corn. The presence of anti-nutritional factors in the food crop decreases the bioavailability of nutrition and has a major impact on health. Hence, reduced erucic acid and glucosinolate in the mustered lead to the development of



single zero and double zero varieties. Genetic bio-fortification is one of the safe and economical ways of enhancing the food value of crop plants.

### **Transgenic biofortification**

Along with the genetic approaches the transgenic approach has also been used to increase the mineral accumulation ability of crops mainly of iron and zinc through increased uptake from the soil, and reduction in the anti-nutritional factors content like phytic acid. Increased provitamin A or  $\beta$  Carotene was achieved by transgenic technology in rice by inserting the genes phytoene synthase (PSY), phytoene desaturase (CrtI), and lycopene  $\beta$ -cyclase ( $\beta$ -lcy) to produce yellow-colored rice called Golden Rice. This technology was also extended to wheat, maize, canola, and cassava to enhance their provitamin content. Transgenic for biofortified Zn in rice has been developed using HvNAS1, phytase, and OsNAS1 genes. The transgenic soybean varieties rich in oleic acid and linoleic acid were developed through transgenic approaches. Transgenic technology can be efficiently utilized to develop biofortified varieties of crop crossing reproductive barriers. However, government regulation and social acceptance is the real question for transgenics.

### **Conclusion**

The consistent effort of governments and other international institutes brought down micronutrient deficiency compared to earlier decades. Food fortification, food supplementation, and biofortification played a crucial role in making this happen. However, micronutrient deficiencies are still a key public health concern, especially in underdeveloped and developing countries. In the coming future biofortification should be more intensively tried for meeting the global sustainable development of human health. Different biofortification strategies need to be implemented in a need-based manner to achieve a nutritious food supply. Biofortified crops are aimed to complement the existing micronutrient deficits and in turn, they should have a visible impact on the health of millions of people suffering from hidden hunger. The use of the biofortification strategy for the improvement of micronutrient contents of staple crops across the world needs strong support and consistent efforts.

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## Uterine Infections – Diagnostic Procedures and Therapeutic Protocols

M. Rajashri, Satish Martha, E. Sunil Anand Kumar

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Uterine dysfunction can have a major impact on the profitability of a dairy operation. Bacterial endometritis is considered to be the most common cause of repeat breeding in bovines. The most commonly involved organisms are Escherichia coli, Staphylococcus, Streptococcus, Corynebacterium, Bacillus, Pseudomonas, Micrococcus and Klebsiella.

### Diagnosis

Clinical endometritis is usually diagnosed by evaluation of uterine discharge detected in the vagina with the aid of

- **Speculum** - should be introduced into the vagina up to the level of the external os of the cervix, and inspection of the discharge is performed with the aid of a flash light
- **Metrichcek tool** - device should be introduced into the vagina up to the level of the external os of the cervix and the discharge should be scooped for evaluation after exteriorization of the device. The mucus score by Vaginoscopy or Metrichcek device is as follows:

Score 0: clear or translucent mucus.

Score 1: mucus containing flakes of white or off-white pus.

Score 2: discharge containing  $\leq 50\%$  white or off-white mucopurulent material.

Score 3: discharge containing  $\geq 50\%$  purulent material, usually white or yellow, but occasionally sanguineous.





### **Metricheck for identifying cows with metritis**

- Detection of fluid in the uterus using ultrasonography.
- Using an electronic nose (DiagNose) - Electronic sensor devices detect vaginal discharge odour substances indicating estrus or pathological infection in cattle

For Subclinical endometritis - Uterine cytology samples can be collected using the cytobrush (At the uterine body, the cytobrush is exposed and the body wall is pressed slightly against the cytobrush while the cytobrush is rolled two or three times) or the low-volume uterine lavage (foley's catheter can be used to perform a low volume lavage) technique



**Cytology tool with cytobrush attached**



White side test is simple and easy test for ruling out the subclinical genital endometritis.

### Procedure

One ml of the cervical mucus was mixed with one ml of 5 per cent NaOH solution in a test tube and heated up to the boiling point and subsequently cooled in running tap water. The appearance of yellow colour was taken as positive indication of infection. Depending on the intensity of colour development the degree of endometritis was classified as 1 – No colour (absence of infection), 2 – mild yellow colour change (mild infection), 3 – intense yellow colour (severe infection).

### Treatment

**Immunomodulators** used in the treatment of endometritis are as follows:

- a. E. coli lipopolysaccharide - 100µg in 60ml PBS
- b. Oyster glycogen -500mg in 60ml PBS
- c. Bacteria-free filtrate
- d. Serum, plasma, or hyperimmune serum
- e. Levamisole - administration of a single dose of levamisole @ 2.5 mg/ kg.bw results in immunomodulatory effect for about 48 h
- f. Leukotriene B4
- g. Granulocyte-macrophage colony-stimulating factor
- h. Human recombinant interleukin-8

### Ozone therapy

Intra-uterine ozone treatment alone (for 10 sec) or combined with parenteral antibiotics

**Hormonal therapy** – prostaglandin therapy – 2 doses of PGF2alpha I/M 8 hrs apart on day 8 of cow suffering with acute puerperal metritis increased first service conception rates by 17% in primiparous cows.

### Miscellaneous

Intrauterine infusion of methanol fraction of neem oil and neem seed powder in treatment of endometritis in cows - higher pregnancy rate (71.42%) was obtained in neem oil fraction–treated cows than control cows (25%) with significant decrease in bacterial load in animal treated with neem–oil (96.02%) and seed–powder fraction (98.70%) compared to control (24.97%).





## Factors Affecting Conception Rates in Dairy Cattle

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Management and environmental factors account for 96% of the variation in conception rates. Herd differences in nutrition, metabolic disorders, reproductive health, heat detection, insemination practices and climate can result in significant differences in conception rates. The remaining 4% of variation in conception rates is due to genetic factors with 3% for the cow and 1% for the service bull.

### Factors affecting conception rates

#### 1. Effect of season:

Monsoon is the best season for highest conception rate of cows having suitable ambient temperature and humidity.

#### 2. Effect of AI sequence number

Sequence of A.I. had significant effect on conception rate it was observed that highest conception rate was recorded in first and second attempt (43.23%) & (43.45%) and after that it declines to (39.67%) & (32.34%) in third and fourth attempt.

#### 3. Effect of body condition

The CR is lower (36%) when cows are inseminated at BCS of 1.0 –2.0 than at 3.5–5.0 (64%)

#### 4. Effect of Bulls on fertility

The effect of bulls on fertility has long been known and CR can vary from 14.3% to 80%. Other factors such as breed of bull, type of semen and semen attributes have significant effect on CR.



### 5. Skill of A.I technician

Magnitude of CR varies by each technician and depends on his degree of skill and experience in the AI technique

### 6. Effect of degree of uterine tone

CR is significantly higher in cows with marked uterine tone, marked vulva swelling and cows with good body condition

### 7. Effect of Parity

Authors reported that CR tended to increase with increased parity number whereas other researchers reported that CR is significantly higher in cows of first than of fourth parity

### 8. Effect of milking and suckling

Lower conception rates were due to draining out of nutrients, thus causing mobilization of body reserves, which could result in negative energy balance and a drop in body condition which will have a negative influence on postpartum reproductive performance resulting in a prolonged calving to conception interval and a reduced CR.

### Treatment:

#### ❖ Hormonal therapy

##### 1. Hormonal Treatment with Bovine Somatotropin (bST) to Enhance Fertility

Bovine somatotropin (bST) increases plasma concentrations of insulin, IGF-I, and growth hormone and by stimulating ovarian function especially after IGF-1 plasma levels are reduced.

##### 2. Treatment with GnRH - One strategy tested for increasing pregnancy rate is to inject GnRH or GnRH analogues at day 11-14 after estrus to increase progesterone secretion and delay luteolysis, thereby increasing the chance for an embryo to initiate its own antiluteolytic mechanism

❖ Nutritional Management: The condition of negative energy balance is similar to under nutrition because it inhibits or reduces pulsatile luteinizing hormone production

#### ➤ Fat feeding to improve energy balance

- Linoleic acid supplemented in the diet prepartum can stimulate arachidonic acid synthesis and lead to higher concentrations of the series 2 prostaglandins.
- Supplementation of the diet with fish meal - Using fish meal to replace soybean meal as a source of protein enhances higher pregnancy and conception rates by reducing PGF<sub>2</sub> $\alpha$  endometrial secretion and aids in establishment of pregnancy rates.



❖ **Administration of antioxidants**

- Treatment of cows with vitamin E and selenium can increase the rate of uterine involution in cows with metritis and improve fertilization rates in ewes and cows
- Injection of vitamin A, a metabolite of  $\beta$ -carotene - Cows fed diets deficient in  $\beta$ -carotene had lower amounts of progesterone in the CL

❖ **Managerial aspects:**

➤ **Environmental temperature:**

- CR is significantly reduced when temperature exceeds 23°C the day after insemination which could heat the embryos and resulted in early embryonic death during cleavage stages. Optimum environmental temperature the day after insemination for conception ranges between 10°C and 23°C.

➤ **Time of A.I in a day:**

- The relatively higher CR in AI carried out before noon indicated a more conducive environment for conception to take place due to its cooler surrounding temperature. Higher CR (78.2%) was achieved in a study when AI is carried out before noon than in the after noon (56.6%).

➤ **Site of insemination**

- Increase in CR when semen is deposited in the uterus than in the cervix. More live spermatozoa can reach the fertilizing site in a shorter time and distance when semen is deposited in the uterus, hence, increases the chances to conceive

- **Thawing** - frozen semen should be thawed in warm water before inseminating into the uterus and this practice improves the CR compared to thawing semen in cold water

➤ **Time of A.I after onset of oestrus:**

- Inseminating cow at 6 h interval from the time heat was detected, was not appropriate. This interval was too short in relation to the time of ovulation. It was suggested that cows should be bred 12–18 h after detection of heat to get higher CR

➤ **Oestrus detection:**

- Close observation for signs of oestrus could result in cows bred at proper times, thus, ensuring better chances for conception.



- **Training of the A.I technicians**
- **“IVETSCOPE”** - first mobile endoscopic unit for everyday veterinary and farm animal use.  
–applicable in Artificial Insemination to visualize the site of semen deposition, optimizes Embryo transfer and for Internal examinations of Vagina. It is a Unique AI Gun Feed through system for training Artificial Inseminators



**IVETSCOPE INSTRUMENT**



## *Hemonchus contortus*: A serious concern in small ruminants

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### *Abstract*

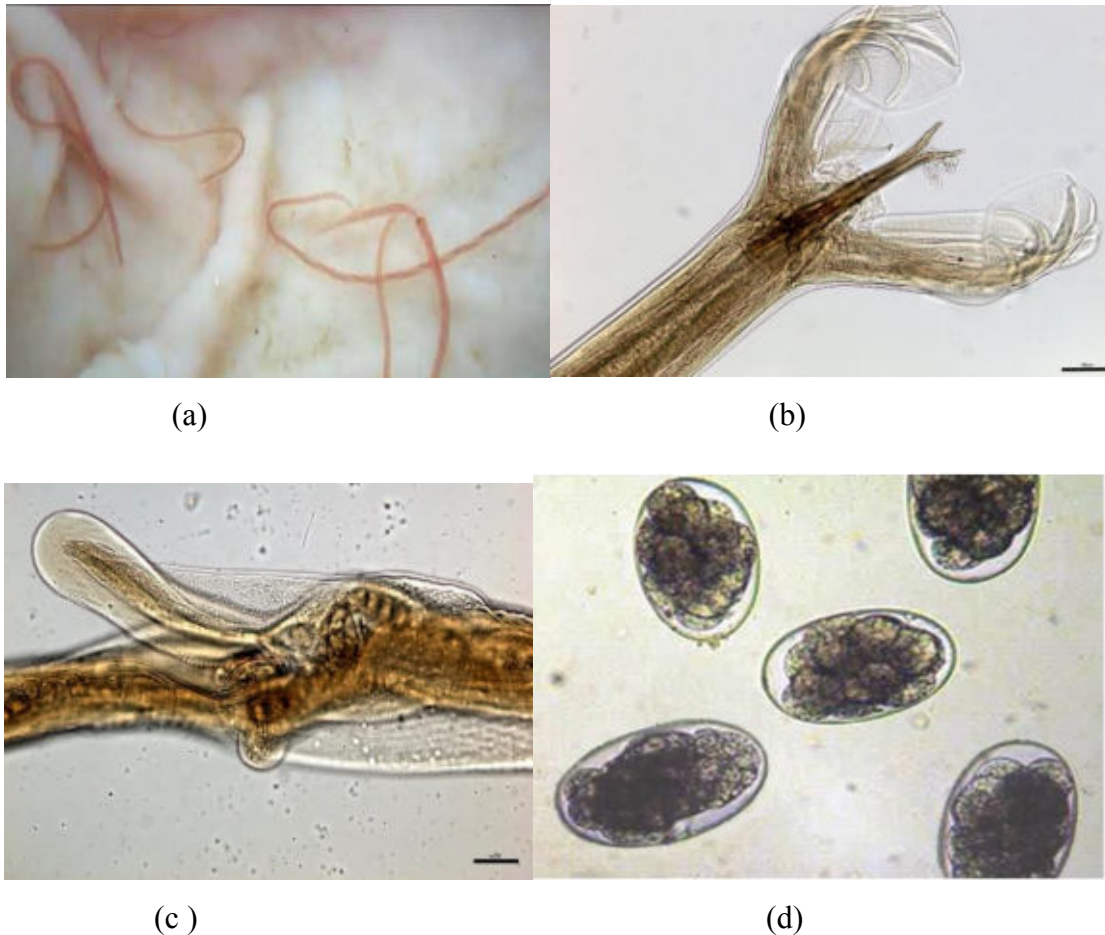
Haemonchosis, caused by the parasitic nematode *Haemonchus contortus*, is a common occurrence in small ruminants. It poses a significant threat to the health and productivity of sheep and goats in tropical and warm temperate zones. The occurrence of hypobiosis (inhibition of the fourth-stage larvae within the host) explains the sporadic outbreaks of haemonchosis in arid and colder environments. The wide climatic distribution of the parasite may also be attributed to the adaptation of local isolates to less favorable ecological conditions. The primary indicators of the disease are related to the nematode's blood-feeding behavior, resulting in anemia, hypoproteinemia, weakness, edema (bottle jaw) and frequent deaths unless treated. While the risk of haemonchosis varies significantly at the local level, even in endemic areas, extensive ecological investigations provide a solid foundation for predicting the relative geographical and seasonal risk based on climatic conditions (Arsenopouloset *al.* 2021).

### **Introduction**

Hemonchosis is a significant and common parasitic infection in Sheep and Goat, prevalent in tropical, subtropical, warm and summer rainfall areas than cooler areas. *Haemonchus contortus*, commonly called as wireworm or stomach worm is a nematode parasite of family Haemonchidae. It is highly pathogenic parasite that localizes in abomasum of affected animals and pathogenicity is mainly due to blood sucking behavior of parasite which adversely affect health and productivity of animals. Diagnosing the disease is difficult as the field evidence can provide suspicion about the infection, but needs to be subsequently confirmed by laboratory tests through parasitological or molecular techniques. Antihelminthic resistance to various drugs like benzimidazoles, imidazothiazole and macrocyclic lactones makes the infection difficult to control.

## Morphology

Males are even reddish in colour while in the female the white ovaries are spirally wound around the red intestine giving the appearance of barber pole. The cervical papillae are prominent and spine like. Buccal cavity contains a dorsal lancet. Male bursa has elongated lateral lobes supported by long slender rays. The small dorsal lobe is asymmetrically situated against the left lateral lobe and supported by Y shaped dorsal ray (fig 1b). The spicules have small barb near its extremity. The Vulva of female has linguiform process (vulval flap, fig 1c). Egg is yellowish in colour and in early stages of cleavage contain 16-32 cells (fig 1d).



**Fig. 1** a- brown worms in abomasum, b- hind end of male showing spicules, c- vulval flap of female, d- eggs of *Haemonchus contortus*

## Pathogenesis

It usually occurs in three stages

**I stage:** seen during 7-25 days of post infection, the packed cell volume (PCV) of the blood decreases but the serum iron concentration remains normal, this is mainly due to time lag between blood loss and compensatory system of body.



**II stage:** seen from 6-14 weeks post infection, PCV is maintained but lower than the normal rate. Infected sheep compensate by threefold increase in erythrocyte production.

**III stage:** characterized by rapid drop in PCV is due to dyshaemopoiesis due to iron deficiency.

### Clinical signs

Disease occurs in three forms

**Hyperacute form:** uncommon form, mostly occurs due to sudden massive invasion, characterized by anemia, passing of dark colored faces and sudden death.

**Acute form:** most commonly seen in young animals, characterized by anemia, hypoproteinemia, oedema, weakness and finally leads to death. Faecal egg counts of 1,00,000 egg per gram(epg) is observed during faecal examination.

**Chronic form:** This is most extreme form of disease and is economically important, here morbidity is 100 % but mortality is low. Animal shows symptoms like weak, unthriftiness, emaciated, anaemia, hypoproteinaemia it may or may not be severe. Faecal egg counts of 2000 eggs are observed during faecal examination.

### Post mortem findings

Pale mucous membrane and skin, blood is watery appearance, internal organs pale in colour, hydrothorax, pericarditis, ascites, extreme cachexia, fat is replaced by gelatinous tissue. Liver is light brown in colour, fragile, fatty changes occur in it, abomasum has reddish brown fluid ingesta and large number of worms present freely moving in warm carcass, abomasal mucosa swollen and bite marks are present.

### Diagnosis

1. Clinical signs – history, examination of body by FAMACHA score, faecal score
2. Faecal egg count and identification- McMaster method, lectin staining, automated egg examination, FLOTAC
3. Larval culture, egg hatch test
4. Immunological detection – ELISA, CFT, indirect immunofluorescence, indirect haemagglutination
5. Visible near infrared spectroscopy- for detection of haemoglobin in sheep faeces
6. PCR, droplet digital PCR – done by DNA extraction from egg in faeces
7. Post mortem examination

### Treatment

Periodical deworming of flock with anthelmintics but the disadvantage with this procedure



is costly, less immune response and development of resistance. Targeted flock and target selective treatment in which only selected animals are treated leaving remaining but these two are labour intensive. Broad spectrum substances like albendazole and macro-cyclic lactones have been known to create resistant parasite strains. the combined use of more than one anthelmintic has been found adequately effective against resistant parasites.

### Control

Due to constantly increasing drug resistance in *H. contortus* and residual effects, the control strategies which employ minimal use of synthetic anthelmintics have gained importance in the sheep industry. The experts have recommended an integrated control mechanism encompassing various approaches instead of relying on a single option to achieve enough control. Management fields like the selection of resistant lines of sheep, adoption of grazing techniques, and vaccines like barberivax, biological control using *B. thuringiensis* act against larvae by releasing toxins (Naeem *et al.*,2021).

### Conclusion

Haemonchosis is a serious infection in small ruminants needed to be a great concern as it decreases productivity of animals and sometimes may leads to death. So effective control measures needed to be taken to control this infection

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# Nanotechnology in Aquaculture

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## Introduction

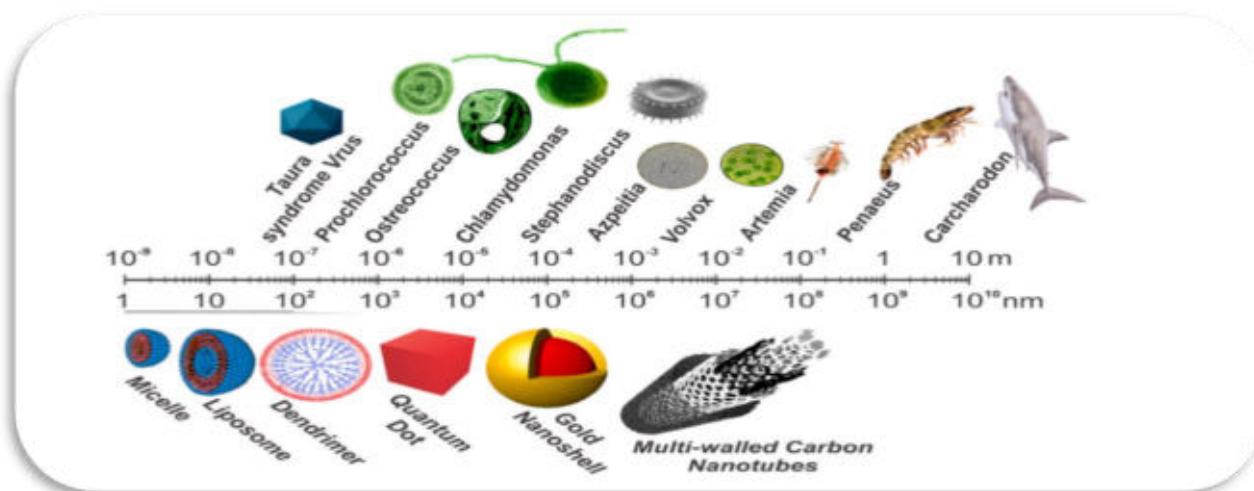
Nano a “*prefix*” derived From Greek word Nano meaning “*Dwarf*”. Which means Extremely small

## What is Technology?

Technology is a branch of Knowledge that deals with creation in the fields of:

- Engineering
- Applied Science
- Pure Science
- Industrial Arts...etc.

Father of Nanotechnology – *Richard Feynman*



Relative size of Nanoparticles compared to different aquatic organisms



The United States National Nanotechnology Initiative (NNI) defined nanotechnology as the “understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications”

- Nanotechnology has enormous potential to provide innovative improvements to aquaculture systems in farm management, increase efficiency and to reduce our impact on the environment, as a necessity impacting our ability to feed the 7 billion plus inhabitants of the planet. Nowadays, nanotechnology is a multi-billion and rapidly expanding industry, exemplified by the more than a thousand products containing nanomaterials currently in the market. Since the past decade, over 300 nanofood products have become available in international markets several dimensions of structural elements, crystallites, molecules and clusters are manifest in nanomaterials, which includes
- **zero dimension** (nanoparticles, nanoclusters, and quantum dots)
- **one dimension** (carbon nanotubes and multiwalled nanotubes)
- **two dimensions** (graphene layers and ultrathin films)
- **three dimensions** (nanostructured materials)

### **Role of Nanotechnology in Aquaculture**

- On the other hand, the utilization of nanoparticles to advance aquaculture and the seafood industry is gaining enormous momentum.
- Aquaculture is the food industry showing the fastest growth and produces more than 50% of seafood used for food.
- However, environmental degradation, chemical contamination, suboptimal nutrition and disease prevalence, are among the factors that negatively impact this sector for the achievement of global food security.

### **Applications**

- Drug delivery for health management.
- Nano sensors for pathogen detection.
- Microbial disinfection.
- Treatment of pollutants in water.
- Delivery of dietary supplements and nutraceuticals.

### **Drug delivery for health management**

- Disease outbreak is one of the main obstacles for the sustainability and development of aquaculture.



- In this context, nanotechnology has an enormous role to play, linked to provide novel perspectives related to disease diagnosis and health management.
- In last few years, controlled release delivery systems and diagnostic sensor based on nanoparticles have been developed to change their properties and structure according to environmental stimulus such temperature, ionic strength, pH, or enzymatic activity.

#### **Nano- sensors for pathogen detection**

- Nano-biosensor systems are currently being developed to allow the detection of very low concentrations not only of parasites, bacteria and viruses, but also of polluting elements in the water (Chen et al., 2016).
- This is particularly important in outbreaks at commercial aquaculture systems, since it can take too much time before the etiological agent causes an impact so that its presence is identified, delaying the treatment to control the pathogen, creating an important economic impact.

#### **Microbial disinfection**

- Many of metal NPs have been used for disease prevention and treatment, such as silver, titanium, copper, among others.
- Metal NPs have different modes of action against bacteria, of which, one of the strongest effects is against the cell membrane and cell wall by attaching to them by electrostatic interaction and being able to disrupt them.
- Metal NPs are also known to trigger a higher oxidative stress state increasing the amount of reactive oxygen species (ROS) which can damage proteins, lipids, and DNA.

#### **Treatment of pollutants in the water**

- Nanotechnology has also been used to treat water pollution, which is one of the main problems in aquaculture.
- Nevertheless, graphene nano-sheets and graphene oxide, linked to removal of several types of pollutants from water, have attracted tremendous attention in last few years.
- Graphene oxide-titanium oxide nanocomposite have been used for adsorption, removal of heavy metal and organic compounds from residual water.

#### **Delivery of dietary supplements and nutraceuticals**

- One of the main underlying concepts behind the idea that nanoparticles can improve the fish development is based on their ability to increase the quantity of nutrients absorbed across the digestive tract.



- Micronutrients, in the form of nanoparticles, incorporated in aquaculture feeds, can penetrate in cells more efficiently, and therefore, rise absorption rate.
- This has been demonstrated in sturgeon and young carp, which showed faster growth rates when fed with iron nanoparticles.

### Use of microelements in the form of nanoparticles to be included in aquaculture feeds

Currently, the potential toxicity of nanoparticles in biological system is becoming a public concern. Nanomaterials may constitute a new source of pollutants to the environment, and research is being focused on the potential negative impact that they could produce. A sustainable approach of the use of nanotechnology into the fisheries and aquaculture industries will require more studies of those issues, including the understanding of how nanomaterials can accumulate and if they could become toxic to aquatic organism or humans.

Many nanoparticles are not biodegradable and tend to accumulate in various organs in host.

Some nanoparticles are extremely combustible and spontaneously burst into flames. Some Nanoparticles such as Nickle, Cobalt and Silicon dioxide are carcinogenic.

### Conclusion

Nowadays, there are many potential applications for nanomaterials in the fisheries and aquaculture industry. Some of the most promising areas in this field are applications related to fish health management, nanoscale ingredient incorporation. Use of nanotechnology in aquaculture feeds and food packaging, as well as applications linked to value-added products, stress reduction and health management. Currently, most of these applications are in an early stage, and high cost is considered the main limiting factor for their wide implementation.

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## Antimicrobial Peptides: from host defence to therapeutics

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### Abstract

Our over-reliance on antibiotics, their overuse and inappropriate prescription has created a serious antimicrobial resistance (AMR) crisis such that the world is on the verge of entering the 'post antibiotics era'. According to CDC, AMR is an urgent global threat affecting health care, veterinary, aquaculture, fisheries, and agriculture industries. The discovery of antimicrobial peptides (AMPs) and the magic bullet 'antibiotics' were contemporary but the former didn't gain attention for therapeutic exploitation. The renewed interest in antimicrobial peptides is a consequence of increasing cases of AMR. This article outlines the diversity, physicochemical properties, mechanism of action, and therapeutic potential of AMPs.

**Keywords:** Antibiotics, Antimicrobial Peptides, Interaction, Potential

### Overview

Antimicrobial peptides (AMPs) are naturally occurring host defense peptides produced by every domain of life ranging from prokaryotes to multicellular organisms including humans and plants as part of their innate defense mechanism. These evolutionarily conserved effector molecules are found abundantly in organs exposed to the external environment or at the passage of pathogen entry. These biological molecules are either expressed constitutively or upregulated during infections. They show broad-spectrum antibiotic activity, targeting bacteria, fungi, viruses, parasites, and even cancerous cells. In most organisms, AMPs are multifunctional molecules that not only provide resistance against pathogen attack but are also involved in other biological functions like immune modulation, wound healing, inhibiting cancer cell growth, and/or functioning as signaling molecules such as hepcidins that are also involved in iron regulations (Shi and Camus, 2006; Raju *et*

al., 2020). These characteristic features make them an attractive candidate for application in medicine, like dermatology, ophthalmology, and cancer therapy.

About 90 years have passed since the discovery of 1<sup>st</sup> naturally occurring AMP viz., Lysozyme, in the human body by Alexander Fleming (Fleming, 1922). Characterization of the first AMP in prokaryotes dates back to 1939 when René Dubos isolated ‘gramicidin’, from a soil bacterium, *Bacillus brevis*, and found that it protects mice from *Pneumococci* infection (Dubos, 1939), and today gramicidins have become one of the most commercialized AMP (Cheng *et al.*, 2014). Hirsch reported the first animal-originated AMP ‘defensin’ in 1956, isolated from the leukocytes of rabbits (Hirsch, 1956). AMPs have also been isolated from plants, for example, purothionin obtained from *Triticumaestivum* and was found to be effective against fungi and some phytopathogenic bacteria (Balls *et al.*, 1942; De Caley *et al.*, 1972). There are a number of databases documenting AMPs classification, prediction, design, and structure, one such database is the antimicrobial peptide database (APD). APD is continuously being updated and currently is in the APD3 version with a total of 3217 AMP entries.

### Structure and Diversity

AMPs are predominantly chains of  $\alpha$ -amino acids (2-100 mer) joined by a peptide bond. This diverse group of oligopeptides displays some standard characteristic features such as amphipathicity, net positive charge (+1 to +5), hydrophobicity (30-70%), and low molecular weight (<13kDa) (Chaturvediet *al.*, 2020; Ganet *al.*, 2021). Its primary structure is simple however, on the basis of secondary structure, AMPs are classified into four structural groups:  $\alpha$ -helix,  $\beta$ -sheet, linearly extended, and loop. They are synthesized biologically, synthetically, or by a combination of both.

### Mechanism of Action

Unlike antibiotics, AMPs attacks multiple target sites on the pathogen. They either work by disrupting the membrane or through immunomodulation. Membrane-disrupting AMPs such as buforin II are selectively cytotoxic to pathogens due to the fundamental difference in cell membrane composition of bacteria and host. In contrast to the host, the bacterial surface is negatively charged and so is the cancer cell (Mahlappuet *al.*, 2016; Gong *et al.*, 2020; Gan *et al.*, 2021). Initially, the cationic AMPs strongly interacts with the negatively charged surface of microbes through electrostatic interaction, followed by amphiphilic and hydrophobic interactions. Once the AMPs adheres to the surface it follows one or a combination of 3 membrane disrupting models, viz., (i) Barrel stave model, (ii) toroidal pore model, (iii) carpet model, ultimately disrupting the membrane integrity, while some may also interfere with the crucial intracellular processes (Kumar *et al.*, 2018). Some AMPs like defensin, cathelicidin, lactoferrin, and anti-LPS factor, exhibit additional



immunomodulatory properties, indirectly killing the pathogen by modulating the host's immune system. They are involved in recruiting APC to the site of infection, inducing the expression of anti-inflammatory cytokines while suppressing the expression of pro-inflammatory cytokines, binds to lipopolysaccharide, influence differentiation of T-cells and beyond (Gan *et al.*, 2021).

### Therapeutic Potential

AMPs are emerging as a potential new class of antibiotics to combat AMR as they display multi-modal, rapid killing mechanisms thus reducing the probability of developing AMP resistance. Some AMPs also display synergistic effects by facilitating the activity of other antimicrobial compounds. Furthermore, AMPs like  $\beta$ -defensin, histatins and LL-37, have also shown efficacy against multi-drug resistant and biofilm-forming microorganisms (Hancock and Diamond, 2000; Le *et al.*, 2022).

Nisin, gramicidin, polymyxins, daptomycin, and melittin are some of the commercialized AMPs. Nisin (From *Lactococcus lactis*), is used as a food preservative or as a treatment for stomach ulcers and colon infections (Gharsallaoui *et al.*, 2016; Dijksteet *al.*, 2021). Polyoxin (from *Streptomyces cacaoi*) is used as a fungicide (El-Naggar, 2021). Lactoferricin B (isolated mainly from bovine milk) is used as a controlling agent of mastitis (Weigel and Shook, 2018) while Gramicidin is a constituent of ophthalmic solutions.

### Conclusion

Antimicrobial peptides are broad spectrum antimicrobial compounds with strong immunomodulatory and anti-cancer properties. They have become an ideal candidate for next generation anti-infective mainly due to its amenability to bioengineering. The field of AMP needs to be further explored and developed with the advancement of science to design AMP-based therapeutic approach in treating bacterial infections and in cancer therapies.

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## Man-Animal Conflict: A Jumbo problem still unresolved

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### Introduction

Human-animal conflict occurs when interactions between humans and wildlife result in undesirable outcomes such as loss of property, livelihood, or even life. Human-animal conflict is described by the World-Wide Fund for Nature (WWF) as "any interaction between humans and wildlife that results in negative impacts on human social, economic, or cultural life, on wildlife population conservation, or on the environment." These encounters cause misery for both humans and wildlife who are directly impacted by the conflict, but they can also have a worldwide influence, with organizations like corporations and sustainable development agencies feeling its aftereffects. The problem has a substantial and truly global scope, yet we are unable to solve it at the necessary scale. As a result of population development and changes in land usage, man- animal conflict have become more frequent in many areas in recent decades. Over the course of three years, elephants killed 1,579 people, including 585 in 2019–20, 461 in 2020–21, and 533 in 2021–22. The greatest fatalities (332) occurred in Odisha, followed by Jharkhand (291) and West Bengal (240). Between 2019 and 2021, 125 people were killed by the tigers in protected areas. Maharashtra accounted for nearly half of all deaths with 61.

### What are the causes of the man- animal conflict?

When Human and wildlife coming into contact with wildlife's natural habitats causes conflict. Crops, for example, are raised by herbivores, while animals are raised by carnivores, prompting farmers who rely on both to take extraordinary efforts to avoid wildlife loss. As a result of population



explosion, with limited land reserves, often humans especially those people living in and around the buffer zones are forced to have frequent interactions with the wild animals. Habitat destruction caused by man-made activities such as deforestation, overgrazing of livestock, expansion of human settlements and agriculture together with any natural calamities also contributes towards the man-animal conflict. The increase in earth's temperature have triggered the collapse of the fragile ecosystems in the nature and have made the wild animals to move into the human habitats in search of food and water. Wildlife population growth as a result of conservation programmes without a subsequent increase in the forest area leads to conflict among the wild animals resulting in the entry of wild animals into the human habitats. Mostly the human habitats neighbouring the forests area are attacked by the aged wild animals as they can easily prey on the domesticated herbivores in the human dwellings. Growing interest in ecotourism and increased access to nature reserves also accounts to the man-animal conflicts.

### **What are the impacts of the man-animal conflicts?**

People, particularly farmers and livestock herders, might suffer large economic losses as a result of human-animal conflict. Wild animals can devastate crops, cause infrastructural damage, and kill cattle, causing financial hardship. Wild animals can endanger human safety, particularly in locations where people and wildlife cohabit. Large predators, such as lions, tigers, and bears, can cause serious harm or death. Human-animal conflict can be harmful to the environment. For example, when humans kill predators, prey populations increase, which can produce ecological imbalances. Human-animal conflict can also hamper conservation efforts by creating false perceptions of wildlife and making conservation measures difficult to adopt. Human-animal conflict can also have psychological effects on people, particularly those who have been attacked or whose property has been damaged. It can cause anxiety, fear, and trauma. In addition to direct losses to communities, human-wildlife conflict has an indirect influence on people all over the world by putting pressure on the global supply chain and agricultural production, resulting in food insecurity and decreasing productivity among farmers.

### **What are the mitigative strategies for reducing man-animal conflicts**

Previously utilized mitigation techniques included relocating troublesome animals from a conflict site to a new area, but current research has revealed that this approach can have harmful consequences on species and is usually ineffective. Building barriers around livestock enclosures, building unique wildlife corridors, and installing beehive fences around farms to dissuade elephants have all shown to be successful and cost-effective human-animal conflict mitigation techniques. In certain cases, government procedures have been set up to give monetary compensation for damages



caused by human-wildlife conflict. Insurance can be provided for the crop damage caused by the human-wildlife conflicts under various crop insurance scheme to compensate the loss incurred by the farmers. These methods aim to lessen the need for animal retaliation and to provide financial incentives for coexisting with wildlife and humans. Across the globe, using guard dogs to keep cattle safe from predators has been successful in lowering human-carnivore conflict. A recent evaluation found that livestock-guarding dogs were adopted as a management strategy in 15.4% of study instances looking into human-carnivore conflict, with animal losses on average 60 times lower than the normal Measures to increase fodder and water sources within forest areas can also be considered as a mitigation measure to prevent the entry of wild animals into human dwellings in search of feed and water.

### **Conclusion**

The conflict between people and nature will always exist. Therefore, the primary goal of human-wildlife conflict management should be to increase the safety of people and wildlife while promoting cohabitation. One health is a collaborative, multisectoral, and trans-disciplinary approach that considers the connections between people, animals, plants, and their common environment in order to get the best results for health and wellbeing. The man-animal conflict management should be recognized as a vital area for preserving biodiversity and fostering sustainable development. In order to attain the 2050 vision of "living in peace with nature," human-wildlife coexistence must be taken into account during the implementation of the SDG frameworks.



# One Health Perspectives on Zoonotic Brucellosis and Effective Mitigation Strategies

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## Abstract

Brucellosis, a resurging zoonotic disease with significant public health implications, necessitates applying the One Health approach, integrating human, animal, and environmental health. The One Health approach integrates human, animal, and environmental health to address the challenges of brucellosis effectively. This article highlights the importance of engagement, assessment, planning, implementation, and monitoring within the One Health framework. By involving stakeholders, mapping communication mechanisms, establishing baseline data, and fostering collaboration, comprehensive strategies can be developed for prevention, control, and treatment. The One Health approach enables holistic brucellosis management, promoting interdisciplinary collaboration and improving surveillance, vaccination, disease control programs, and community engagement.

## Introduction

Brucellosis, caused by gram-negative bacteria of the genus *Brucella*, is a prevalent re-emerging bacterial zoonotic disease with bioweapon potential (Zhou et al., 2020). It poses a severe global public health threat, affecting humans and animals. The World Health Organization (WHO) has recognized brucellosis as the leading neglected non-malarial febrile zoonotic illness (WHO, 2020). To prevent and control brucellosis, focusing on risk factor management, disease transmission prevention, and surveillance is essential. Culling of infectious animals is identified as the most effective control strategy (Durrani et al., 2020).

The One Health approach promotes collaborative efforts across various local, national, and international disciplines, integrating the health of people, animals, plants, and ecosystems. In

combating brucellosis using this approach, it is crucial to identify infection sources and develop targeted animal management methods accurately. Implementing a proper strategy and successful interventions can lead to a decrease in brucellosis cases. Strict surveillance, data collection, compilation, and reporting are vital in utilizing the One Health approach to navigate a developing public health crisis. Monitoring various disease strains with zoonotic potential and timely data reporting is essential. Existing surveillance programs in veterinary and medical fields, such as the Integrated Disease Surveillance Project (IDSP) and the National Animal Disease Reporting System (NADRS), can collaborate with environmental sectors by exchanging data, comparing reports, and analyzing issues. These partnerships effectively reduce the severity of disease outbreaks in humans and animals.

Additionally, evaluating the future pandemic potential of brucellosis pathogens and screening potential vectors and hosts are necessary. Understanding the environmental factors, reservoir hosts, transmission modes to humans, and the possibility of human-to-human transmission requires studies on temperature, ecological factors, and pathogen dynamics. Disease prevention through immunization, rapid and practical diagnostics, and improved laboratory infrastructure are crucial. Raising public awareness about zoonotic diseases, antibiotic resistance, and maintaining clean environments is significant.

The One Health approach recognizes the interconnectedness of human, animal, and environmental health and emphasizes interdisciplinary and multisectoral collaboration to tackle complex health challenges. In brucellosis, the One Health approach involves coordination and collaboration among veterinarians, public health officials, epidemiologists, wildlife biologists, and other experts. Several examples of how the One Health approach can be applied to brucellosis include:

1. **Improved surveillance:** One Health surveillance systems gather and analyses data from various sources, such as veterinary clinics, hospitals, laboratories, and wildlife monitoring programs. This comprehensive approach facilitates early detection and response to animal and human brucellosis outbreaks.
2. **Cross-species vaccination:** Developing cross-species vaccines that protect both animals and humans can be a solution for preventing brucellosis. Identifying shared antigens among different species of brucellosis-causing bacteria can aid in developing effective vaccines.
3. **Integrated disease control programs:** One Health-integrated disease control program for



brucellosis encompasses strategies like vaccination, quarantine, testing, and culling infected animals. Coordinated implementation across sectors and jurisdictions maximizes the impact on disease control.

4. **Risk communication and community engagement:** Engaging communities and stakeholders in disease prevention and control efforts through education, awareness campaigns, and culturally appropriate interventions is vital.

Overall, the One Health approach recognizes the complexity of brucellosis and underscores the importance of collaboration and integration across sectors and disciplines to effectively prevent, control, and treat the disease.

The One Health strategy encompasses the following components:



#### 1. **Engagement:**

- One of the key steps in the One Health initiative is to involve stakeholders. This can be achieved by conducting a scoping mission to assess the prevalence and incidence of the disease in the community. This assessment helps in understanding the specific context and challenges related to the disease in that community.
- Additionally, it is important to gain insights into the sociocultural and traditional customs of the people in the community where the disease has previously occurred. This understanding helps in tailoring interventions and communication strategies that are culturally sensitive and effective.
- To ensure the sustainability and effectiveness of the One Health program, it is crucial to establish long-term government support. This support should recognize One Health as a critical component in preventing and controlling zoonotic diseases. Government support provides the necessary resources, coordination, and policy framework to implement comprehensive and integrated approaches to disease prevention and control.

#### **Assessment:**

- The assessment involves utilizing infrastructure mapping to create network maps that illustrate the formal and informal communication, collaboration, and coordination mechanisms within and between sectors.



- It also includes establishing baseline data on the current status of zoonotic illness burden and its epidemiological situation. This data will be used to design preventative and control plans at various levels, including local, sub-national, national, and regional.
- Another crucial step is conducting a gap analysis to identify capacity deficiencies within and between sectors responsible for managing the system or program once the baseline data is established.

### **3. Planning:**

- The planning phase focuses on developing plans and protocols that incorporate and leverage the involvement of all relevant One Health sectors.
- It also involves establishing a global One Health alliance/network/partnership and creating a comprehensive business plan.

### **4. Implementation:**

- During the implementation stage, projects are carried out to validate concepts and address the causes of emerging diseases. This may include organizing annual or semi-annual international One Health conferences.
- Gathering information on One Health experiences worldwide is essential to enhance knowledge and conduct human development and training initiatives.

### **5. Monitoring and Evaluation:**

- The monitoring and evaluation phase includes engaging in information exchange, knowledge management, and dissemination activities.
- Implementing a communication strategy is crucial, which includes formal communication channels, targeted programs, and messaging to facilitate resource sharing and maximize public support.

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## Artificial Insemination in Mare

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### Introduction

Inseminating mares with chilled semen is a common method of breeding, allowing owners to keep their mare at home and increasing the range of stallions available. The aim of breeding mares with chilled semen has always been to inseminate the mare zero to 24 hours before ovulation. This is achieved by daily palpation and ultrasound examinations to detect a large, soft follicle and a marked endometrial oedema pattern. Occasionally, mares do not ovulate as expected, or there may be problems with the courier service transporting the semen from the collection center to the mare owner's premises or AI center where the mare is being monitored.

Semen is collected from stallions using an artificial vagina, cooled to extend the lifespan of sperm and then shipped to the location of a mare awaiting insemination. Mares can be monitored for estrus, or heat, and semen can be ordered and scheduled to arrive before the mare ovulates an oocyte, or egg. Semen usually arrives in less than 24h by air or 48h if shipped by ground. Upon arrival, a veterinarian or trained technician can inseminate the mare prior to ovulation.

### History

The horse is the first animal species for which the use of artificial insemination (AI) was recorded. Bowen (1969) cites a report from 1322 in which stallion semen was taken to inseminate mares belonging to an Arab chief. The Russian biologist, Ivanov is credited to be the first person to

develop the technique. He later organized an equine AI center and developed the improved methods of collecting, diluting and transporting the stallion's semen (Tischner, 1991).

Research specifically into artificial insemination (AI) in equids has been limited, as a number of breed societies will still not accept for registration progeny conceived in this way, but many set strict regulations, such as a limit on the number of foals that can be registered per stallion per year. In Europe, Australia, China, South Africa and the USA, equine AI is now widespread in its use.

### **Advantages of Artificial Insemination**

1. Removal of geographical restrictions.
2. Minimization of disease transfer, both venereal and systemic
3. Reduction in injury risk both to handlers and horses. The risk is further reduced if the stallion can be encouraged to mount a dummy mare.
4. Increasing the number of mares that can be inseminated per ejaculate.
5. Improvement of native stock through semen importation.
6. Development of gene banks for future reintroduction of genetic material.
7. Breeding of difficult mares – those with physical abnormalities, especially caused by accidents, infection, poor perineal conformation, psychological problems, etc. However, care must be taken to ensure that such problems are not heritable.
8. Breeding from difficult stallions – those with physical problems, injury, infection, inadequate semen characteristics, psychological problems, etc.
9. Reduction in labor costs.
10. Semen sexing

### **Selection of stallion**

Research studies suggest that  $100-250 \times 10^6$  progressively motile sperm per insemination are optimal for conception. In other words, there are only a certain number of spermatozoa available each day therefore, care must be taken to make intervals between breeding's (in natural cover) long enough to ensure maximum conception rates. Once ejaculation frequency exceeds every other day, sperm output per ejaculate decreases. This is not to suggest, however, that are insufficient spermatozoa to impregnate a mare.

Stallion semen should be evaluated under a microscope regularly during the breeding season to document fertility. Besides frequency of ejaculation; stallion age, testes size, libido, and seasonality must also be considered when evaluating fertility. Both anabolic steroids and winter daylengths render stallions less fertile by reducing gonadotropin secretion which in turn decreases testicular size, sperm output, and libido. Older stallions produce less spermatozoa than younger



stallions. Libido can also be reduced by overuse, misuse, and lack of teasing by an estrous mare.

## **Semen Collection**

### **Methods of semen collection**

1. Easiest method is the collection of dismount samples. The drips of semen are collected from the stallion after withdrawal from the mare into a sterile jar.
2. Condoms have been developed for use with horses.
3. The artificial vagina (AV).

### **Preparation of AV**

They provide a warm, sterile lumen, surrounded by a water jacket, under some pressure, with a collecting vessel at the end, in an attempt to mimic the natural vagina. Most AVs consist of a solid outer casing with two rubber linings, an outer and an inner. The outer lining and the casing form a jacket, into which warm water and/or air is passed by means of a tap or valve.

### **Procedure of semen collection**

The stallion is allowed to mount and the collector diverts the penis, when erect, towards the AV. The stallion should be allowed to gain intromission and enter the AV of his own free will and not have the AV forced upon him. The AV can be stabilized by being held against the hindquarters of the mare, if present, or the back of the dummy

The occurrence of ejaculation is noted, as in natural covering, by the flagging of the tail or by feeling for the contractions of the urethra and the passage of the semen along the ventral side of the penis.

After collection, the collecting vessel must be carefully removed from the AV and the semen evaluated as soon as possible. If it is not possible to carry out semen assessment immediately, it can be extended and stored at 4–5°C for up to 24 hr without appreciable reduction in its viability, and a reasonably accurate evaluation of the semen can still be obtained (Malmgren *et al.*, 1994).

### **Use of semen**

The method used depends on the stud system and the location of the mare(s) to be inseminated. Once the semen has been evaluated, it can be considered for insemination. Semen can be used in one of four ways:

1. Used immediately undiluted to inseminate a single or possibly two mares, depending on the volume collected.
2. Diluted and used immediately for insemination into several mares.
3. Diluted and refrigerated for use over the next 72 hrs.
4. Diluted and frozen for use at a later date.



### Raw semen

The best results are obtained using undiluted semen inseminated immediately. However, such use of semen defeats two of the main objectives of AI – increasing the number of mares that can be fertilized with one ejaculate and transportation – though it may be of use as a veterinary or management aid.

### Chilled semen

If semen is to be extended prior to use, it should be done so immediately. A vast array of diluents has been developed, based normally upon milk, gelatine or egg yolk plus antibiotics).

The acceptable range for a normal stallion's semen parameters.

Parameter	Acceptable range
Volume of sperm produced	30–250 ml
Sperm concentration	30–600 × 10 <sup>6</sup> ml <sup>-1</sup>
Morphology Minimum	40–50% physiologically normal
Live: dead ratio	6.0:4.0
Motility	Minimum 40% progressively motile sperm
Longevity at room temperature	45% alive after 3 h
10% alive after 8 h	
Ph	6.9 – 7.8
White blood cells	<1500 m <sup>-1</sup>
Red blood cells	< 500 ml <sup>-1</sup>

All these extenders give acceptable fertilization rates for chilled semen. Extended semen can be stored for up to 2–3 days if extended in a ratio 2:1 (semen: extender) and cooled slowly to 4–8°C Over 4 h and kept at this temperature until use. Such treatment allows limited semen transportation and storage in a refrigerator or Equitainer.

### Frozen semen

Prolonged storage can only be achieved by freezing, but the techniques of freezing horse semen is nowhere near as refined as those for cattle. Numerous extenders have been tried, most are similar to those used for chilled insemination, but with the addition of a cryoprotectant, such as glycerol. The major problem is identifying a suitable cryoprotectant that can be used to prolong the formation of ice crystals either within the sperm head, so reducing physical damage, or within the surrounding solution, reducing sperm desiccation. Glycerol is used as such an agent in cattle, but



appears to be toxic to equine sperm. However, in the absence of any other successful agent, low concentrations of glycerol continue to be used. Detergents and a combination of sugars has also been used as cryoprotectants. In addition to variation in results with extenders, there is great variation between and within stallions, with pregnancy rates of 10–70% being reported.

### **Semen Dilution**

The extent of semen dilution depends on the initial concentration of the sample and the motility of the sperm (Magistrini *et al.*, 1987). Insemination of diluted semen containing  $100 \times 10^6$  progressively motile sperm (PMS) per insemination gives good results, but normally  $500 \times 10^6$  sperm per insemination is recommended in order to allow a margin of error (PMS required =  $100-500 \times 10^6$ ). The insemination of  $800 \times 10^6$  sperm is advised when using frozen semen, in order to compensate for loss occurring during the freezing process.

### **Insemination Volume**

The volume of inseminate varies from 10 to 30 ml for fresh semen, 10 to 60 ml for chilled and 0.5 to 5 ml for frozen (British Equine Veterinary Association, 1997). It has been suggested that volumes in excess of 100 ml or less than 0.5 ml are detrimental to conception rates.

### **Sperm numbers**

Conventionally, mares are inseminated with a minimum of 500 million progressively motile (live) sperm. This is known as the insemination dose. For semen that is to be shipped as chilled semen, double this amount should be sent (one billion motile sperm) to ensure that there are at least 500 million motile sperm at the time of insemination. Semen should be mixed with pre-warmed ( $37^\circ\text{C}$ ) extender immediately after collection. A final concentration of 25 million to 50 million spermatozoa/ ml in extended semen is usually the ideal concentration for shipping chilled semen samples. This means a typical volume of 20ml to 40ml of extended semen to inseminate should be sent.

### **Insemination Technique**

Mares are inseminated non-surgically. When using fresh or chilled semen, this should occur, as with natural service, on either day 2 or day 4 of estrus. Frozen semen requires better synchrony with ovulation, ideally to within 6 hr. Semen, both diluted and undiluted, is deposited into the uterus by means of a plastic sterile pipette, with syringe attached, or by an insemination gun, guided in through the cervix to the uterus, using the index finger. Alternatively, the pipette can be guided in through the cervix as per rectal palpation, the cervix being felt through the rectum wall. The syringe is loaded with semen held between two air bubbles and then attached to the end of the pipette. Once through the cervix, the insemination pipette is pushed into the uterus about 2 cm. When it is in place,



the semen is slowly expelled by depressing the plunger.

### **Conclusion**

In many parts of the world, equine AI is widespread in its use. The UK, though a leader in many aspects of the equine industry, lags behind, largely due to the failure of the Thoroughbred industry to recognize and hence register progeny conceived by AI. Until it can be persuaded that AI is an acceptable means of breeding the horse, the application of AI will be restricted to use in other breed societies. Despite this, it is evident that equine AI is here to stay and will continue to expand, opening up with it exciting opportunities in the selection and breeding of the equine species.



## Genetic Improvement of Trees Used in Wind Breaks

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The genetic quality of the nursery stock used in windbreaks has been a concern of many people responsible for windbreak planting for about as long as other people have been concerned about the genetic quality of loblolly pine (*Pinus taeda* L.). In 1923 Johnson (1923) cautioned tree planters in the Great Plains of North America to use only tree species proven to be hardy in their area. He also described within-species variation in Siberian elm (*Ulmus pumila* L.) noting that "strains" coming from different localities in China were quite variable in hardiness when grown in the northern Great Plains. Progress in improving trees and shrubs for windbreaks has not been as dramatic as the gains have been with timber species. The benefits of windbreaks are substantial, but they are often indirect and long-term. There has been little pressure on government and university research administrators to devote more of their resources to the genetic improvement of windbreak species. Private industry has not become involved in producing genetically-improved seed of windbreak species because of the limited market for such seed.

Despite the meager allocation of money, resources and people to the effort, the genetic improvement of windbreak species is continuing at a steady pace in many parts of the world.

1. The types of improvement being sought for windbreak species
2. The importance of improving these traits
3. Methods or programs being utilized to achieve these improvements
4. results of these improvement efforts
5. Improved cultivars currently available for windbreaks
6. Future needs.

## **Traits of Importance to Windbreaks**

### **Survival**

Traits that affect tree survival are of greatest importance in windbreak establishment. Windbreaks are most commonly planted in regions of climatic and edaphic extremes where plant life is subjected to periodic stresses from limited water, poor soil, high winds, cold winter temperatures, and attack by insects, animals and diseases. Establishment of planting stock is often difficult, uncertain and expensive. Replanting for several years is often necessary to achieve satisfactory stocking rates. To be effective, a windbreak must maintain a minimum density to provide the benefits for which it was designed. Other improvements in a tree are meaningless if it does not survive. Significant differences among origins in rates of survival have been reported for most of the provenance tests that have been conducted on windbreak tree species.

### **Cold hardiness**

Cold hardiness may take several forms: resistance to winter injury, frost tolerance or frost avoidance. Common forms of winter injury are branch-tip die-back, frost cracks, winter sunscald, cambial injury, root injury and needle burning of conifers. That resistance to low winter temperatures is especially important for trees and shrubs of the Canadian prairies. Selection of cold hardy genotypes is generally carried out after episodes of late spring frosts, early autumn freezes or unusually severe winter weather. The problem of relying on such episodes in the selection process is their sporadic, unpredictable nature and the difficulty of observing uniform conditions on a repeatable basis.

### **Drought hardiness**

Drought hardiness or drought resistance describes the relative abilities of different plant species to survive extended periods of reduced precipitation. There are three major types: drought escaping, drought tolerating and drought avoiding. Drought-escaping plants complete their life cycles before severe water deficits occur. Drought-tolerating plants possess the ability, through the properties of their protoplasm, to survive desiccation. Drought-avoiding plants are able to postpone dehydration through structural features affecting water absorption and loss.

Genetic variation in drought resistance has been reported for several angiosperm windbreak tree species. Lack of research concerning drought resistance among coniferous species may be the result of fewer problems with this trait among these species

### **Pest resistance**

The importance of breeding for pest resistance depends upon the availability, suitability and cost of other methods of pest control. If chemical or biological control methods are effective,





environmentally safe and inexpensive, then it makes little sense to devote major resources and time to breeding for resistance to a particular pest. Unfortunately, there are few tree species used in windbreaks for which this is the case. Most often other control methods are too expensive, ineffective or not compatible with other farming operations that must be carried out at the same time control measures should be applied. In these situations, breeding for pest resistance may offer the only practical means of controlling pest damage.

Several cultivars of windbreak tree species with proven resistance to specific insects or diseases are available. Hybrid poplars (*Populus* sp.) resistant to leaf rusts (*Melampsora* and *Marssonina* species) and Austrian pine (*Pinus nigra* Arnold) resistant to *Dothistroma pini* Hulb. are the most widely planted examples of such cultivars.

### **Adaptability to poor soil**

Many of the sites where windbreaks are needed have soils that make tree establishment and growth difficult. Alkaline soils, saline soils, sandy soils, blow sand and soils with high water tables have soil properties that may limit the choice of tree and shrub species. Very little information exists concerning within-species variation in adaptability to poor soils. As more provenance tests are established on a greater variety of sites, the information on adaptability **to poor soils will be increased.**

### **Growth rate**

The height of a windbreak is a major determinant of its effectiveness. For maximum effect, it is best to choose trees that will grow as tall as the site will allow. Fast-growing trees that reach maximum height quickly will reduce the time required for the windbreak to produce its maximum benefits. After the species have been chosen, the next decision affecting growth rate is choice of seed source or cultivar. It is at this point that tree improvement activities can benefit the tree planter by providing planting stock capable of faster growth than common, nursery-run planting stock. Within-species variation in growth rate has been reported in most windbreak species for which provenance tests have been conducted.

### **Crown Form**

Most windbreaks are planted to accomplish what their name implies; a reduction in wind velocity or a change in wind flow patterns. The distance protected and the effectiveness of wind reduction are governed by windbreak height, density and orientation to prevailing winds, (George, 1971). The capacity of a windbreak to furnish protection depends on its structure, i.e. the sum total of all tree and shrub foliage contributing to its effective height, density and continuity (Read, 1968). Windspeed reduction patterns are determined primarily by the porosity and distribution of pores in



the barrier ( Skidmore, 1976). The primary purpose for which a windbreak is established will determine its optimum permeability, e.g., windbreaks designed to distribute snow may be more porous than those designed to control wind erosion. Windbreak density can be controlled by adjusting the number of tree rows, spacing between trees in-the-row, spacing between rows and the choice of tree or shrub species used in each row.

On an individual tree basis, crown density is determined by component factors of branch angle, branch diameter, number of branches, live branch retention, and kind and amount of foliage (Dawson and Read, 1964). Crown density may be estimated and subjectively scored as very sparse, sparse, moderate, dense or very dense. Because wind protection is important during the entire year, the density of deciduous species is best judged when the trees are without foliage. In coniferous trees, foliage production, years of needle retention and relative needle length contribute to the overall density rating.

The heritability's of traits influencing crown density and form are largely unknown for most windbreak species. Only a few provenance studies of windbreak species have measured variation in crown characteristics

### **TREE IMPROVEMENT METHODS FOR WINDBREAK SPECIES**

There are seven basic steps used for the genetic improvement of most tree species:

1. Assess the range of genetic variation in major traits among populations (species trials, provenance tests)
2. Select individual phenotypes superior for traits of interest
3. Intermate the selected trees to combine favored traits (wind pollination, controlled pollination, breeding)
4. Evaluate the progeny of these matings on a variety of test sites (progeny testing)
5. Select among progeny for phenotypes exhibiting the best combination of traits
6. Propagate selected phenotypes by vegetative propagules (grafts, cuttings or tissue culture) or by seed
7. Release improved cultivars.

The first and most basic practice that should be instituted by all nurserymen and tree planters is labeling seed and planting stock as to seed source as well as species. To understand the geographic variability of the many species of trees and shrubs being planted in windbreaks, tree planters must keep track of the seed sources they are planting. If problems arise such as plantation failures, pest damage, die-back or lack of winter hardiness, they may be linked to the use of a poorly adapted seed source. On the positive side, such information may document the wide adaptability of particular seed



sources over a variety of sites and climates.

The second low-cost practice that can produce immediate results is the use of local seed sources. If good information is not available on the potential performance of a particular seed source, e.g., one available from a commercial seed dealer, then avoid it and try to collect or purchase seed from a local source. A local seed source of a native species should be well adapted to the planting site. For an exotic species, try to collect seed from plantations or windbreaks that have survived and performed well for at least half their expected rotation-age. The use of planting stock grown from such seed should pose less risk of failure than planting stock grown from seed for which there is no past history. Determining whether a seed source can be considered 'local' may be facilitated by the use of seed zoning maps. Seed zones are subdivisions of land areas established to identify areas of relatively homogeneous soil types, climate and other factors that influence tree survival and growth (Cunningham,1975). Planting stock grown from seed collected from trees growing in one seed zone should perform well when planted in the same seed zone.

A third practice that can be instituted quickly and relatively inexpensively is the collection of seed from phenotypically superior trees or stands. Superior stands used for this purpose are called seed production areas. This practice can be beneficial even if the seed trees have not been proven superior by provenance or progeny tests. Even if such selections eventually prove to have been superior only because of favorable environmental conditions and are unable to pass that superiority on to their offspring, it is unlikely that they are genetically inferior to an unselected seed source of unknown performance potential. Trees that are larger, healthier and more vigorous than adjacent trees, for reasons that cannot be ascribed to site quality, are potentially genetically superior, and should be utilized as interim sources of improved seed. In North America, seed-production areas have been established for at least 49 species of trees and shrubs commonly planted in windbreaks. Many of these seed production-areas furnish seed used to grow seedlings of cultivars that have been "officially released" by state experiment stations or federal agencies such as the Soil Conservation Service and the Agricultural Research Service of the United States Department of Agriculture. Release of named cultivars is an important method to promote the use of genetically improved trees and shrubs used in windbreaks. Plant breeders have used this procedure to identify and promote the use of improved varieties of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), flax (*Linum usitatissimum* L.), corn (*Zea mays* L.) and many other crops. Varieties, also called cultivars, are the culmination of the breeder's efforts to improve a particular crop. A cultivar represents the best plant material available at that time for a particular use. Cultivars of tree and shrub species are tested over a variety of sites for several years to ensure that they perform above the average for that



species and are worthy of release. Table II lists the tree and shrub cultivars that have been released for use in windbreaks and other conservation-type plantings. Planting stock of these cultivars is generally available from nurseries operated by state agencies or associations of soil conservation districts.

### **The Future**

Most of the future work in the genetic improvement of windbreak species will continue the traditional methods and techniques that are currently being used. Seed orchards will be established for many of the major windbreak tree species, such as ponderosa pine (*Pinus ponderosa* Dougl. ex-Laws.), Scotch pine, blue spruce, Black Hills spruce (*Picea glauca*), Rocky Mountain juniper (*Juniperus scopulorum* Sarg.), eastern redcedar (*Juniperus virginiana* L.), Austrian pine, Siberian elm, green ash, hackberry (*Celtis occidentalis* L.), silver maple (*Acer saccharinum* L.), honey locust (*Gleditsia triacanthos* L.) and possibly bur oak (*Quercus macrocarpa*)

Several tree species that are normally propagated by vegetative means will be genetically improved by "packaging" clonal mixtures. Hybrid poplars, native cottonwoods (*Populus* sp.) and perhaps even some of the willow species (*Salix* sp.) will benefit from this approach. Clones used in the mixtures will be selected on the basis of clonal tests on a variety of sites and will exhibit high levels of pest resistance, increased rate of growth, adaptability to adverse sites and compatibility with other clones in the mix. Composition of the clonal mixtures will be dynamic and substitution of clones based on new information will be a standard operating procedure. The introduction of genetic variability into field plantings by the use of clonal mixtures is a key ingredient for improving species such as the hybrid poplars. The impact of many of our disease and insect problems can be significantly reduced by the use of clonal mixtures. This approach brings with it special problems for the nurseryman because it requires extra effort and special care in lifting, sorting, labeling and packing the trees for distribution. Those researchers and tree improvement specialists responsible for determining the composition of the clonal mixture will have to test each clone rigorously, paying particular attention to competition between clones and choosing very carefully clones that are compatible and that will complement each other when planted together.

In the last few years, several new techniques have developed which tree breeders can use for tree improvement. These techniques include tissue culture, electrophoresis and genetic engineering. With tissue culture, trees can be vegetatively propagated, but more importantly, superior trees can be selected in a test tube. Tissue from trees can be grown in a test tube and screened for resistance to diseases and insects, tolerance to high pH or resistance to herbicides. Electrophoresis can be used by tree breeders to identify superior seed sources, detect pollen contamination in seed orchards or predict



long-term seedling performance. Genetic engineering may allow tree breeders to transfer superior genes from one organism into another. For example, if genes for drought tolerance are isolated in a grass species, these genes might be incorporated into trees, allowing better survival after planting. Tree breeders will benefit from these new techniques more than any other group of plant breeders. Long generation intervals and prolonged field testing will no longer be the obstacle to tree improvement they once were.



## Botanical studies of Neem

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### Botanical Names

*Azadirachta indica* A.Juss

Syn. *Melia azadirachta* , *Melia indica* Hooker, *Melia indica* Brandis

### Common Names

English : neem, Indian lilac

Hindi : neem, nimb

Tamil : vembu, veppan

Sanskrit : nimba, nimbou, arishtha (reliever of sickness)

### Classification

Family : Meliaceae, sub-family: Melioideae, Tribe: Melieae

Genus : *Azadirachta* A.Juss

Species : *indica* A.Juss

### Origin

According to Gamble (1902), the center of origin of *A. indica* is in the forests of Karnataka (south India) or the dried inland forests of Burma (Myanmar). Other authors were of the opinion that this tree originated in the forests of the Shivalik hills (foothills of the western Himalayas) or on east coast of south India. The great variety in the shape of the leaves and other morphological features support the theory of the origin of *A. indica* in upper Myanmar (Schmutterer, 1995); later it became naturalized in the forests of central and western India. The subject of origin is still controversial. Roxburgh (1874) gave its origin in India when Myanmar (Burma) was a part of India. Brandis (1921)

and Jacobs (1961) describe its origin in dry regions of Upper Myanmar (Irrawady valley, upper region of Prome). Some authors also suggest the place of origin of neem tree in parts of South India, such as Karnataka (Troup, 1921; Vartak and Ghate, 1990).

### **Ethnobotanical Studies**

In some of the recent botanical surveys in India, some more information has been collected and reported that it as an Ayurvedic plant. In some surveys, stress was laid on finding the cure for certain diseases. It is reported neem has been used for jaundice and malaria. The use of neem in leather technology at the village level for preservation of leather goods is well known. It is also noted that it was also used for curing snake skin.

### **Etymology**

The present popular name “neem”, also spelled earlier as “nim”, has been derived from the Sanskrit word “nimba” which means sprinkler, which is the short term for “sprinkler of nectar (ambrosia)”. The other Sanskrit synonyms for the tree, as given in the chapter on Ayurveda, refer to its habitat and the use of it in ancient India.

The meaning of the generic name *Azadirachta* does not appear to be interpreted properly in most of the literature. It is often said that it is from the Persian words *azad*—free, and *drakhat*—tree, i.e., free tree, and when the specific name *indica* is added to it, the meaning of the botanical name becomes the free tree from India, which does not convey any specific significance of the name.

The views expressed by Watt (1889) appear to be more convincing, according to which the Persians were well conversant with the allied tree *Melia azedarachh* also commonly known as the China berry, but in Persian as “*Azadarakhat*” (the corrupted form of it in most of the north Indian languages is *Dharek*). As discussed with a Persian scholar in Panjab University, India, *aza* means bitter in Persian and *drakhat* means tree, so the name of the China berry in Persian stood for “bitter tree”. When the neem was introduced into Iran, to distinguish it from the China berry, which it resembled to a major extent, neem was called *Aza-drakhat Hindi*, i.e., the bitter tree from India, which led to the present botanical name *Azadirachta indica*,

### **Distribution**

In India the tree is most widely used. It is grown from the southern tip of Kerala to the Himalayan hills, in tropical to subtropical regions, in semiarid to wet tropical regions, and from sea level to about 700 m elevation. It is now well established in at least 30 countries, particularly those in the regions along the Sahara's southern fringe, where it has become an important provider of both fuel and lumber. Although widely naturalized, it has nowhere become a pest. Indeed, it seems rather well "domesticated": it appears to thrive in villages and towns. Over the last century or so, the tree



has also been established in Fiji, Mauritius, the Caribbean, and many countries of Central and South America. In some cases, it was probably introduced by indentured laborers, who re-membered its value from their days of living in India's villages. In other cases, it has been introduced by foresters. In the continental United States small plantings are prospering in southern Florida, and exploratory plots have been established in southern California and Arizona.

### **Description**

Neem trees are attractive broad-leaved evergreens that can grow up to 30 m tall and 2.5 m in girth. Their spreading branches form rounded crowns as much as 20 m across. They remain in leaf except during extreme drought, when the leaves may fall off. The short, usually straight trunk has a moderately thick, strongly furrowed bark. The roots penetrate the soil deeply, at least where the site permits, and, particularly when injured, they produce suckers. This suckering tends to be especially prolix in dry localities. Neem can take considerable abuse. For example, it easily withstands pollarding (repeated lopping at heights above about 1.5 m) and its topped trunk resprouts vigorously. It can also freely coppices after repeated lopping at near-ground level. Regrowth from both pollarding and coppicing can be exceptionally fast because it is being served by a root system large enough to feed a full-grown tree. The small, white, bisexual flowers are borne in axillary clusters. They have a honey like scent and attract many bees. Neem honey is popular, and reportedly contains no trace of azadirachtin. Previous botanic names were *Melia indica* and *M. azadirachta*. The latter name (not to mention neem itself) has sometimes been confused with *M. azedarach*, a West Asian tree commonly known as Persian lilac, bakain, dharak, or chinaberry. The taxonomy of all these closely related species is so complex that some botanists have recognized as many as 15 species. The fruit is a smooth, ellipsoidal drupe, up to almost 2 cm long. When ripe, it is yellow or greenish yellow and comprises a sweet pulp enclosing a seed. The seed is composed of a shell and a kernel (sometimes two or three kernels), each about half of the seed's weight. It is the kernel that is used most in pest control. (The leaves also contain pesticidal ingredients, but as a rule they are much less effective than those of the seed.) A neem tree normally begins bearing fruit after 3-5 years, becomes fully productive in 10 years, and from then on can produce up to 50 kg of fruits annually. It may live for more than two centuries. Neem is a large-sized evergreen tree, but younger trees in dry localities may become leafless for a short period, and new leaves may appear in March-April, which are pinkish green in color. The tree may grow up to a height of 20m and a girth of 2.5m.

#### **a) Stem**

The color of the bark varies according to the part of the plant, its age and locality. The younger branches have a lighter color bark but in a mature trunk it may be grey to greyish black,





rough, feebly fissured, and exfoliating. The inner surface of the bark is fibrous and pinkish brown. Small deposits of gum may be present on the stem in some places, but occasionally in some trees, which are quite old and in a humid climate, a fetid sap may be exuded from the trunk.

#### **b) Root**

It is normally dicotyledonous in nature, but in more than half of the population, vesicular—arbuscular mycorrhizal (VAM) infection is present due to *Glomus* and *Cigaspora* at 250 cm length. The intensity of infection varies with the availability of water. Neem appears to be a highly mycorrhizal-dependent species. It was concluded that a deep-rooted growth habit along with VAM infection may be a survival mechanism when competing for nutrients and water with shallow-rooted and fast-growing plant species. It appears that VAM not only increases the nutrient uptake of the plant but also makes the tree tolerant to root diseases, transplant shock, toxicity of heavy metals and seasonal extremities like drought, etc. It has also been said that *Phytophthora tinnamoni* which is destroying neem worldwide, may be attacking those trees that lack mycorrhiza, as VAM forms a cover on the root and thus protects the plant from pathogens.

#### **c) Leaves**

These are alternate, exstipulate, on a long slender petiole, dorsal side darker green, ventral light in color, leaves 20–40 cm long, dense at the end of branches, alternate, leaflets 7–15, sometimes up to 17, variable in shape, particularly with respect to the central axis. The leaves appear smooth but closer examination of young leaves near the shoot apex reveals the presence of resin-secreting glands. The lower portion of the leaf stalk is covered over with extra floral nectaries. Leaves are bitter to the taste. It was observed that the flattening of twigs and crowding of leaves with prominent ridges and furrows with a disturbed phyllotaxy. The leaflets are 2–7 cm long and 1–4cm broad, imparipinnate, lanceolate, upper side bigger than the lower but it may vary within a population, often alternate, obliquely falcate, coarsely and bluntly serrate. The breadth of lamina and the degree of dentation on the margin of leaflets vary from locality to locality. In general, leaflets from dry arid areas have narrow lamina and sharp teeth along the sides.

#### **d) Flowering**

Generally, the tree starts flowering at three to five years of age and becomes fully productive at the age of about ten years. The reproductive phase lasted 3–4 months. In general, the beginning of flowering was marked by the simultaneous appearance of a new flush of leaves and the inflorescence primordia during February/ March. Blooming became evident in the



second week of March and the peak-time of blooming was in the third/ fourth week of April. Fruiting started 2 weeks after the commencement of blooming. Flowering culminated at the end of May in all populations. Fruits became mature by the third week of June. By the last week of July, all fruits had dropped under the canopy and seeds germinated *en masse* during rains in July/August. Trees growing in areas with a warm winter bloom first, followed by areas where the winter is comparatively cooler. Depending on the locality, flowering may range from January to May. Sporadic flowering in September-October has been observed quite often, in addition to that in February-March. An abnormal seedling was reported during December from these trees and suggested that these trees may be used as germ plasm. In the Murshidabad area of West Bengal (India), the tree flowered throughout the year.

#### e) Inflorescence

The inflorescence is an axillary or a terminal panicle  $16.6 + 2$  cm in length ( $n = 100$ ). Each flowering branch had  $12 + 5$  (range = 8–18) panicles and a panicle produced  $91 + 21$  bisexual flowers that opened acropetally. Anthesis occurred between 16.00 and 07.00 hours and open flowers were retained for 3–4 days. During the peak-time of flowering, a panicle presented  $10 + 7$  (range = 3–21) open flowers in a day.

#### f) The Flower:

The buds are small, hermaphrodite, numerous, stalked, arranged in long, slender, very lax elongated axillary panicles, shorter than the leaves, bracts minute, deciduous. Flower buds open in the evening and are more scented at night. These buds give rise to 4–5 mm long whitish pink flowers.

- *Calyx*: Sepals 3 to 5 wide, imbricate, rounded, blunt, ciliate, sepals smooth and thin.
- *Corolla*: Petals 5, imbricate and oblong, oval in the bud, spreading, spatulate, somewhat twisted with a conduplicate claw, smooth outside, finely pubescent within. The number of petals may be 4–8, which is not genetically based.
- *Androecium*: stamens 10, situated at the base of a hypogymous disk, the stamina filament combined into a long, cylindrical, erect tube, somewhat dilated below as well as at the top, furrowed and smooth externally, hairy within, terminating above in 10 blunt, thick, recurved trifid lobes, anther smooth erect, closely placed, introse, oblong, two celled. The pollen grains are 3–4 corporate, prolate-spheroidal or sub-prolate, apocolpium medium, exine smooth, slightly thickened at the aperture.
- The pollen grains are tetra-colporate, shed at the two-cell stage and have suborbiculate to smooth exine. On average, the pollen grains measured  $43.7 + 2.4$  mm ( $n = 100$ ) in diameter and contained



starch grains as reserve material. The viability of freshly released pollen grains was  $71.4 \pm 1.6\%$  ( $n = 400$  flowers) and pollen became completely non-viable 10–11 days after anthesis. A flower produced  $3763 \pm 374$  pollen grains ( $n = 100$  flowers) and invariably six ovules in an ovary; the pollen:ovule ratio was  $\sim 627$  ( $520\text{--}813$ ;  $n = 100$  flowers).

- *Gynoedum*: carpels, 3–5, syncarpous, superior, as many locule with 2 ovules in each loculus, style about the length of the staminal tube, stigma 5 lobed, placentation parietal. The ovary is trilocular at the base, becoming unilocular at the ovule-bearing region. The pollen tube is monosiphonous, and enters the ovule through a micropyle. One of the synergids is destroyed. Syngamy precedes triple fusion, resulting in an enlarged zygote. Twin embryos occur commonly; the number of seed per embryo may be 1–3. The gametophyte develops in the usual way; the embryo sac is of Polygonum type. Study of premature fallen fruits indicated that embryo abortion is common.
- The stigma belongs to the dry-papillate category. Each stigma has an apical, papillate, non-receptive and a trilobed crown supported over a distinctly papillate rim of receptive zone. Receptivity is attained 12 h after anthesis and is retained for up to 2 days after anthesis. A receptive stigma supported significant pollen germination and increased peroxidase activity. The papillae of the crown are round-tipped and measure  $60 \pm 10.8$   $\mu\text{m}$ .
- Pollen grains do not adhere to the non-receptive papillae and usually fall onto the receptive papillae underneath. The papillae in the receptive zone were  $78.4 \pm 20.8$   $\mu\text{m}$  ( $n = 100$ ) long, densely crowded, unicellular, unbranched, and pointed at the tip.
- Lipids are present in the peripheral region of the receptive papillae. The style is hollow-type and lined with glandular stylar canal cells and forms a continuum between the stigma and the ovary. The canal cells at the stigma-style interface are papillate and glandular.
- *Pollination*: The flowers are cross-pollinated in general, in spite of bisexual flowers and the absence of self-incompatibility. Pollination is occasionally entomophilous but usually anemophilous. Freshly opened flowers of Neem are white and sweet scented. Nectar is secreted in traces during anthesis and is usually seen glistening at the base of flowers between 06.30 and 18.00 hours. Foraging activity started from 05.30 to 06.30 hours. During the entire foraging period in a day (06.30 to 10.30 hours),  $287 \pm 46$  insects visited a flowering branch ( $n = 50$ ). The peak time of visitation was between 07.30 and 08.30 hours and 08.30 and 09.30 hours, during which  $95 \pm 12$  insects were recorded. A total of 13 insect species (seven Hymenoptera, three Lepidoptera and one species each from Diptera and Thysanoptera) were identified as regularly foraging on the flowers. The most dominant Hymenoptera was *Apis*. Honeybees carried out



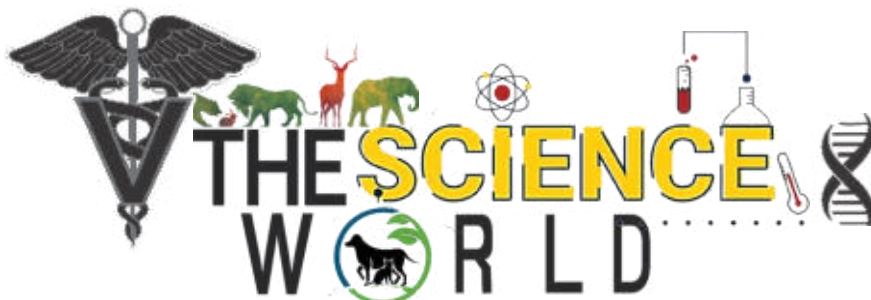
occasional pollination between 16.30 and 18.00 hours, but the frequency was lower than that in the morning (6 + 3 honeybees observed per hour in the evening). Butterflies (*Danaus chrysippus*, *Junonia almanac*) made a few visits (3 + 2 per hour) to the flowers. Thrips were mostly confined to the staminal tube and carried 4 + 1 ( $n = 20$ ) pollen grains on their body. Honeybees (*Apis indica*, *Apis mellifera*, *Apis dorsata*) were the most effective floral foragers. Experiments ( $n = 300$ ) conducted to ascertain the role of wind in pollination showed 1.8 + 0.5% fruit set. The density of airborne pollen grains in the canopy was 14+2  $\text{cm}^{-2}$  and it declined completely 8 m away from the canopy.

- **Seed Development:** A study of seed development has shown a steady increase in fruit/seed length, breadth, fresh and dry weight up to 12 weeks. The moisture content after this period started declining, with an increase in seed oil, protein and carbohydrates. The ovary is syncarpous, superior, and three-celled with 1–2 ovules per cell. The fruit is a glabrous, olive-like drupe, 1–3 cm in diameter, varying in shape from elongate oval to roundish. It is yellow when ripe and comprises a sweet pulp enclosing a single seed (rarely 2–3 seeds). Neem has a strong root system with a deep tap root and extensive lateral roots. Suckers can be produced following damage to the roots.
- **Fruit:** It is an ovoid drupe, bluntly pointed, 1–2cm long, when young and unripe smooth and green with white milky juice, yellow to brown when ripe, epicarp thin, mesocarp with scanty mucilaginous sweetish pulp, endocarp hard enclosing the seed. The fruit gets darker in color and wrinkled on maturity. The seed length varied between 11 and 18mm, width 4.5–8.5mm, and weight 100–530 mg. Among a three-year-old population it was observed that 92.3 percent of the trees had 1–100 fruits/tree, 4.36 percent 101–200 fruits/tree and 0.49 percent (one only) had more than 400 seeds/tree.
- **Seed Dispersal:** Most of the seed fall on the ground under the tree, where at that time the soil is water logged or there may be rain streams. The fruit may remain in moist conditions under the tree or occasionally may travel some distance with rain water. Since there is no dormancy, most of the seed may germinate immediately, but perish because of lack of conditions for further seedling growth. Occasionally, some fruits are swallowed by birds for their sweet pulp and the seeds are passed out of the body, undigested, because of the hard endocarp. The seeds so dropped are far away from the trees; if they germinate, the seedlings have much better chances of surviving and producing plants, as compared to undispersed seed.
- **Seedling Morphology:** Germination is epigeal. The fresh mature seed, if in humid conditions, may start germinating within a day or so, but fully dry seed may germinate from the 7th day of



sowing and complete it in 25 days. Cotyledons are plano-convex, sub-opposite, lowest one sessile, blade obovate-oblong, stem erect. A few seedlings have an alternate cotyledon; in these, the lower cotyledon has a unilacunar two-trace node and the other a trilacunar three-trace node. In those cases where cotyledons are opposite, both cotyledons have a unilacunar two-trace or trilacunar three-trace condition. During the further course of development, the cotyledon along with the endocarp is pushed above the soil because of the elongation of the hypocotyl in the lower region. The plumule emerging from the cotyledon dislodges the endocarp. The top of the seedling at this stage is green, glabrous or with minute odorless glands. These glands are common in younger leaflets but become fewer in number as the leaf matures. The phenomenon of twin seedlings has also been observed, which may be as high as 11.27 percent. This may be due to the development of one or more than one ovules, out of five of the ovaries (pentacarpellary), giving rise to more than one seed under the same endocarp, but it may be also due to polyembryony, with a frequency of 1 out of 800.





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Popular Article

## Breeding strategies in Neem

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### Strategies for long-term breeding

Breeding strategies are normally evaluated in terms of genetic gain expected for traits of importance, usually over a period of time. Expected gain could be utilized by a proposed selection and propagation system. Several long-term breeding strategies are now available, which are designed to retain sufficient genetic variability to counteract the risks of inbreeding in future generations. In recent years, different strategies and methods have been proposed and used to widen the genetic base of breeding populations for long-term breeding. The Multiple Population Breeding Systems (MPBS) and Hierarchical Open-Ended systems (HOPE) have been suggested for breeding allogamous tree species (Namkoong 1989). In the former, intensive recurrent selection is practiced within multiple independent populations, to create broad differences among them in relation to their source germplasm, their traits and adaptabilities. The latter case, HOPE, is maintained as a very large base population, which is open-ended as far as receiving new materials is concerned. MPBS may be the better choice for neem because it is better suited for combining the highest possible genetic gains and highest possible genetic diversity. It also gives the breeders more options for shifting breeding goals with changing environments and demand (Namkoong 1989; Eriksson et al 1993).

Tree breeders evaluate the genetic resources available for improvement, and select genes of great utility and economic importance and package them in genotypes that can be used to establish commercial plantations. Tree breeders also identify superior genotypes in existing provenances and propagate them clonally to tap both additive and non-additive genetic effects governing commercial traits. In the study, the neem tree has been shown to be an allogamous species. For long-term progress

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in neem breeding, a general flow chart may be suggested. The following major approaches may be included in a long-term improvement program.

- 1 Introduction of provenances (40-50 trees/provenance)
- 2 Establishment of seed production areas (SPA)
- 3 Genetic testing and establishment of seed orchards (SO) include
  - selection of plus trees in the provenances
  - progeny testing of plus trees and
  - establishment of seed orchards for long-terms genetic gains
- 4 Clonal strategies include
  - clonal propagation for selected plus trees for large genetic gains
  - establishment of clone trials and clone banks
- 5 Conservation of genetic diversity

From the above long-term breeding population, short-term breeding populations (5-100 individuals) could be drawn, which are adapted to the local climate having the desired traits. Some promising genotypes may be possible to select for clonal tests directly. The long-term and short term breeding populations will be maintained by continuous population improvement, which would ensure a wide genetic base and long-term progress in neem breeding. The essential features of this long-term breeding population are that the improvement will result from mild selection, which increases the frequency of desirable genes without the loss of neutral genes.

### **Breeding objectives and methods**

Breeding strategies differ considerably with the aims and objectives of the tree improvement program. The neem can be bred for many purposes such as higher fruit yield and other desired agronomic traits. The first category includes the uses as medicine, pesticides, fungicides, nematicides, cosmetics, fodder and organic manure. The latter category includes timber and fuelwood, agroforestry species, shelterbelts, avenue trees, drought and disease resistance.

Multiple stems that produce high biomass, high wood density, and large quantities of fruit with high limonoid and oil content, should be the criteria for selecting provenances for fuel wood production, charcoal, pharmaceutical and pesticide industries. For early establishment of a plantation, especially in dry areas, provenances of neem that show high survival rate and fast growth may be the best choice. In the long-term for utilization as timber, provenances that have a straight stem and more promising intraspecific hybrids could be among the selection criteria. For agroforestry species, a narrow crown with deep-rooted habits; for shelterbelts, resistance to high wind-run and persistent leaf habits; for avenue trees, a larger crown with evergreen features could be selection



criteria.

Net photosynthesis is also an important trait affecting yield. Leaf and leaf stomatal characters, stomatal density, and total guard cell length per unit leaf area, as reliable indicators of stomatal conductance, were suggested as important components for yield. Leaflet ratio, shoot: root ratio, and leaf area could be easily measured traits in breeding for drought tolerant neem.

The strategy to improve several characters simultaneously depends on whether they are controlled by a single major gene or groups of genes. If the characters are positively correlated, improving of one character would improve the other and selection becomes efficient. Independent characters could be selected successively or simultaneously. Strongly negatively correlated traits should be carefully estimated, since improving one character always amounts to lowering the level of other(s). To improve several characters simultaneously, index selection is probably the best choice.

In neem breeding, most of the characters mentioned are probably quantitative in nature. The recent studies indicate that seed diameter, leaflet ratio, and leaflet area were all independent. These traits could be improved without having negative effects on other. In the preliminary studies, no significant negatively correlated traits were observed. The economic traits of interest in this species could be growth, straight bole, crown form, and seed oil, including limonoid contents.

A natural hybrid (*A. siamensis* × *A. indica*) found in Thailand indicates that hybridization among related species is possible and promising for further neem improvement. Natural or artificial hybrids could be introduced both in long- and short-term breeding programs. When breeding neem for biochemicals it may be advantageous to advance to the F<sub>2</sub> generation and select in the recombinant generation.

The usefulness of polyploid and mutation breeding in neem are still questionable. Gene transfer techniques could be useful for producing transgenic neem (Naina et al. 1989). Genes that control drought tolerance and genes for high 'azadirachtin-A' contents could be usefully exploited. The establishment of seed production areas (SPO) from provenance trials is featured as the most effective and time-efficient strategy for short-term neem improvement. For long-term sustainable tree production as well as fruit yield, seed orchards (SO) are suggested.





## Neem and Its Importance

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Neem has also been called “Heal all”, “Divine Tree”, “Village Pharmacy” and even “Nature’s drugstore”. The ancient Indian found many therapeutic uses for the tree and also observed that the tree could survive in very dry and arid conditions. In due course of time, the name and fame of neem spread, not only in the remote areas of the Indian subcontinent but also in the adjoining countries in Asia, now known as Sri Lanka, Malaysia, Indonesia and Thailand. Since ancient times, India has had cultural and commercial relations with the people of these countries. Whereas in folklore mainly the leaves and to some extent the oil was used in Ayurveda (the Indian system of medicine), Siddha (the system of medicine practiced in some parts of south India) and Unani Tibb (the Greco-Persian system of medicine), polyherbal preparations containing one, two or all five parts of the plant, i.e. leaves, bark, flower, fruit and root, called *panchang* in Ayurveda, were used. In the traditional systems of medicine, some of the preparations were for internal administration, while others such as nasal drops, medicated oils or fats were for external application.

European colonizers, on their arrival in India in the sixteenth century, also noticed this important tree and they called it *Margosa*. This term has been widely used in the subsequent literature and until recently neem was called *Margosa indica* and neem oil was known as *margosa* oil. European physicians in India, as well as Indian physicians trained in the orthodox system of medicine (allopathy) and in homoeopathy, saw great virtues in the nineteenth century in the bark of neem both from the stem and the root, but mainly stem bark was used, because of its easy accessibility. The

bark was considered a substitute for cinchona, widely prescribed for malaria and other fevers at that time. Neem bark was included in the Indian Pharmacopoeia, the Indian Homoeopathic Pharmacopoeia and even in the British Pharmaceutical Codex.

At one time it was in the US National Formulary, but it is doubtful if the source of this drug was neem or the closely allied *Melia azedarach*, also called China berry, with which neem has very often been confused. Keeping in view the importance of neem in Indian culture, some studies were carried out in the earlier part of the twentieth century to establish the therapeutic efficacy of the various claims made about it in the traditional systems of medicine. The researchers showed that neem lacked profound pharmacological activity, which was considered important at that time for a herb to be a source of a drug. Neem was also not found effective against any disease, as compared to the other drugs available at that time. The oil with its foetid odor was not acceptable in any form, even for external application.

During the Second World War, because of the scarcity of various raw materials and war needs, research work on the industrial utilization of neem oil started again. These workers filed patents for the pharmaceutical use of neem bitters and for refining the oil.

Ketkar (1976) in the organization Neem Mission, tried to popularize neem products, keeping in view the large number of trees growing in India, and the amount of oil and seed cake they can yield. The Neem Mission propagated the idea of making neem soap from the oil at the village level as a small-scale cottage industry, and the utilization of seed cake, left after the extraction of oil, as a manure and as a denitrifying agent for nitrogenous fertilizers. Due to this effort and that of other agencies, neem soap for toilet purposes became a household name in India.

It was well known to Indian farmers that during invasion avoid the neem tree and that it has an antifeedant property. The research on neem got a new stimulus, when out of 2000 plants investigated for their action against insects, only neem gave promising results. It was found that it was not only effective against insects but also quite safe for human beings and other warm-blooded animals. The active compound was later isolated and identified as azadirachtin. Azadirachtin attracted the attention of workers all over the world, and various studies were published on it.

The commercialization of azadirachtin under the trade name *Margosan-O* and its clearance by the Environmental Protection Agency (EPA) of the USA (Larson, 1987) started a new era of non-hazardous insect controlling agents from plants. It was observed that neem could adapt itself to a dry, harsh and hostile climate and degraded soil, particularly in the dry arid regions of the world, where availability of water is quite poor. It could also be planted for soil reclamation. The tree could provide



much-wanted shade to cattle and man in scorching heat and support undergrowth vegetation. The leaves could be used as fodder for ruminants, particularly at times of scarcity. During a recent drought in Gujarat, a west Indian state, a large number of cattle were saved by feeding them neem leaves. It acts as a wind-breaker, an avenue tree, and the dry leaves that fall on the ground provide organic matter for the soil to support vegetation. The wood can be used as fuel, so scarce in arid regions, and also as timber for household furniture, and for agricultural implements. The seed can provide oil for use in household lamps for illumination, as a lubricant for agricultural machinery, against various pests and diseases and for soap. The oil when applied to leather goods prolongs their life and is also useful as a first-aid medicine for healing wounds and skin diseases of man and domestic animals. The seed cake, after washing, can be used in small amounts in poultry and cattle feed. It may be used as such as organic manure. It not only provides nutrition to plants, but helps in the conservation of nitrogenous fertilizers and the elimination of nematodes.



## Bovine Fasciolosis: A Liver Fluke Disease

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### *Abstract*

Fasciolosis is a disease caused by parasite known as *Fasciola*. The disease mainly seen in ruminants like cattle, buffaloes, sheep and goat and also elephants. Depending upon the animals there are various species of the parasite. The trematode fluke mainly affects the liver of the animal and produces various pathological conditions and as a result the animal become emaciated. In cattle, the chronic liver fluke disease is more common than the acute stage. The disease should be diagnosed and the treatment should be provided with proper anthelmintic. Otherwise, it will lead to serious economic loss to the farmers. Faecal sedimentation technique is the most commonly used diagnostic method for diagnosis of fasciolosis in field condition. Prevention of the disease is done by regular deworming of the animals and the control of snail intermediate hosts.

### **Introduction**

Fasciolosis is basically a parasitic disease caused by a trematode of the family Fasciolidae. There are different species of *Fasciola* found in different animals which causes various pathological conditions particularly affecting the liver of infected animal. *Fasciola* have indirect life cycle which means they require second intermediate host for their development. The adult forms the parasite leave the intermediate host and encyst on plants to become the infective stage. The animal gets the infection by ingestion of contaminated grass. The infected animal will show clinical signs and symptoms depending upon which it is possible to diagnose the disease. On the other hand, there are some other diagnostic procedures also available which can be used to diagnose the disease. Treatment is done by using anthelmintic drugs specific for trematodes.



## Etiology

The primary etiological agent of Fasciolosis in cattle is a trematode parasite named as *Fasciola sp.* There are two species of family Fasciolidae namely *Fasciola hepatica* and *Fasciola gigantica*. They are mainly flatworms having a dorsoventrally flattened body. They are also known as Diastomes. They possess anteriorly situated oral sucker or anterior sucker and posteriorly situated ventral sucker or acetabulum. The mouth is situated at the center of the oral sucker which leads to the pharynx and then to the esophagus. Esophagus ends in two blind ceca which are highly branched. These parasites do not have anus and they excrete their waste materials by regurgitation through mouth. The outer covering is known as tegument which possess spines. The excretory organ is known as Flame cells. *Fasciola hepatica* have broader shoulder than *Fasciola gigantica*. The ventral sucker is present at the level of shoulder and as large as oral sucker. Trematodes are hermaphrodites which means they have both the sexes in single individual. The fine follicular structures fill the lateral fields of the body of the parasite known as vitelline glands. The uterus lies anterior to the testes.

## Life Cycle

*Fasciola* have indirect life cycle. They require intermediate host to become mature fluke. The eggs of the parasite are excreted along with the faeces of the host. The eggs hatch when the temperature is about 26°C. The time required for hatching of egg is about 10-12 days which produces the first larval stage called miracidium. Miracidium is broad anteriorly with a small papilliform protrusion. The tegument of miracidium is ciliated and the organism possess a pair of eye spot. After that the miracidium is ingested by the intermediate host. In case of *Fasciola*, the intermediate host is a snail of genus *Lymnaea*. There are various species of *Lymnaea* which acts as intermediate host of *Fasciola* in different geographical locations. In Europe, Asia, North America and Africa the species is *Lymnaea truncatula* and in Australia it is *Lymnaea tomentosa*. The intermediate host for *Fasciola gigantica* is *Lymnaea auricularia var. rufescens*. The further development of the parasite occurs inside the snail intermediate host. The miracidium further develops into sporocyst. Each sporocyst give rise to five to eight numbers of redia. The redia contains circular thickening behind the level of the pharynx and at the beginning of posterior quarter there is a pair of blunt process. The redia will finally become cercariae. They possess a tail of length twice than that of the body and there is no eye spot. In an around two hours, the cercaria settle on the blades of the grass and the tail casts off from the body of the cercaria. The secretions from the cystogenous glands of the cercaria will form the



cyst of diameter 0.2mm. These cercaria now become infective which are ingested by the definitive host like cattle.

Following ingestion by definitive host, the excystation of the cercaria occurs in the duodenum of the definitive host. After excystation gradually the cercaria migrate into the abdominal cavity and then to the liver. Migration of the flukes in the liver parenchyma occurs in an around five to six weeks of infection and in seven weeks they begin to enter in the main bile duct. Gradually the cercaria reaches sexual maturity and starts producing eggs. In eight weeks of infection eggs can be detected in bile followed by in faeces. The favourable temperature for the development of the egg is 10-26°C. Below 10°C there is no development of the eggs. For the development of snail intermediate host, the requirement is clear stagnant or slow-moving water with high oxygen content and abundant aquatic vegetation.

### **Pathogenesis**

The pathological conditions that are produced in fasciolosis are related to the liver. The pathogenesis can be divided into acute or subacute and chronic type. The types of pathogenesis will depend upon the numbers of metacercaria ingested. The clinical signs and symptoms are different in both the cases.

### **Pathogenesis of Acute and Sub-Acute Fasciolosis**

Acute fasciolosis is less common than that of chronic type. The main pathological condition that can be seen in acute fasciolosis is traumatic hepatitis occurs due to the migration of large numbers of immature flukes in the liver parenchyma which makes haemorrhagic tracts in the liver. If the numbers of immature flukes are more then it may cause rupture of liver capsule and bleeding occurs into the peritoneal cavity.

When the animal survives the acute fasciolosis then it may develop subacute fasciolosis in which the animal lose weight rapidly and the abdomen distended many folds.

### **Pathogenesis of Chronic Fasciolosis**

Chronic fasciolosis is more common in cattle. When the infected animal consumes less numbers of metacercaria for a longer duration of time it causes chronic fasciolosis. The pathogenesis that may develop in chronic fasciolosis are of two types namely, Hepatic Fibrosis and Hyperplastic cholangitis.

When the immature flukes produce migratory tracts in the liver parenchyma during acute phase of the disease, with the advancement of time thrombus formation occurs in the hepatic veins



and liver sinusoids and it produces a state of ischaemia followed by coagulative necrosis of liver parenchyma. Healing and regeneration of the affected area occurs via growth of collagen tissue and lead to Hepatic fibrosis. The hepatic architecture is destroyed when the scar tissue starts its contraction. To restore the normal architecture of liver fibrous tissue connects the migratory tracts to the normal tissues which divides the hepatic parenchyma into many lobules. In an around seven days after infection, lymphocytes, eosinophils and macrophages start migrating from hepatic vein into the surrounding tissues.

The other condition which will arise in chronic fasciolosis is known as hyperplastic cholangitis. This condition is caused by the presence of adult flukes in the bile ducts of the definitive host. Hyperplasia of the bile duct epithelium occurs and infiltration of eosinophils and monocytes in the lamina propria is a characteristic feature. The inflammatory reaction occurs in the bile duct epithelium as the suckers and spines of the adult fluke denude the epithelium. The parasite deposits its eggs in the smaller bile ducts and with the passage of time granulomatous reactions will occur to such eggs. Due to hyperplasia of the biliary system, the ducts become permeable to the plasma proteins and there is a leakage of proteins, particularly albumin. This is reason why hypoproteinaemia develops chronic fasciolosis. The other condition which is seen in chronic fasciolosis is the calcification of the bile duct wall. Due to the calcification the duct protrudes markedly from the surface which is difficult to cut with a knife and resemble the stem of a clay pipe. This phenomenon is known as Clay pipe stem liver. This is a characteristic finding of chronic fasciolosis in cattle. Moreover, *Fasciola hepatica* has been reported to increase the susceptibility of cattle to Salmonella Dublin infection.

### Clinical Signs of Fasciolosis

Clinical signs are the major indications for the diagnosis of the disease. In cattle the disease may produce three types of clinical signs namely acute and chronic. In acute condition the animal may die suddenly and blood-stained froth may appear at the natural orifices similar to anthrax. The abdomen may be distended which is painful and there is severe damage to the liver. Anaemia is also produced due to blood sucking activity of the parasite.

In cattle the chronic case of fasciolosis is more common than the acute one. The major clinical signs that are developed in chronic fasciolosis are mainly anaemia, unthriftiness, anorexia. The mucous membrane become pale and skin become dull. Due to hypoproteinaemia submandibular oedema develop and this condition is known as **bottle jaw** which is a characteristic sign of chronic



fasciolosis. General debility and emaciation are also produced due to the liver involvement. Constipation is common in cattle in which the animal passes faeces that are hard and brittle in nature. Diarrhoea is evident in extreme case. In calves' emaciation and weakness may lead to prostration.

### **Diagnosis of Fasciolosis in Cattle**

The diagnosis of the disease is possible with the help of history and clinical signs. Moreover, faecal sedimentation test is done in the field condition to detect the eggs of the parasite in the faeces of infected animal with help of a microscope. The eggs should be differentiated from the eggs of paramphistomes. The *Fasciola* eggs have yellow shell with indistinct operculum and they are smaller than the eggs of paramphistomes. The shell in the eggs of paramphistomes are transparent and distinct operculum and they are large the differential diagnosis should be done with diseases like Haemonchosis, infectious necrotic hepatitis.

### **Treatment of Fasciolosis in Cattle**

For the treatment of Fasciolosis there are some anthelmintic used which is giving excellent results. The drug of choice to treat the disease is Triclabendazole which is active against both mature and immature flukes. Oxylozanide is an another anthelmintic which is effective against mature flukes. The other anthelmintic which can be used to treat fasciolosis are Rafoxanide, Nitroxynil, Albendazole. Along with the anthelmintic liver supportive should also be provided.

### **Prevention and Control of Fasciolosis in Cattle**

Prevention of fasciolosis is essential as it causes economic loss to the farmers. Fasciolosis can be controlled by regular deworming of cattle. Moreover, the animals should not be allowed to graze in swampy areas as it is a major source of infection. Another method of control of snail intermediate host is the biological control where ducks and frogs are used which can eat the snails and thereby breaks the lifecycle of the parasite. Molluscicides can also be used to control the intermediate host but care should be taken so that it doesn't infect the waterbodies which is dangerous to the aquatic flora and fauna. Moreover, physical destruction of the snail can be done.

### **Conclusion**

Fasciolosis is a common parasitic disease in ruminants especially which may cause serious economic loss to the farmer. So, prevention of the disease is better than treating it after the infection. To prevent veterinarian should be able to create awareness among the farmers regarding the disease and its consequences. The proper deworming schedule should be followed in every livestock farm





to prevent the disease. Also, care should be taken so that animal can no graze on swampy areas where there is a great chance of getting the infection. The infected cattle should be treated as early as possible.

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## Innovations in Veterinary Care: Unleashing the Potential of Regenerative Medicine for Tissue Repair and Pain Management

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### *Abstract*

Regenerative medicine has emerged as a transformative field in veterinary care, offering innovative approaches for tissue repair, pain relief, and enhanced animal well-being. Stem cell therapy, platelet-rich plasma (PRP) treatments, and other regenerative techniques using autologous serum products have shown great promise in promoting healing and reducing discomfort. By harnessing the body's healing mechanisms, regenerative medicine provides non-invasive and effective alternatives to traditional therapies. Stem cells possess remarkable regenerative potential, capable of differentiating into various cell types to facilitate tissue regeneration. PRP therapy utilizes concentrated platelets and growth factors to accelerate healing and reduce inflammation. These regenerative medicine techniques have wide-ranging applications, including orthopedic injuries, degenerative joint diseases, skin wounds, and gastrointestinal disorders. The benefits extend to older animals with compromised healing processes. As research advances, the future holds immense potential for customized regenerative therapies tailored to individual animals.

### **Introduction**

In recent years, veterinary medicine has witnessed remarkable advancements in regenerative medicine. Stem cell therapy, platelet-rich plasma (PRP) treatments, and other innovative techniques are revolutionizing how we approach animal healing and pain relief. With the ability to aid in tissue repair, promote regeneration, and alleviate discomfort, regenerative medicine is reshaping veterinary care and offering new hope for our furry companions.

## **Understanding Regenerative Medicine**

Regenerative medicine focuses on harnessing the body's natural healing processes by delivering trophic and growth factors to restore damaged tissues and organs. Stem cell therapy, in particular, has gained significant attention. Stem cells are unique mesenchymal cells with the remarkable ability to differentiate into various cell types, aiding tissue regeneration and repair. By injecting these cells into injured areas, veterinarians can promote healing and alleviate animal pain.

### **The Power of Stem Cells**

Stem cells have shown tremendous potential in veterinary care, offering a non-invasive and effective treatment option for various conditions. These remarkable cells can differentiate into tissue types, including bone, cartilage, tendons, muscle, corneal, and even nerve cells. This versatility allows them to target specific areas of damage and promote healing.

### **Platelet-Rich Plasma (PRP) Therapy**

Another regenerative medicine technique making waves in veterinary care is platelet-rich plasma (PRP) therapy. PRP is derived from the animal's blood, containing many platelets and growth factors. It can be administered to the injured area through liquid injections or topical platelet gel to promote tissue repair, reduce inflammation, and accelerate the endogenous healing process. It is particularly effective in musculoskeletal injuries like ligament tears, osteoarthritis, and tendonitis. Due to its tendency to promote the formation of massive amounts of granulation tissue, PRP treatment is highly suited for chronic wounds or in cases of considerable tissue loss.

### **Autologous conditioned serum (ACS) Treatment.**

ACS are protein-rich serums isolated on the centrifugation of the animal's blood. These are primarily used for intra-articular administration in horses to treat osteoarthritis. Treatment using ACS consists of an injection into the affected area every 1-2 weeks must be repeated three to five times when necessary.

### **Benefits for Animal Patients**

Regenerative medicine techniques offer numerous benefits for animal patients. First and foremost, these treatments provide a non-surgical and minimally invasive alternative to traditional therapies. They can significantly reduce pain and inflammation, prevent scar tissue formation, and enhance the overall quality of life for animals suffering from chronic conditions. Moreover,



regenerative medicine approaches can restore functionality, allowing pets to regain mobility and enjoy an active lifestyle.

### **Wide Range of Applications**

The applications of regenerative medicine in veterinary care are diverse. From treating orthopedic injuries, degenerative joint diseases, and tendon/ligament damage to addressing skin wounds and gastrointestinal disorders, these innovative therapies can potentially transform treatment outcomes for various conditions without any adverse effects. They are particularly beneficial for older animals, whose healing processes may be slower or compromised.

### **Looking Towards the Future**

As research in regenerative medicine continues to progress, veterinary care is poised for further breakthroughs. Scientists are exploring new sources of stem cells, refining treatment protocols, and expanding the range of conditions that can be effectively treated. The future holds immense potential for customized regenerative therapies tailored to each animal's needs.

### **Conclusion**

Regenerative medicine has ushered in a new era of hope and healing in veterinary care. Stem cell therapy, PRP treatments, and other innovative techniques are providing effective, non-invasive alternatives for promoting tissue repair, reducing pain, and enhancing the well-being of our beloved animal companions. As these technologies advance, we can look forward to a brighter future where regenerative medicine plays an increasingly central role in veterinary care, allowing our furry friends to lead healthier, happier lives.

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## Internal Parasites in Poultry

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### *Abstract*

A parasite is an organism that lives in or on another organism (referred to as the host) and gains an advantage at the expense of that organism. The two types of internal parasites that affect poultry are worms and protozoa. Usually, low levels of infestation do not cause a problem and can be left untreated. Clinical signs of a parasite infestation include unthriftiness, poor growth and feed conversion, decreased egg production and in severe cases death. Parasites can also make a flock more susceptible to diseases or worsen a current disease condition.

### **Introduction**

#### **Round worms**

Roundworms (nematodes) are common in poultry, waterfowl and wild birds. Species of round worms that affect poultry include large roundworms (*Ascaris* spp., also known as ascarids), species of small roundworms (*Capillaria* spp., also known as capillary worms or threadworms), and cecal worms (*Heterakis gallinarum*). Roundworms can cause significant damage to the organs they infest. Most roundworms affect the digestive tract; others affect the trachea (windpipe) or eyes (Tellez *et al.*, 2012).

Large roundworms are the most damaging of the worms common to backyard flocks. A severe infestation can cause a reduction in nutrient absorption, intestinal blockage and death. Easily seen with the naked eye, large roundworms are about the thickness of a pencil lead and grow to 4-5 inches long. Occasionally, they migrate up a hen's reproductive tract and become included in a developing egg. The life cycle of a round worm is direct; that is, worm eggs are passed in the

droppings of infected birds and then directly to birds that consume contaminated feed, water, or feces. Also, worm eggs may be picked up by snails, slugs, earthworms, grasshoppers, beetles, cockroaches, earwigs, and other insects. Known as intermediate hosts, these insects carry the eggs and when eaten by a bird pass the eggs to the bird. Identifying and minimizing the number of intermediate hosts that poultry have contact with helps prevent the birds from being infected with worms. Medication containing the active ingredient piperazine is available for use against large roundworms in poultry but is not effective against other internal parasites of poultry.

**Small round worms** can affect the different parts of birds and cause a variety of symptoms. Species that infect the crop and esophagus cause thickening and inflammation of the mucus membranes located there. Turkeys and game birds are most commonly affected by such species, and producers can suffer severe losses due to these parasites. Other species of small roundworms are found in the lower intestinal tract and cause inflammation, hemorrhage, and erosion of the intestinal lining. Heavy infestations result in reduced growth, reduced egg production, and reduced fertility. Severe infestations can lead to death. If present in large numbers, these worms can be seen during necropsy (examination after death). Small roundworm eggs are very small and difficult to see in bird droppings without a microscope. Medications that contain levamisole are effective in treating small round worms.

Cecal worms are commonly found in chickens. As the name implies, they grow in the ceca (two blind pouches at the junction of the small and large intestines). Although cecal worms typically do not affect chickens, the worms can carry *Histomonas meleagridis*, a species of protozoan parasite that causes histomoniasis (blackhead) in turkeys. Turkeys can contract histomoniasis by eating chicken manure containing infected cecal worm eggs or earthworms that have ingested infected cecal worm eggs. So, although chickens generally are immune to problems caused by cecal worms, controlling the worms is still important for turkey health. Levamisole is effective in controlling cecal worms (Kassem *et al.*, 2021)

#### Tapeworms

Several species of **tapeworms** (cestodes) affect poultry. They range in size from very small (not visible to the naked eye) to more than 12 inches long. Tapeworms are made up of multiple flat sections. The sections are shed in groups of two or three daily. Each section of tapeworm contains hundreds of eggs, and each tapeworm is capable of shedding millions of eggs in its lifetime. Each



species of tapeworm attaches to a different section of the digestive tract. A tapeworm attaches itself by using four pairs of suckers located on its head. Most tapeworms are host specific, with chicken tapeworms affecting only chickens, and so on. Tapeworms require an intermediate host to complete their life cycle. These intermediate hosts include ants, beetles, houseflies, slugs, snails, earthworms and termites. For birds kept in cages, the most likely host is the housefly. For those raised on litter, intermediate hosts include termites and beetles. For free-range birds, snails and earthworms can serve as intermediate hosts, so controlling the intermediate hosts of tapeworms is vital in preventing initial infections and reducing the risk of reinfection.

### **Protozoa**

Protozoa are single-celled organisms found in most habitats, and they include some parasitic pathogens of humans and domestic animals. Protozoan parasites that are important to backyard poultry growers are coccidia (species of the *Eimeria* genus), cryptosporidia (*Cryptosporidium baileyi*) and histomonads (*H. meleagridis*). By far, the most common protozoan parasites of chickens and turkeys are **coccidia**. Nine species of coccidia affect chickens, and seven affect turkeys. Coccidia are species-specific, meaning that coccidia that affect chickens, for example, do not affect turkeys or other livestock. Coccidia live and reproduce in the digestive tract, where they cause tissue damage. This damage reduces nutrient and fluid absorption and causes diarrhea and blood loss (Agriculture Victoria, 2011).

**Coccidiosis** (infection with or disease caused by coccidia) can increase a bird's susceptibility to other important poultry diseases, such as necrotic enteritis. Coccidia are in nearly all poultry. Chicks develop immunity to coccidiosis over time, with most severe cases occurring when chicks are three to six weeks old. Signs of coccidiosis include bloody diarrhea, watery diarrhea, abnormal feces, weight loss, lethargy, ruffled feathers and other signs of poor health. Most store-bought feeds contain medication that controls but does not eliminate coccidia. Eating such feed allows young birds to develop resistance to the coccidia prevalent in their environment. However, if the birds are exposed to a different species of coccidia, they will not have immunity, and disease symptoms may result. A common medication for controlling coccidiosis in birds not fed medicated feed is amprolium. As mentioned above, following the instructions for administration is important for proper drug delivery and bird recovery. Vaccines are currently available that give newly hatched birds a small amount of



exposure to coccidia, allowing them to develop immunity without developing the disease. With proper vaccination and management, routine anticoccidial medications are not necessary.

Cryptosporidiosis is infection with or disease caused by **crypto sporidia**. Crypto sporidia are not specific to chickens and can infect other birds and even mammals. Crypto sporidia frequently spread from flock to flock on the feet of animals and people and can be carried by wild birds. Intestinal cryptosporidiosis is common, and symptoms are usually mild. Frequently, the only symptom is pale skin in yellow-skinned breeds. Cryptosporidiosis also can be contracted by inhalation, resulting in a respiratory infection that is more severe than the intestinal form. There is no treatment for this form of cryptosporidiosis. Providing supportive therapy and guarding against secondary infection are the only courses of action. Once recovered, birds are immune to future infection.

As mentioned previously, **histomoniasis** is a disease of turkeys caused by **histomonads**, protozoan parasites carried by cecal worms. Histomoniasis is a serious, even deadly disease and is most common in range-raised birds. Turkeys raised with access to chicken fecal material or earthworms that have ingested cecal worm eggs pick up histomonads and develop the disease. There is no effective treatment for histomoniasis. The only effective control is to control cecal worms, thereby reducing the spread of histomonads. Also, you should not house or range turkeys with chickens or in areas where chickens recently have been.

### **Prevention Of Parasitic Infection in Poultry**

- Infection with all internal parasites in poultry can be controlled by keeping birds in clean conditions and stopping them wandering around free.
- Cages and houses should be kept clean with droppings removed every week.
- Feed and water containers should be cleaned out every day.
- Do not allow wet muddy areas to develop around water containers or anywhere else.
- Cages and houses should be thoroughly cleaned before new birds are placed in them.
- Do not keep birds on the same area of ground year after year as contamination of the soil will increase.
- Purchase chickens as newborn chicks. If you purchase adult birds, quarantine them for a minimum of two weeks to monitor their health and assess for potential disease and parasite symptoms.





- Do not place young birds, especially under 3 months old, in runs where older birds have recently been kept as they may develop infection with coccidian
- One sick bird can infect the rest of the flock simply by contaminating the waterers. Test and sanitize the water, and keep waterers and feeders cleaned to help control or reduce the chance of spreading infection.
- Provide regular deworm protocols in all the birds to control the parasitic infection
- Use all-natural supplement to help breakdown the natural defenses of intestinal worms and their eggs. This makes parasites more susceptible to attack by the bird's immune system, stomach acids and bacteria in the gut.

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## Hypomagnesemia in Sheep

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### Introduction

Hypomagnesemia is an acute metabolic disease of sheep, characterized by a variety of clinical signs like excitability, grinding of the teeth, salivation, ataxia, recumbency, and tetanic muscle spasms and is caused by a depressed supply of metabolizable magnesium from prolonged consumption of lush immature grasses. It is also known as grass tetany or grass staggers or wheat pasture poisoning. Adult lactating animals are most susceptible because of the loss of Mg in milk. Due to its wide geographic distribution and as a causative of extensive economic losses, it has major importance and high concern for the entire sheep industry. Economic losses result from deaths, determent of full utilization of pasture feed and the cost of preventive programs.

### Occurrence

Hypomagnesemia occurs in all breeds of lactating sheep grazing early spring pastures. Although most cases develop in ewes during the first 4 wks of lactation, other classes of sheep occasionally develop the disease. Most cases occur during spring and autumn following rains and rapid growth of grasses.

### Etiology and pathogenesis

- Continuous and exclusive grazing on lush grasses causes Hypomagnesemia.
- In normal sheep, the plasma levels of magnesium and calcium are 2-3 mg/dl and 8-12 mg/dl, respectively. Grazing lush grasses may depress the plasma levels of Mg and Ca to pathogenic levels and result in the disease.

- Lush green plants growing on ammonium fertilized soil, contain high concentrations of protein, potassium and aconitate.
- Absorption of ammonia by plants results in reduced uptake of Magnesium and Calcium with little effect on potassium and produces high amide concentrations in plants with carbohydrate depletion. These factors combine in the animal to create high conc. of free ammonia in the rumen, an increase in rumen pH and depletion of carbohydrate and further reduces the availability of Mg and Ca.
- Mobilization of Mg from its reserve in bone to extracellular fluid commences when plasma Mg level falls below 1.8 mg/dl. When this reservoir is exhausted the plasma level of Mg falls; when the conc. reaches about 1.0 mg/dl, the plasma Ca levels also declines. When it reaches 0.7 mg/dl, irritability develops, and when it reaches 0.5 mg/dl, fatal tetany and convulsions ensue. Death probably results from respiratory failure. Recovered animals are susceptible to recurrence of the disease.

### **Clinical signs and Postmortem lesions**

In acute cases:

- Affected sheep which may appear to be grazing normally, suddenly throw up their heads, gallop in a blind frenzy, fall, and exhibit severe paddling seizures
- chomping of the jaws
- frothy salivation
- fluttering of the eyelids and nystagmus
- Seizures may recur at short intervals, and death usually occurs within a few hours
- In many instances, animals at pasture are found dead without observed illness.

In less severe cases:

- Hyperesthesia
- Twitching of ears
- Grinding of teeth
- Small muscle fasciculations
- Breathing acceleration
- Tremors, walking and running movements
- Muscular incoordination



- Mild cases may recover spontaneously and episodes may recur.
- Some fall to a recumbent position, convulse, pass into coma and finally die

**Post mortem lesions:** At necropsy, petechial and echymotic hemorrhages over serosal surfaces of heart and intestines

### Diagnosis

- Based on clinical signs and laboratory findings
- History of excitement and convulsions among lactating ewes grazing lush grasses strongly suggest the disease.
- Plasma Mg levels of 0.50 to 0.25 mg/dl in blood

### Differential diagnosis

should be differentiated from hypocalcemia and enterotoxaemia.

- Hypocalcaemic animals respond to intravenously administered Calcium boro gluconate and their blood analysis shows plasma Ca levels as low as 3 mg/dl and Mg levels near normal values.
- Enterotoxaemia causes sudden deaths and affected animals contain identifiable toxin in the small intestine.

### Prevention and treatment

- It can be Prevented by oral feeding of crude magnesium oxide either by individual dosing with 15 g dissolved in water and given at 2day intervals or by feeding in a mineral mix containing 20% of the compound.
- Fertilizers rich in potassium and nitrogen reduce the availability of magnesium from the pasture, and increase the risk of grass tetany. Hence, animals should not be allowed for grazing these pastures soon after fertilizer application.
- Treatment includes parenteral administration of 50 ml of a 20% solution of calcium boro gluconate and 25 ml of 50% solution of magnesium sulfate.
- Daily oral supplements of Mg oxide (10 g) should be given in the danger period

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## Important Tips of Animal Disease Outbreak Management In Field Conditions

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### *Abstract*

Investigations of disease outbreaks in livestock always considered as a great challenge to the field veterinarians owing to limited access of sophisticated diagnostic tools as well as lack of availability of suitable laboratory facilities. This article envisages a spectrum of activities like clinical diagnosis to sampling as well as vaccination, epidemiological risk factor assessment, strengthening bio-security measures related to outbreak management which will refresh the knowledge of all stakeholders including veterinarians, animal owners and Govt. officials.

### **Introduction**

An animal disease outbreak occurs when a specific infectious disease affects a significant number of animals within a particular population, region or species over a particular period of time. The occurrence of disease cases surpasses the normal expectancy. There is rapid and widespread transmission of the disease resulting in a higher incidence of illness and mortality among the effected ones. Animal disease outbreaks can fuel significant economic, social and public health implications. Therefore, it necessitates the requirement of a systemic and organized approach to control the spread of the disease and minimize its impact on animal populations. This article outlines some key steps and considerations for managing a veterinary disease outbreak in field conditions.



## **1. Early detection and reporting**

Early detection of an outbreak is crucial for prompt intervention. There should be strong surveillance system to promptly monitor for any signs of illness or disease in the animal population and report to the appropriate authorities.

## **2. Rapid Response**

Once a disease outbreak is suspected or confirmed, it is crucial to respond quickly. This involves mobilizing resources, activating an emergency response team comprising veterinarians, animal health professionals, and relevant stakeholders, as well as implementing control measures.

## **3. Quarantine and isolation**

Strict quarantine and isolation measures to prevent the spread of the disease should be implemented. Isolate infected animals from the healthy ones. Healthy animals should be moved to a different geographical location and restrict the movement of the affected hosts.

## **4. Disease diagnosis**

Accurate diagnosis is crucial to confirm the field outbreak diseases timely and take appropriate control measures accordingly. Field diagnostic tools, on site laboratory facilities, rapid diagnostic tools through haemato-biochemical evaluations and techniques can be utilized for this purpose.

## **5. Control of vectors, intermediate host and reservoir**

Vectors, intermediate host and reservoirs play a crucial role in transmission of the disease. In order to control vectors insecticides can be applied to vector breeding sites. Introducing natural predators and parasites that target them can eliminate vectors by competitive exclusion. Sterile male technique and other biotechnological methods that alter factor at genetic level can be introduced to control their population. Proper and timely vaccination, quarantine and isolation of the animals suspected of carrying the disease, surveillance and early detection of disease in intermediate hosts can allow for prompt intervention and control measures.

## **6. Ecological Niche filling**

This practice is prevalent in the poultry industry where endogenous intestinal microbes are fed to day old chicks so that no space is available for the growth of pathogenic microbes such as salmonella, campylobacter and e coli.



## 7. Strengthen biosecurity measures

It refers to set of measures and protocols to prevent the introduction and spread of pathogens from infected animals to susceptible ones. Control access to farm premises. Visitors should be restricted, and proper entry and exit procedures should be in place for staffs, vehicles and equipment. Strict hygiene practices including cleaning and disinfection of facilities, feed, water, equipment, vehicles should be practiced regularly. Personnels should use appropriate protective clothing, footwear and follow hand hygiene protocols. Testing before and after entry to the herd, isolation and quarantine of incoming animals, effective vaccination and prophylactic treatment should be implemented strictly.

## 8. Vaccination

Ring vaccination, barrier vaccination and dampening down protocols should be followed.

- ❖ **Ring vaccinations** -It is a strategy used to control the spread of disease by vaccinating animals in a ring or buffer zone covering a radius up to 3 KM around an outbreak area. It involves vaccinating susceptible animals that are in close proximity to infected or at-risk animals. It aims to create a barrier of immune animals around the outbreak area, preventing spread of the disease to unaffected populations.
- ❖ **Barrier vaccination:** It is a strategy used to prevent the spread of diseases by vaccinating animals along natural barriers, at the boundary of geographical areas or man-made barriers. The primary goal is to create a vaccinated buffer zone between infected areas and susceptible populations, blocking the transmission of the disease.
- ❖ **Dampening down**-It is a strategy where vaccination is done in the infected zone to reduce the chance of transmission of the ailment.

## 9. Slaughter and culling

Mass slaughter is practiced during bird flu to avoid the risk of contamination of the environment and transmission of the highly infectious disease.

## 10. Mixed, Alternate and Sequential grazing

Mixed grazing involves the simultaneous grazing of different livestock species such as cattle, sheep and goats within the same pasture. It helps control pathogenic parasites as some livestock are less susceptible to some parasites than others. Alternate grazing also known as Rotational grazing or paddock grazing involves dividing a pasture into several smaller sections called paddocks. Livestock are then rotated between these paddocks at regular intervals allowing the parasitic life



cycle to break as they die off in vacant pastures without host. Sequential grazing also known as strip grazing involves moving livestock through a series of sequentially arranged pastures or strips.

### **11. Hygienic disposal of carcass**

Carcass should never be disposed off at or near water source and should not be kept for long in shed to avoid contamination of soil and prevent vector approach. Unless approved by veterinarian, it is not safe to open a carcass. Deep burial method which is the most common method of carcass disposal should be practiced. Burning and incineration method have advantage over burial method as it completely destroys pathogens and carcass.

### **12. Communication and coordination**

Establish efficient communication channels and coordination mechanisms among all relevant stakeholders, including veterinary authorities, farmers, veterinary professionals, and public health officials. Regular updates on the outbreak situation, control measures, and any necessary precautions should be circulated widely.

### **13. Education and Training**

Proper educations should be given to animal owners, farmers, veterinarians, technicians and related stakeholders regarding the disease, its signs and symptoms, mechanism of transmission, prevention and control measures to combat the disease effectively. Training programs can enhance the capacity to detect, respond to, and manage outbreaks.

### **14. Continuous Monitoring and Surveillance**

Even if the outbreak comes under control, ongoing surveillance is necessary to monitor the disease situation and track the effectiveness of control measures. It also helps to detect any new cases and prevent resurgence.

### **15. post-outbreak evaluation**

Once the outbreak is under control, conduct a comprehensive evaluation to assess the efficiency of the response and identify areas for improvement. This will help strengthen future outbreak management strategy.

### **Conclusion**

When an animal disease outbreak occurs, it is crucial to quickly identify the source of disease, potential risk factors, understand its transmission dynamics and epidemiological risk factors, and implement appropriate measures to control and prevent further spread. The above control measures and tips including early detection, reporting, quarantine measures, effective vaccination, biosecurity





measures, continuous monitoring and surveillance system should be kept in mind and action should be taken immediately to combat the situation.

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## A brief focus on cell culture and its techniques

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### *Abstract*

Cell culture involves a complex of processes of cell isolation from their natural environment (*in vivo*) and subsequent growth in a controlled environmental artificial condition (*in vitro*). Cells from specific tissues or organs are cultured as short term or established cell lines which are widely used for research and diagnosis, most specially in the aspect of viral infection, because pathogenic viral isolation depends on the availability of permissible cell cultures. Cell culture provides the required setting for the detection and identification of numerous pathogens of humans, which is achieved via virus isolation in the cell culture as the “gold standard” for virus discovery.

### **Introduction**

Cell culture is a fundamental technique used in various fields of biological research and biotechnology. It involves the growth and maintenance of cells in a controlled laboratory environment, providing an artificial system that allows researchers to study cellular behavior, function, and responses under controlled conditions. Cell culture has revolutionized our understanding of cell biology, disease mechanisms, drug development, and tissue engineering.

The process of cell culture typically begins with the isolation of cells from a tissue or organism of interest. These cells are then cultured *in vitro*, meaning outside of their natural environment, in specialized containers called culture dishes or flasks. The cells are placed in a suitable growth medium, which contains all the necessary nutrients, growth factors, and signaling molecules required for their survival and proliferation.

The growth medium is carefully formulated to mimic the physiological conditions that cells would experience in their natural environment. It usually consists of a balanced mixture of salts,

amino acids, vitamins, sugars, and other components necessary for cell growth. Additionally, serum, such as fetal bovine serum, may be added to provide essential proteins and factors that support cell growth.

Maintaining a sterile environment is crucial in cell culture to prevent contamination by bacteria, fungi, or other microorganisms that could compromise the integrity of the culture. Therefore, strict aseptic techniques are employed, including working in a laminar flow hood, using sterilized equipment, and regularly testing for contamination.

Cells in culture require a controlled environment to thrive. This typically involves maintaining specific temperature, humidity, and carbon dioxide (CO<sub>2</sub>) levels to mimic physiological conditions. Specialized incubators are used to provide these optimal conditions, ensuring the cells' viability and growth.

Cell culture techniques can be classified into two broad categories: primary cell culture and cell lines. Primary cell culture involves culturing cells directly derived from tissues or organs, providing a more representative model of the *in vivo* system. On the other hand, cell lines are immortalized cells that have undergone genetic modifications or acquired the ability to proliferate indefinitely in culture. Cell lines are widely used due to their convenience and availability, although they may not fully recapitulate the characteristics of the original tissue.

Cell culture is a versatile tool with numerous applications in biomedical research. It allows scientists to investigate cell behavior, cellular responses to stimuli, cell signaling pathways, and the effects of drugs or toxins. It also plays a vital role in producing biological molecules, such as therapeutic proteins or vaccines, through large-scale production of cells in bioreactors.

In summary, cell culture is a powerful technique that enables the growth and study of cells in a controlled laboratory environment. It has revolutionized our understanding of cellular biology, disease mechanisms, and drug development, and continues to be an essential tool in various scientific and medical fields.

## Cell Culture Technique

There are several types of cell culture techniques, each serving different purposes and applications. Here are some common types of cell culture:

1. **Adherent Cell Culture:** Adherent cell culture involves growing cells that require attachment to a surface for growth and proliferation. These cells adhere to the culture vessel, such as a petri dish or flask, and form a monolayer. Examples of adherent cell types include fibroblasts, epithelial cells, and endothelial cells.
2. **Suspension Cell Culture:** Suspension cell culture involves growing cells that can thrive and proliferate in a suspended state without requiring attachment to a surface. These cells are typically grown in culture vessels such as flasks or bioreactors that allow for efficient mixing and aeration



of the culture medium. Suspension cell culture is commonly used for culturing immune cells, lymphocytes, and certain types of cancer cells.

3. **Primary Cell Culture:** Primary cell culture involves the isolation and culture of cells directly derived from tissues or organs. These cells maintain their original characteristics and closely resemble the in vivo environment. Primary cell cultures are often used to study cell behavior, tissue development, and disease models. However, they have a limited lifespan and can undergo senescence after a few passages.
4. **Cell Line Culture:** Cell line culture involves the growth of immortalized cells that have undergone genetic modifications or acquired the ability to proliferate indefinitely. These cell lines are derived from primary cells but have been altered to bypass senescence and possess an unlimited growth potential. Cell lines are widely used in research and biotechnology due to their convenience and availability. Well-known examples include HeLa cells, HEK293 cells, and CHO cells (Chinese Hamster Ovary cells).
5. **Organoid Culture:** Organoid culture involves the growth of three-dimensional structures that resemble specific organs or tissues. Organoids are derived from primary cells or stem cells and can recapitulate the architecture and functionality of the original organ to a certain extent. They are valuable tools for studying organ development, disease modeling, and drug screening.
6. **Co-Culture:** Co-culture involves culturing two or more different cell types together in the same culture system. This technique allows researchers to study cell-cell interactions, cell signaling, and complex cellular responses in a more physiologically relevant context. Co-culture can involve different cell types from the same tissue or different tissues altogether.
7. **3D Cell Culture:** 3D cell culture techniques aim to recreate the three-dimensional architecture and microenvironment found in vivo. These cultures can involve scaffolds, hydrogels, or other support structures that provide a matrix for cells to grow and interact. 3D cell cultures are advantageous for studying cell behavior, tissue development, and drug screening, as they better mimic the in vivo environment compared to traditional two-dimensional cultures.

## Cell Culture Application

Cell culture has numerous applications across various fields, including biomedical research, biotechnology, pharmaceutical development, and regenerative medicine. Here are some common applications of cell culture:

1. **Basic Cell Biology Research:** Cell culture is extensively used in basic research to study fundamental cellular processes, such as cell division, differentiation, metabolism, and gene expression. Cultured cells provide a controlled and reproducible system for investigating cellular mechanisms.



2. **Drug Discovery and Development:** Cell culture plays a crucial role in drug discovery and development. Researchers can use cultured cells to test the efficacy, toxicity, and side effects of potential drug candidates before conducting animal or clinical trials. Cell culture models can provide valuable insights into drug absorption, distribution, metabolism, and excretion.
3. **Disease Modeling:** Cell culture allows scientists to create disease models by culturing cells derived from patients with specific diseases. These models, known as "in vitro disease models," enable researchers to study disease mechanisms, screen potential drugs, and develop personalized medicine approaches. Examples include cancer cell lines, neuronal cell cultures for neurodegenerative diseases, and hepatocyte cultures for liver diseases.
4. **Vaccine Development:** Cell culture is essential for vaccine production. Viruses or bacteria used in vaccines are often grown in cell cultures to generate large quantities of the infectious agents. Cell culture systems, such as Vero cells for polio vaccine or chicken embryonic cells for influenza vaccine, provide a controlled environment for efficient viral replication.
5. **Tissue Engineering and Regenerative Medicine:** In tissue engineering, cell culture is used to grow and manipulate cells outside the body to create functional tissues and organs. Researchers can seed cells onto biodegradable scaffolds in the culture dish, allowing the cells to proliferate and differentiate into specific tissue types. These engineered tissues can potentially be used for transplantation or as models for studying tissue development and disease progression.
6. **Toxicity Testing:** Cell culture is employed in toxicity testing to assess the potential adverse effects of chemicals, drugs, or environmental factors on living cells. By exposing cultured cells to various substances, scientists can evaluate cell viability, proliferation, and specific cellular responses, helping to identify and understand potential hazards.
7. **Bioproduction:** Cell culture is used for large-scale production of biopharmaceuticals, such as recombinant proteins, monoclonal antibodies, and vaccines. Cultured cells, such as Chinese hamster ovary (CHO) cells or human embryonic kidney (HEK) cells, are genetically modified to express the desired protein or vaccine antigen and are grown in bioreactors to produce high yields of the desired product.

### **Difficulties and their prevention in cell culture: -**

Cell culture is a fundamental technique used in various fields of research, including biomedical, pharmaceutical, and biotechnological applications. However, there can be several difficulties associated with cell culture, which can impact the success and reproducibility of experiments. Here are some common difficulties encountered in cell culture and prevention measures:



1. **Contamination:** Contamination with bacteria, fungi, yeast, or other cell lines is a significant challenge in cell culture. It can lead to unreliable results and the loss of valuable cell lines. To prevent contamination:
  - Practice strict aseptic techniques, including proper handwashing, wearing sterile gloves, and working in a laminar flow hood.
  - Regularly clean and disinfect the cell culture hood, incubators, and other equipment.
  - Test and quarantine new cell lines or reagents before introducing them into the cell culture environment.
  - Use antibiotics, antifungal agents, or other appropriate agents in the culture media, if necessary.
2. **Cell line misidentification:** Accidental misidentification or cross-contamination of cell lines can occur, leading to erroneous results and wasted efforts. To prevent cell line misidentification:
  - Authenticate cell lines regularly using techniques such as DNA profiling, short tandem repeat (STR) analysis, or other reliable methods.
  - Maintain a detailed record of the origin and passage history of each cell line.
  - Handle and store cell lines separately to avoid cross-contamination.
3. **Cell adhesion and growth issues:** Certain cell lines may have difficulties adhering to the culture vessel or growing properly. To address adhesion and growth issues:
  - Optimize the culture conditions, including the type of culture vessel, coating materials (e.g., extracellular matrix proteins), and culture media formulation.
  - Adjust the seeding density to achieve an optimal cell density for growth.
  - Periodically check and maintain the health of the cell lines, ensuring they are free from viral infections and genetic alterations.
4. **Cell death and apoptosis:** Cells can undergo cell death or apoptosis due to various factors, including incorrect culture conditions, oxidative stress, or suboptimal handling. To prevent cell death:
  - Optimize the culture conditions, including temperature, pH, humidity, and gas composition (e.g., CO<sub>2</sub> levels).
  - Use appropriate culture media and supplements to support cell survival and growth.
  - Handle the cells gently during passaging, avoiding excessive pipetting or mechanical stress.
5. **Cross-contamination between cell lines:** Cross-contamination can occur when cells from different lines come into contact, leading to the contamination of both cultures and compromised experimental outcomes. To prevent cross-contamination:
  - Strictly adhere to proper aseptic techniques and avoid mixing cells from different lines.
  - Designate separate areas and equipment for different cell lines, minimizing the risk of accidental cross-contamination.
  - Regularly monitor and confirm the identity of cell lines to ensure their purity.



6. **pH shifts and osmotic stress:** Fluctuations in pH or osmolarity can affect cell viability and functionality. To prevent pH shifts and osmotic stress:
  - Maintain proper pH and osmolarity levels in the culture media by regularly monitoring and adjusting as needed.
  - Use appropriate buffering systems to stabilize pH.
  - Carefully calculate and adjust the concentration of osmolites (e.g., salts, sugars) in the culture media.
7. **Nutrient depletion and waste accumulation:** Over time, cell culture media can become depleted of essential nutrients, while waste products accumulate, adversely affecting cell growth and function. To address nutrient depletion and waste accumulation:
  - Regularly change the culture media to ensure an adequate supply of nutrients and remove waste products.
  - Optimize the feeding regimen based on the specific requirements of the cell line (e.g., batch culture, fed

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## Canine Ehrlichiosis – An Overview

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### Introduction

Canine ehrlichiosis is caused by gram-negative, obligate intracellular, pleomorphic bacteria of the genus *Ehrlichia* (order Rickettsiales, family Anaplasmataceae). *Ehrlichia* spp. infect primarily leukocytes, forming intracytoplasmic, membrane-bound bacterial aggregates, called morulae (Sainz et al., 2015). At least five tick-transmitted *Ehrlichia* species have been documented to infect dogs, potentially causing the clinical disease (Sainz et al., 2015; Harrus et al., 2012). *Ehrlichia canis* was the first species recognized to infect dogs and is the principal cause of canine monocytic ehrlichiosis (CME) [Sainz et al., 2015, Donatien et al., 1935, Neer et al., 2002]. However, currently, this disease has a global distribution mainly in tropical and subtropical regions. (Kukreti et al, 2018). *Ehrlichia* spp. infect primarily leukocytes, forming intracytoplasmic, membrane-bound bacterial aggregates, called morulae (Sainz et al., 2015). The course of infection is divided into acute, subclinical and chronic phases (Harrus et al., 2012).

### Taxonomy

Three different *Ehrlichia* species can cause canine ehrlichiosis: *E. canis*, *E. chaffeensis* and *E. ewingii* (see Tab. 1). *E. canis* causes canine monocytic ehrlichiosis (CME). This disease, also known as tropical canine pancytopenia, canine rickettsiosis or canine hemorrhagic fever, was first described in Algeria in 1935 by Donatien and Lestoquard. CME has since been reported in many





parts of the world, mainly in the tropical and subtropical regions. However, the geographical distribution of *E. canis* is expanding alongside that of its main tick vector, the Brown Dog tick, *Rhipicephalus sanguineus*. *E. canis* form microcolonies within a membranelined intracellular vacuole (so-called morula), primarily in monocytes and macrophages of mammalian hosts. The pathogen replicates only in the cytoplasm of monocytic cells, and the formation of morulae is a defining characteristic that can be used for diagnosis.

**Table 1: Three different *Ehrlichia* species can cause canine ehrlichiosis**

Species	Common name	Host	Cells most commonly infected	Primary vectors
<i>Ehrlichia canis</i>	Canine Monocytic Ehrlichiosis (CME)	Dogs and other members of the family Canidae, cats, humans	Primarily mono -nuclear cells (Monocytes and lymphocytes)	<i>Rhipicephalus sanguineus</i> , <i>Dermacentor variabilis</i>
<i>E. chaffensis</i>	Human Monocytic Ehrlichiosis (HME)	Humans, deer, horses, rodents	Monocytes, macrophages	<i>Amblyomma americanum</i> , <i>Dermacentor variabilis</i>
<i>E. ewingii</i>	Canine Granulocytic Ehrlichiosis (CGE), Human Monocytic Ehrlichiosis (HME)	Dogs, humans	Primarily neutrophils and eosinophils	<i>Amblyomma americanum</i> , <i>Otobius megnini</i>

### Life cycle

- Infection is transmitted to dog through bite of infected *R. sanguineus*
- Transmission in tick occurs transtadially
- Larvae & nymphs become infected while feeding on rickettsaemic dogs and transmit infection to host after moulting to nymph & adult respectively.

### Pathogenesis

- Incubation period - 8-20 days; organism multiply in macrophage of mononuclear phagocytic system by binary fission
- Thrombocytopenia
- CME has 3 phases
- Acute phase
- Subclinical phase
- Chronic phase



### Acute phase:

- Develops 1-3 weeks after tick bite & lasts for 2-4 weeks
- Fever, anemia, depression, loss of appetite
- Stiffness and joint pain
- Liver, lymph node, spleen enlarged

### Sub-clinical phase:

Animal may appear normal or show slight anemia

- Dogs remain persistent carriers in this phase
- Platelets subnormal

### Chronic phase:

- Tropical pancytopenia occurs due to bone marrow hypoplasia
- Affected animals develop extensive serosal and mucosal haemorrhage
- Epistaxis, bleeding from inner aspect of thigh & forearm also occurs
- Animals prone to renal infection

### Clinical signs:

- Fever, lethargy, weight loss, loss of appetite, abnormal bleeding
- Enlarged lymphnode, enlarged spleen, pain & stiffness
- Anterior uveitis, chorioretinitis, & retinal hemorrhage

### Diagnosis:

Blood smear examination  
Examination of buffy coat  
Lymphnode aspirate

### Treatment:

- Oxytetracycline @7.5-10 mg/kg every 8hrs for 21-28 days or doxycycline @ 5mg/kg every 12hrs for 21-28days
- Supportive treatment
- Severe cases- blood transfusion

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## Role of Probiotics in Aquaculture

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### Introduction

- The world “probiotics was coined by Parker (1974), and defined as “Organisms and substances that give to intestinal microbial balance.
- Fuller (1989) revised the definition as “live microbial feed supplement which beneficially affects host animal by improving its intestinal microbial balance”.
- Probiotics are often termed as “friendly”, “beneficial”, “good” or “helpful” bacteria, because they help keep the gut healthy. More recently, the probiotics are defined as “live microorganisms” that when administered in adequate amounts confer a health benefit on the host (FAO / WHO, 2001).



### Why we have to use probiotics



- During the last decades, antibiotics used as traditional strategy for fish diseases management and also for the improvement of growth and efficiency of feed conversion. An alternative approach to manage fish and shrimp health, that is fast gaining attention in aquaculture industry is, “probiotics”, a microbial intervention approach for disease prevention and control, high survival and growth by enhancing the feed conversion efficiency

**Generally, probiotics refers bacteria belonging to gram positive especially**

<b>Lactobacillus sps,</b>	<b>Weissella sps.</b>
<b>Bifidobacterium sps</b>	<b>Carnobacterium sps.,</b>
<b>Streptococcus,</b>	<b>Enterococcus sps.,</b>
<b>Lactococcus sps.</b>	<b>Microalgae (Tetraselmis) and Yeasts (Debaryomyces)</b>
<b>Streptococcus sps.</b>	<b>Lactobacillus sps.,</b>

### Types of Probiotics

They are 3 types of probiotics, as mentioned below:

1. Water Probiotics – Applied to pond water
2. Soil Probiotics – Mixed with sand and applied to pond bottom
3. Feed / gut Probiotics – Given as feed supplement



**Water probiotic**



**Soil probiotic**



**Gut probiotic**

### Water Probiotics

These are marked in 2 forms

I) Dry forms

II) Liquid forms.

- Liquid forms give positive results in lesser time, when compared to the dry and spore form bacteria, though they are lower in density
- These play major role in improving the water quality of culture pond.
- Dose 1kg or 1 litre per hectare



### Soil probiotics

- Bacteria like Rhodococcus, Nitrobacter, Nitrosomonas and Sulphur reducing bacteria clean the bottom of aqua ponds.
- Dose 20-25 litre per hectare

### Feed / gut probiotics

Lactic acid bacteria.

- Probiotics act as a microbial nutritional medicine that benefits the host health condition by reducing mucosal and systemic immunity and improving the physiological and nutritional actions. These enhance the fish and shrimp feed efficiency by stimulating digestive enzyme and maintain the balance of intestinal microbes, resulting in improved nutrient absorption, utilization and ultimately the survival, growth of fish and shrimp.
- Dose 3-5gm/kg feed



### Role of Probiotics

- The three types of probiotic bacteria can be directly applied to soil, water of the farming pond and also as an additive to feed.
- Various commercial probiotics are available in the market in different combinations and bacterial counts.
- Reports inform that use of probiotic bacteria reduces mortality rate. However, the quantity of cells presents in the probiotic, given with feed plays a major role in the survival of animals.
- The bacterial count of  $10^9$  g-l is ideal than  $10^{12}$  g-l and it indicates that the increasing of bacterial count does not offer protection to the animals.
- Mixed probiotic bacterial species yields better result by enhancing lysozyme activity, migration of neutrophils, and plasma bactericidal activity, than probiotic with single species.
- Now a days, probiotics like yucca, glucans etc are also included in the probiotic preparation which are non-digestible ingredients and help in stimulating the growth of probiotic bacteria especially in colon region of fish.
- Probiotic bacteria are isolated from the pond, sediment, soil, water and animal. The potential effect of probiotic relies on the source from which the bacteria are isolated and the way of application. So, it cannot be considered that all the commercial probiotics available in the market are potential one and they many vary based on the source and type.

The effect of probiotic is depending on various factors like mainly the type of probiotic, the dosage, method of application, duration of application, frequency of application. The commercial probiotics available in market are either in liquid or powder form. Instead of applying these products directly in the pond water or soil, it may be allowed for further fermentation mixing with jaggery for a period of 4-6 hrs which would improve the viability and functionality of microbes for better performances.



The probiotics are also prepared in encapsulated form through various processes like emulsification, extrusion, spray drying and adhesion to starch etc. This encapsulation aids to prevent the damage of probiotic bacteria from low pH and other digestive enzymes of GIT. If the probiotic bacteria survive well in the intestine, then the performance of bacteria will be effective against the infectious. The viability of probiotic depends on the method of production, storage temperature, survival and stability in the intestinal tract.

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## Environmental DNA - As a Technology for Assessment of Biodiversity

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### Introduction

- **Environmental DNA** or **eDNA** is DNA that is collected from a variety of environmental samples such as soil, seawater, snow or air, rather than directly sampled from an individual organism.
- As various organisms interact with the environment, DNA is expelled and accumulates in their surroundings from various sources.

#### A brief history of eDNA

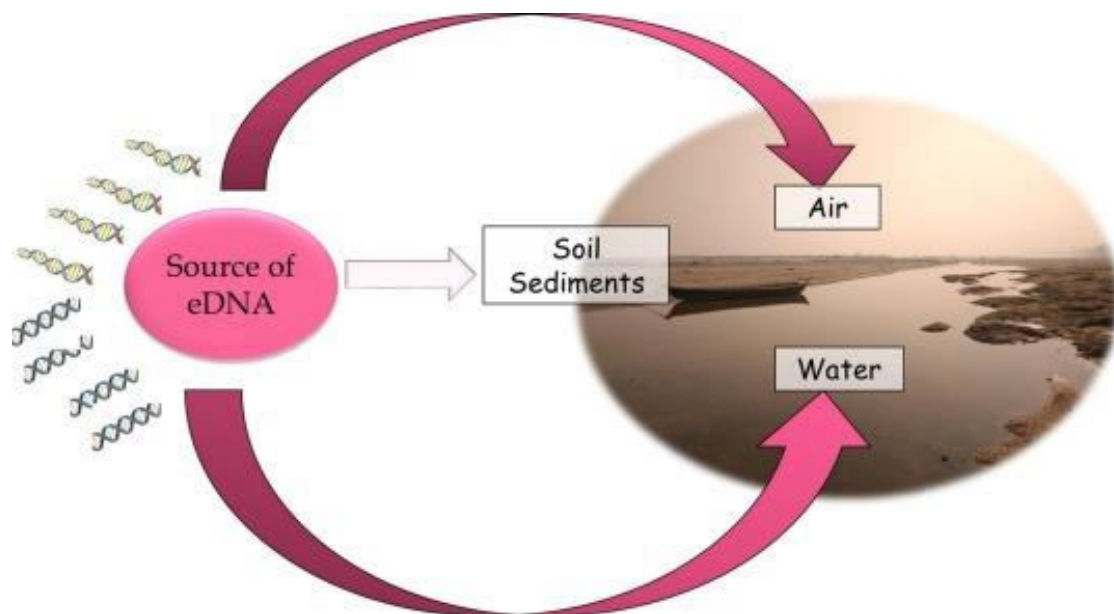
- Over the last few decades, the development of eDNA and sequencing techniques have resulted in an increasing interest in its use as a tool for both targeted species detection and assessments of the biodiversity of any ecosystem.
- The term “Environmental DNA” was first coined in microbiology to describe a technique for collecting DNA from a soil environmental sample without first isolating the target microorganisms.

#### eDNA:

- Environmental DNA (eDNA) is an effective conservation tool that allows scientists to discover the presence or absence of specific species in an ecosystem.
- Ecologists, geneticists, and data scientists work together to improve this new technique. Environmental DNA (eDNA) is mitochondrial or nuclear DNA that was discharged into the environment by animals.

### Source of eDNA

- The cells and waste that they shed and excrete, such as faeces (poops), urine, mucus, gametes, shed skin, hair, blood, epidermal cells, and corpses, are the source of eDNA in soil, organic sediments, and water.
- In this precise meaning, eDNA is thought to be a combination of trace amounts of intact cells (intracellular DNA) and DNA fragments (extracellular DNA) released into the environment by animals that are no longer present, and may be detected by sampling the environment alone.
- Extracellular DNA from injured tissues frequently degrades into smaller fragments, while intracellular DNA is obtained from cells or organisms present in the sample and is more likely to be of excellent quality.



The major sources of eDNA in [aquatic environment](#).

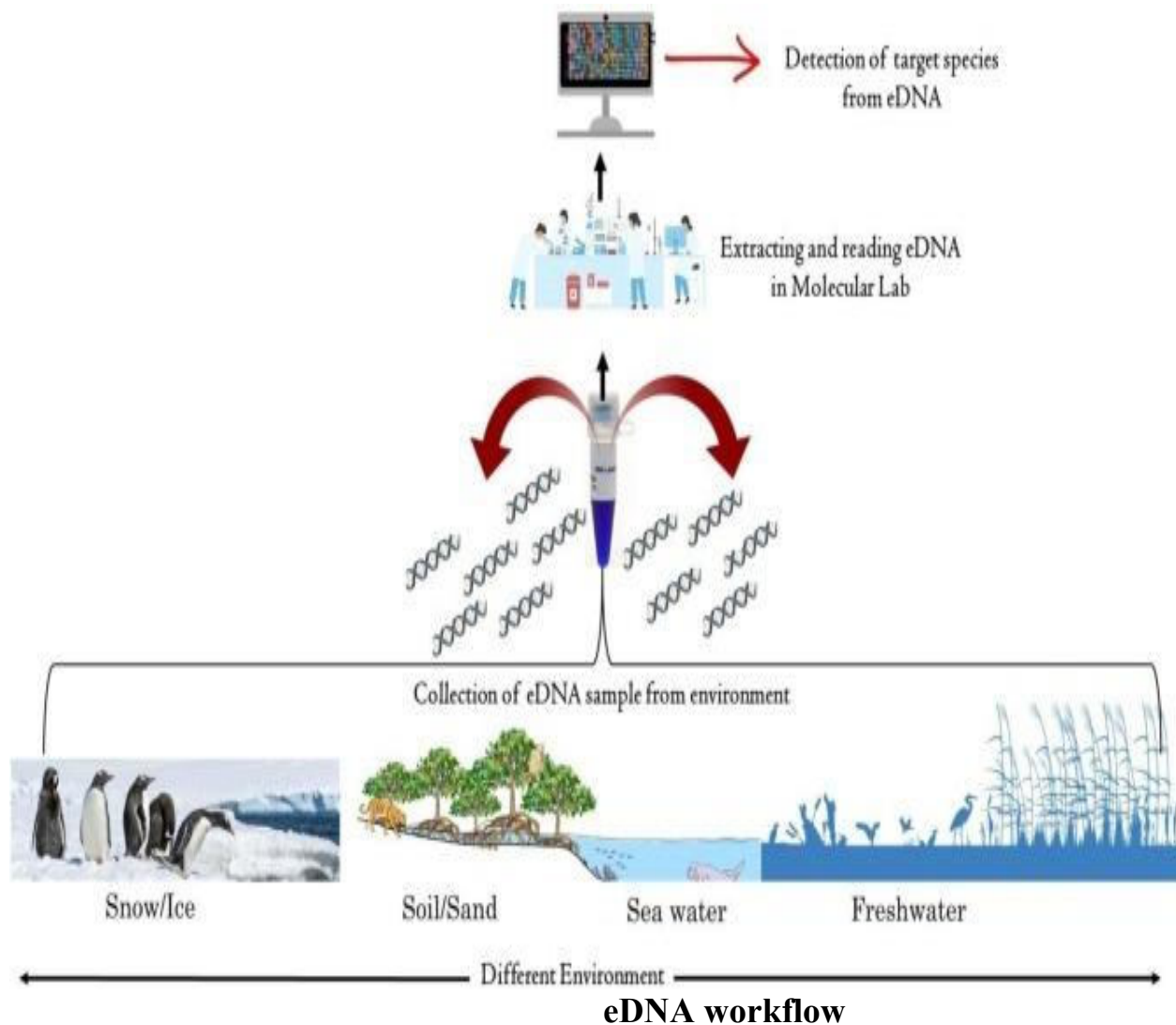
### eDNA WORKFLOW

Environmental DNA assessment of aquatic microorganisms (referred to as “eDNA analysis” from this point forward) from water samples involves some basic steps are:

- sample collection,
- eDNA capture,
- eDNA extraction,
- PCR and sequencing,
- Sequence analysis







### Abundance estimates using eDNA

- The quantitative study of eDNA provides a substantial opportunity to measure a species presence–absence in natural ecosystems and its relative abundance (Jerde et al., 2011).
- eDNA has the ability to record not only the number of species present, but also the number of individuals living within any given environment, allowing ecological inquiries to change from species richness to species diversity.
- According to one study, electrofishing took 93 days to identify a single example of a rare species of fish, while eDNA took only 4 h to detect the species (Jerde et al., 2011).
- This provides advanced data for biodiversity and ecosystem monitoring, allowing for the tracking of changes in ecosystems over time, the observation of variations between habitats and ecosystems, and the understanding of ecosystem health.



- Recent studies positively correlated eDNA concentration from qPCR with broad categorical variables of high/low density of like frogs in ponds (Ficetola et al., 2008), and Asian carp in different waterways (Jerde et al., 2011).

### Uses of eDNA methods

#### ➤ Biomass estimation:

- Species distribution and biomass estimation are essential components for the conservation of populations. Species distribution can be determined by absence/presence data.
- This novel method has ability to detect single species to the targeting of many species from ecosystem.

### Advantages of eDNA as an assessment tool

- **High sensitivity:** The eDNA analysis is sensitive enough to detect low-abundance or low-detection rate species.
- **Non-invasive:** No organism handling, capturing or distribution is required.
- **Cheap:** The present technique is cheaper than the conventional environmental sampling technique as it requires the least field work or equipment.
- **Time-saving:** eDNA sampling and analysis is a fast and rapid method that gives us results within a day or two.
- **Universal:** The present technique is universally accepted in the study of ecosystems, wildlife conservation and other fields.

### Disadvantages of eDNA as an assessment tool

- The eDNA approach only gathers information on the presence or absence of the target species. It does not provide any information on factors such as a species' life stage, reproduction, or fitness.
- Based on eDNA, hybrids cannot be identified from their maternal species because most eDNA studies focus on mitochondrial DNA, which is transmitted only from the mother.

### Type 1 and type 2 errors:

- There are two types of error which are false positive (type 1) and false negative (type 2) error rates.
- Traditional methods used to sample species in aquatic habitats are prone to type 1 error (i.e., recording a species when it is absent; generally, through misidentification of observation or possible cross-contamination)
- Type 2 errors (i.e., failing to detect a species when it is actually present). Environmental DNA approaches are also subject to these potential errors; however, eDNA methods have a reduced likelihood for type 2 errors as PCR analysis methods are highly sensitive.



## Factors affecting eDNA and survey

- Various abiotic and biotic factors affect fish eDNA and their surveys. eDNA degrades during transport, either through abiotic or biotic processes such as sedimentation and Biofilm.

## Conclusion

- Environmental DNA analysis has been used in targeted species detection studies with PCR and qPCR assays, and in community (i.e., multi-species) studies using metabarcoding.
- The eDNA has the potential to make significant contributions to the detection of rare or threatened species as well as invasive species, community and ecosystem biodiversity, functional diversity, wildlife, and conservation\_biology.
- Since 2012, there has been a wide range of research on eDNA metabarcoding which is used in biodiversity conservation, fish community identification, fisheries management, the target of invasive species, and fish biomass/abundance estimations.
- The use of eDNA is escalating, and it is currently being used to monitor biodiversity and improve environmental probabilities to possibilities, as well as to monitor benthic organic enrichment, community assessment in microcosm research to detect anthropogenic contamination, coastal\_sediment structure, and to study the most diverse ecosystems.

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## Murrel culture

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### Introduction

- Murrels are commonly called as snakeheads belong to family channidae. They are air breathing fishes which inhabit permanent shallow lentic waters.
- Murrel culture contribute important freshwater fishery in India and in great demand for its pleasant tasty flesh and less spines.
- There are about 33 species of murrels distributed in tropical Asia including north China and Africa.
- Over 15 species are reported from Asia and Africa.

### Species of culture importance:

*Channa striatus* (striped snakehead) – state fish of Andhra Pradesh & Telangana

*Channa marulius* (bullseye snakehead)

*Channa punctatus* (green snakehead)



*Channa striatus*  
*Channa marulius*



*Channa marulius*



*Channa punctatus*

### Production

- Total inland fish landings in India (2019-2020) - 2.01 Lakh Tonnes.
- Of that Andhra Pradesh is highest with 0.58 lakh tonnes landings.
- Followed by Bihar (0.45), Telangana (0.38), Assam (0.25)

### Food and feeding habits

- Juveniles eat mostly aquatic insects while spawn and early fry nearly entirely devour zooplankton.
- While adults eat medium-sized fish fry, minnows, shrimp, worms, and insects, fingerlings primarily eat shrimp, annelids, and small fish.
- Additionally, they are very piscivorous.
- Piscivorous tendencies in *C. striatus* appear in the size group of 151-250 mm.
- The majority of larger adults are piscivores, with small sized murrel, weed fish, and carp minnows make up the bulk of their diet.
- True cannibalism is also seen in the juvenile and fry stages.

### Age at maturity

- One and two years.
- Length:
  1. *C.striatus* – above 25cm.
  2. *C.marulius* – above 36cm.
- Even though rainy season is *C. striatus*'s peak mating season, the species appears to reproduce all year round.

### Breeding

#### 1. Natural

The brood fish, which range in size from 100 to 250 g, are stocked in a small pond with shallow water depth. only after the month of March, the fish begin to move in synchrony. They build a nest with their tails near the border of tanks and eat the weeds that grow there. This species typically lays its eggs on shallow margins of weed-infested waters where the weeds have been removed in a small circular area. This clearing of the weeds occurred probably during the breeders'



aggressive spawning movement, as the weeds assisted in keeping the floating eggs from dispersing. Both parents guard the egg and larvae. Fertilization is external.

## 2. Induced breeding:

- The ideal spawning temperature is between 25 and 28 ° c HCG must be supplied at a dosage of around 2000 IU/kg of body weight for females and 1500 IU/kg of body weight for males. For male and female fish, the recommended doses of carp pituitary extract are 20–30 mg/kg and 30–40 mg/kg, respectively, of body weight.
- Injection is administered intramuscularly (at the base of pectoral fin).
- Typically, the spawning lasts 16 to 18 hours.
- A 1 kg female fish lays between 10,000 and 15,000 eggs. Unfertilized eggs are opaque, while fertilised eggs are translucent.
- The diameter of a fertilised egg is between 1.2 and 1.5 mm.
- Utilizing a plankton net, the fertilised eggs are gathered and moved to a FRP container for hatching.
- The hatching process takes 20 to 24 hours. The average hatchling rate is between 70 and 90 percent, and the fertilisation rate ranges from 80 to 98 percent.
- The size of the newly hatched larvae is between 3.0 and 3.5 mm.

## Culture

### 1. Fry production:

- The digestive tract is visible as a straight tube connected to the yolk sac after hatching, but it lacks any accessory digesting organs (liver, pancreas and gall bladder).
- After 1–2 days of hatching, the digestive tract is fully established, and the yolk sac absorption is quite quick. Fry begin consuming the meal, such as zooplankton, after three days (protozoans, rotifers, and cladocerans).
- They keep doing this until they reach a size of 20 to 30 mm.
- At 20 to 25 days, a survival rate of 50 to 60 percent is anticipated.
- Cannibalism and heterogeneity are the two processes that have a significant impact on the development and survival of striped murrel fry.
- The artificial feeds are provided once the digestive tract has fully developed and contain between 40 and 50 percent protein.



### 2. Fingerlings production

- The fingerlings eat minute crustaceans and zooplankton (especially insect).



- For hatchery-reared fingerlings, live feed like tubifex and earthworms are a good source of nutrition.
- The stocking rate varies greatly, from 20,000 to 4,60,000 ha each year.
- A stocking density of 15000/ha has been associated with a higher survival rate of 76.67 percent.
- Low market value fish and rice bran are combined in a 3:1 to 8:1 ratio to feed fish.
- Fish are fed between 6 to 8 percent of their body weight.
- To increase survival rates if cannibalism persists, it is advised to increase feeding rates.
- About 40 to 45 percent of striped murrel fingerlings' dietary requirements are for protein.
- A 30 to 40% chance of survival is expected.



### 3. **Grow out**

- For striped murrel to grow, a pond with a surface area of 0.1 to 0.2 hectare and a depth of 1 to 1.5 m is optimal.
- At a feeding rate of 5% of body weight, the striped murrel is stocked at a density of approximately 10,000/ha.
- The suggested stocking size for grow out is greater than 10 g.
- The annual growth is predicted to be 600–800 g, and the annual fish yield is 2.0–2.5 tons/ha.
- Aquatic weeds are beneficial to striped murrels because they offer shade during the hotter months, act as a deterrent to poachers, and promote the proliferation of insects.

### **Refernces**

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Murrels and murrel culture – N.M.Chakrabarty

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## Seaweeds In India

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### Introduction

Seaweeds are macroscopic non flowering marine plants, that live either in marine or brackish water environment. Like the land plants, seaweeds contain photosynthetic pigments and with the help of sunlight and nutrient present in the seawater, they photosynthesize and produce food. All most 10,000 species have been identified marine algae are reported in the world.

Seaweeds are also termed as the ‘Medical Food of the 21st Century’ as they are being used as laxatives, for making pharmaceutical capsules, in treatment of goiter, cancer, bone-replacement therapy and in cardiovascular surgeries. Seaweeds are wonder plants of the sea.

Seaweeds are found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available.

### Resources

Seaweeds are abundant where rocky or coral formations are there. The luxuriant growth of several species of green, brown and red algae occurs along the southeast coast of Tamil Nadu from Rameswaram to Kanyakumari, Gujarat coast, Lakshadweep and Andaman Nicobar Islands. Fairly rich seaweed beds are present in the vicinity of Mumbai, Karwar, Ratnagiri, Goa, Varkala, Visakhapatnam and in coastal lakes like Pulicat and Chilika.

**Some of the commercially important seaweeds of India are as follows:**





**1. Agarophytes (Agar yielding Seaweeds):**

Gracilaria edulis, G. fergusonii, G. arcuata, G. indica, G. obtusa, G. crassa, G. corticata, G. corticata var. corticata, G. corticata var. cylindrica, Gelidiella acerosa, Gelidium, Pterocladia

**2. Alginophytes (Seaweeds which Yield Alginic acid):**

Sargassum wightii, S. longifolium, S. ilicifolium, S. myriocystum, Turbinaria conoides, T. ornata, T. decurrens, Acanthophora spicifera

**3. Carrageenophytes (Seaweeds which Yield Carrageenan):**

Hypnea, musciformis, Hypnea valentiae, Kappaphycus alvarezii (non-native)

**Potential:**

Of the 10,000 species have been identified marine algae are reported in the world, around 844 species of seaweeds have been reported from Indian seas, their standing stock is estimated to be about 58,715 tons (wet weight). Out of the 844 seaweed species, India possesses around 434 species of Red Algae, 194 species of Brown Algae, and 216 species of Green Algae.

**Classification of Seaweeds: -**

- Three groups
  - 1) Chlorophyceae (Green algae)
  - 2) Rhodophyceae (Red algae)
  - 3) Phaeophyceae (Brown algae)

**1. Chlorophyceae (Green algae):**

- b) Green algae are found in the fresh and marine habitats. They range from unicellular to multi-cellular, microscopic to macroscopic forms
- c) cell wall consisting of an inner cellulose and outer pectin layer.
- d) photosynthetic pigments such as Chlorophyll a & b, contained in the special cell structure known as chromatophores.
- e) Photosynthetic product is Starch.
- f) Green algae can produce sexually (by isogamous, anisogamous or oogamous type) and asexually by forming flagellate and sometimes non-flagellate spores. The vegetative propagation is achieved through fragmentation.
- g) Include Ulva, codium, cladophora, spirogyra, chlorella, Caulerpa, Enteromorpha, monostroma etc. are green seaweeds. They are used as fresh cooked foods and soups.



## 2. Rhodophyceae (Red algae):

- Except for few species they are exclusively marine.
- They inhabit intertidal to subtidal to deeper waters.
- Inner cell wall is of cellulose and outer cell wall with amorphous matrix of mucopolysaccharides (i.e., agar, porphyrin, furcellaran, carrageenan). Cells are uninucleate /multinucleate with a large centric vacuole.
- The colouration of Rhodophyta is due to water-soluble pigments, the red phycoerythrin and blue phycocyanin. Other pigments present are chlorophyll a & b, carotene etc.
- The photosynthetic product of this group is Floridian starch.
- All the members of this group produce one or more kinds of non-flagellated spores that are either sexual or asexual in nature.
- These are agar and carrageenan yielding seaweeds. So, these are called agarophytes and carragenophytes.
- Includes gracilaria, gelidium, hypnaea, eucheuma and chondrus crispus.



**Red Seaweeds**

## 3. Pheophyceae (Brown algae): -

- Brown algae are exclusively marine forms.
  - The cell wall is two layered. Outer layer is mucilaginous and sticky due to the presence of alginate, The inner layer is of cellulose (microfibrils).
  - Brown algae vary in coloration from olive –yellow to deep brown. The coloration is due to the accessory carotenoid pigment and fucoxanthin. The amount of fucoxanthin varies in different species of brown algae.
  - other photosynthetic pigments of the brown algae are Chlorophyll a & c, B-carotene and xanthophylls.
- The photosynthetic products of the brown algae are Laminarian and Mannitol. These are called kelps. The product is called alginin or alginic acid. So, these plants are also called as alginophytes.

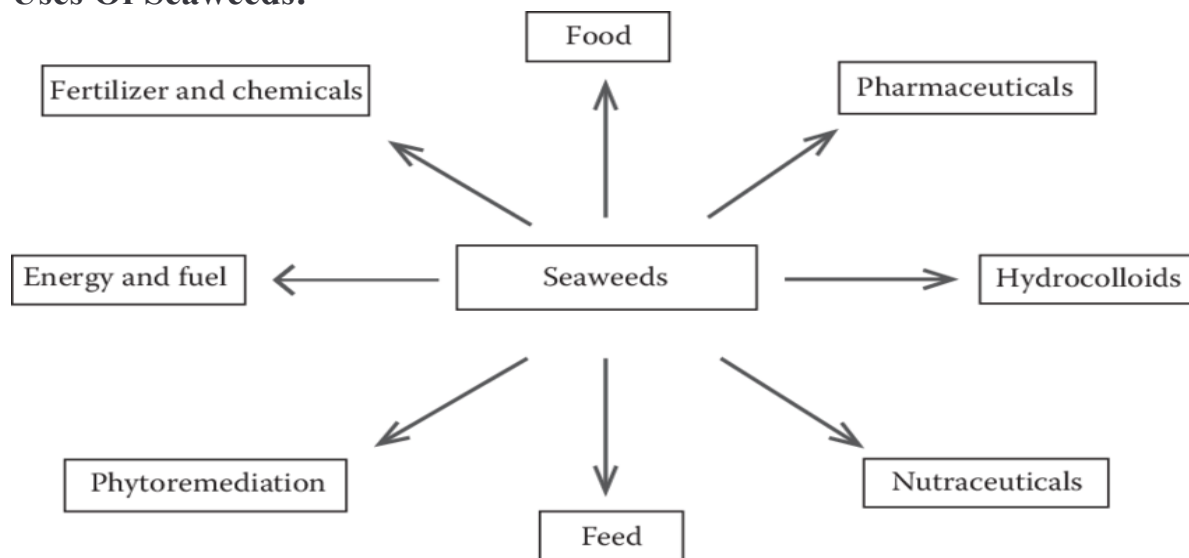


- Includes Laminaria, Undaria, Turbinaria, Sargassum, Macrocystis



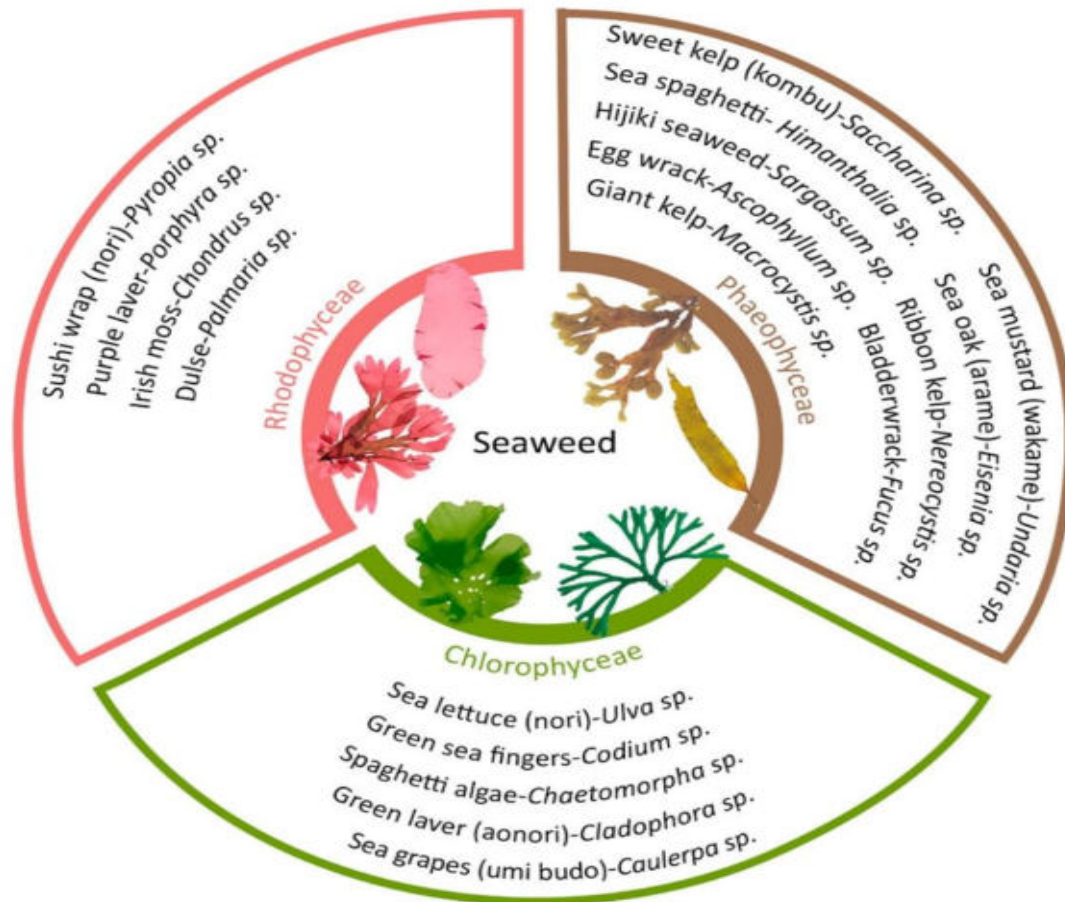
### Brown Seaweeds

#### Uses Of Seaweeds:



#### List of seaweeds used for Direct Consumption:





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## Microbial consortium based bioinoculants for crop production and waste management

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### Abstract

Microorganisms-based consortial technology in the current era, encompasses liquid biofertilisers for major nutrients such as Nitrogen, Phosphorous, Potassium, and also for micro nutrients like Zinc, Iron, Sulfur *etc.*, and biocontrol agents for plant pest and disease control, decomposers for crop waste management. There are various products released by both governmental and private companies. In India, there are few agencies that work on microbial consortium technology that operates under Indian council of Agricultural Research, New Delhi. Also, National Centre of Organic Farming (NCOF), Ghaziabad had developed few technologies for agrowaste management. Some of the commercial formulations includes BioNPK, Bio Phos, BioZn, Azophosmet, BioGrow, Bio pulse, CSR-BIO, Goa Bio 1, Arka Microbial consortium, NCOF waste decomposer, PUSA decomposer *etc.*, The farmers should be motivated and created awareness for use of these products for organic farming.

**Key words: Microbial consortium, biofertilizers, waste decomposers, organic farming**

### Introduction

Microorganisms-based consortial technology offers good substitutes to replenish crop nutrients. In the agricultural ecosystem, microorganisms have a crucial role in fixing/ solubilizing/ mobilizing/ nutrient recycling. These microorganisms occur in soils naturally, but their populations are often scarce. To get bumper crop yield, the desired microbes from the rhizosphere are isolated and artificially cultured in the adequate count and mixed with suitable carriers or as they are in suitable combinations by artificial culturing. In replacing the chemical fertilizers and carrier-based

biofertilizers, liquid microbial consortium-based technologies developed by governmental institutes that are technically validated in multiple cropping trials had wide avenues for soil health restoration (Pindi and Satyanarayana, 2012)

### **Microbial consortium-based bioinoculants for Nitrogen, Phosphorous, Potash and Zinc**

There are many validated technologies developed by ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh on the microbial consortium and commercialized through Agrinnovate India Ltd.

BioNPK is a liquid-based biofertilizer bioformulation consisting of bacteria such as nitrogen-fixing *Azotobacter* sp., Phosphate solubilizing *Paenibacillus* sp., and potash solubilizing *Bacillus* sp. The shelf life of the formulation is up to 24 months without loss of viable cell numbers and tolerates high temperature. BioNPK can be used for cereals, millets, pulses, vegetables and oil seed crops. Seed treatment, root dip for seedlings and soil application for tree plants are the application methods recommended for this formulation.

### **Phosphorus solubilizing bacterial consortium**

Bio Phos+ and Bio Phos are liquid formulations of Phosphorus solubilizing bacteria containing *Paenibacillus* sp. and *Kluyvera* sp. respectively. It has a longer shelf life of 12 months with good viability and capable properties to withstand high temperatures. Bio Phos and Bio Phos+ can be used for multi various crops through seed treatment, root dip for nursery seedlings and even for soil application to tree crops. BioGrow is a consortium of different bacterial species possessing properties such as phosphorus solubilization, production of Indole acetic acid (IAA) and siderophores. BioGrow is a recommended alternative nutrient for vegetable crops especially solanaceous crops such as tomato, brinjal, potato and floriculture crops such as marigold.

### **Bio Potash and Bio Zinc**

Bio potash is a liquid formulation with efficient K-solubilizing bacteria viz., *Bacillus decolorationis* that can be used in different types of soils.

For crops like maize, paddy, wheat, mustard and potato. Application is done through seed treatment, root dip for seedlings and soil application for tree plants.

Bio Zinc is a liquid formulation containing highly efficient zinc solubilizing bacteria suitable for different types of soils. *Bacillus endophyticus* in the formulation solubilizes zinc very effectively and has a longer shelf life of 12 - 24 months without loss of microbial populations and properties upon exposure to high temperatures. Bio Zinc is used for cereals, millets, pulses, vegetables and



oilseed crops.

Iron and zinc fortification can be achieved by endophytic microbial inoculants viz., *Arthrobacter sulfonivorans* DS-68 and *Enterococcus hirae* DS-163 have been identified and validated to increase iron content in wheat grains. Endophytes *Bacillus subtilis* DS-178 and *Arthrobacter* sp. DS-179 increases zinc content in wheat grains.

### **Azophosmet**

Beneficial bacteria such as *Azospirillum*, phosphobacteria and Methylobacterium colonize the rhizosphere region and have the ability to fix nitrogen, solubilize phosphorus and stimulate plant growth. The bio-fertigation precisely delivers the bioinoculants in the root zone. The microbial consortium of Azophosmet experimented in cotton resulted in increased plant growth, biomass and number of bolls per plant compared to individual bioinoculant. It further reduced the cost of bioinoculants and eased the technology of application with one bioinoculant 'Azophosmet'.

### **Arka microbial consortium**

Arka Microbial Consortium (AMC) is a carrier-based formulation that contains nitrogen fixing, phosphorus and zinc solubilizing plant growth-promoting microbes as a single product. The uniqueness of this formulation is that the end user need not apply bioinoculants individually as nitrogen fixers, phosphorus solubilizers and plant growth-promoting rhizobacterial inoculants. This single microbial mix can be conveniently applied to seed, soil, water and nursery rooting media like coco-peat in protrays. The synergistic effect of the formulated microbial consortium favours sustainable production and precision farming, especially in horticultural crops. The consortium promotes early seed germination and improved seedlings establishment rate. Seedlings become ready for early transplantation by 3-4 days with increased seedling vigor. In vegetables, the cost of nitrogen and phosphatic fertilizer requirements can be reduced by 25 - 30 percent with a 15% yield increase.

### **Methods of Application**

- Seed Treatment: 10-20 g inoculum for 100-200 g of vegetable seeds.
- Coco peat enrichment: One kg AMC enriches 1000 kg (1 tonne) of coco peat.
- Soil drenching: AMC @ 20 g/ liter of water. Applied nearer to the root zone on the 10<sup>th</sup> day after transplanting of crops.
- Main field application: AMC @ Five kg per acre can be mixed with 500 kg of farm yard manure and applied near the root zone of the standing crop.



### **Bio-priming techniques for rice**

An equal cultural proportion of mixed cyanobacterial inoculants such as the culture of *Plectonema boryanum*, *Anabaena doliolum* and *Nostoc commune* used for coating rice seeds is called the bio-priming technique. Germination of bio-primed seeds was 18-20% higher than non-coated seeds and an increase in grain yield of 5.3 to 7.9% was observed by this technique.

### **Microbial consortium as soil conditioners**

A potential bio-growth enhancer and a low-cost bio-formulation based on the integration of the dynamic microbial consortia (*Bacillus* sp. and *Trichoderma* sp.) with dynamic culture media for increasing the productivity of crops grown in sodic soils is called "CSR-BIO.". It comprises *Bacillus pumilus*, *B.thuringiensis* and *Trichoderma harzianum* act as a soil conditioner and improves the nutrient availability in problem sodic soils. It is available in the market in the form of liquid concentrate for foliar and drenching applications with a dosage recommendation of 2 liters per acre (Damodaran et al., 2013).

### **Microbial consortium for management of biotic stress**

Drought stress can be managed by a special type of Archaea bacterial formulation called Grow-Sure technology. ICAR-Central Soil Salinity Research Institute, Karnal, Haryana had developed a unique bio-stimulant developed using the highly efficient salt-tolerant bacterial strains obtained from high-stress rhizosphere such as *Bacillus licheniformis*, *Lysinibacillus fusiformis*, *Lysinibacillus sphaericus*. It is highly recommended for vegetables like tomato, chilies, potato, brinjal, and for fruit trees such as banana, mango, etc., CSR-BIO Bioraj, ICAR BIO Bioraj, CSR-BIO growth enhancers, etc. are the other products marketed through Agrinnovate India Limited (AgIn) today.

### **Goa Bio - 1 for plant growth promotion of paddy in coastal regions**

Soil biological activity in salt-affected soils can be improved by augmenting the saline-tolerant plant growth-promoting microorganisms. ICAR-Central Coastal Agricultural Research Institute, Goa, India identified a salt tolerant plant growth-promoting bacterial consortium with a standardized stable formulation namely Goa Bio-1 with a shelf life of  $>10^8$  CFUg<sup>-1</sup> for 18 months. It is targeted for better nutrient mineralization and alleviation of salinity stress for improved paddy yield in the saline areas of coastal regions. The formulation is suited for paddy cultivated under saline soils and in nurseries of vegetable crops, black pepper, fruits and plantation crop.





### **Microbial consortia for bio-control**

Talc-based formulation of *Trichoderma harzianum* called a 'Green fungicide' had been successfully tested in papaya and tomato nursery at farmers' field in Begusarai, Bihar. They had been proven to be effective against *Pythium*, *Phytophthora*, *Rhizoctonia*, etc.

Three bio-formulations of namely talc-based eco-pesticide *Pseudomonas fluorescens*, eco-green fungicide namely vermicompost based bio-formulation of *Trichoderma viride* and green fungicide called talc-based bio-formulation of *Trichoderma harzianum* were developed successfully and found effective against some soil and seed-borne pathogens like *Rhizoctonia*, *Sclerotium*, *Sclerotinia*, *Fusarium*, *Pythium*, *Ralstonia*, *Macrophomina*, *Bipolaris*, *Phoma*, etc.,

Bio-Pulse consortia technology is a fly ash-based formulation containing *Trichoderma harzianum* and *Bacillus amyloliquefaciens* for bio-control of *Fusarium* wilt in pulses and had been tested and verified in chickpea. Wilt disease in chickpea can be suppressed to 40% with increased grain yield by treatment with bio-pulse.

### **Microbial consortium for degradation of lignocellulosic agrowastes**

The microbial formulation with four hyper lignocellulolytic fungal consortium such as *Trichoderma viride*, *Aspergillus niger*, *Pleurotus florida* and *Phanerochaete chrysosporium* is recommended for composting of lignocellulosic biomass of agro-wastes obtained from paddy and wheat straw, pearl millet, soybean, and maize residues very effectively. A carrier-based inoculum is commercially available in packs of 1 kg and is sufficient for the conversion of 1 tonne of biomass. Mature compost can be obtained within 65-70 days by pit or windrow methods. By this process, enriched compost can be prepared from various crop residues in a period of 70-75 days.

### **Pusa decomposer technology**

Ligno-cellulolytic enzyme-producing fungal consortium developed by the scientists of the Indian Agricultural Research Institute, IARI, Pusa, New Delhi is called Pusa decomposer. Currently, Pusa decomposer is being provided in the form of capsules and liquid formulation and can decompose the agri residue *in-situ* within 25 days. Decomposer mixture involves making a liquid formulation using decomposer capsules and fermenting it over 8-10 days and then spraying the mixture on fields with crop stubble to ensure speedy bio-decomposition of the stubble. For one hectare, four capsules of Pusa decomposer mixed with jaggery and chickpea flour, 25 liters of spray solution can be prepared. It takes around 20 days for the degradation process to be completed. Pusa decomposer converts harvested paddy straw to decompose at a much faster rate than usual. This



practice is aimed at an economical and effective solution to the menace of rice straw burning and the management of agri-horticultural crop residues. Bio-degradation of crop residue and farm waste helps in the improvement of soil biological health by increasing the organic carbon in the soil.

Inoculation of PUSA decomposer @ 4 capsules per tonne of municipal solid bio-waste proved to be an effective microbial source for rapid degradation. This reduces the duration of composting from 60 days to 35 days. Further, the compost produced by the inoculation of the PUSA decomposer recorded significantly high quality and nutrient contents which can be effectively recommended for crop production.

### **Organic Waste Decomposer**

National Centre of Organic Farming (NCOF), Ghaziabad has developed a bio-waste decomposer formulation using a consortium of microorganisms extracted from desi cow dung. The technology is also validated by ICAR for quick composting of organic waste, soil health improvement and as a plant protection agent. Waste decomposer is available in powder/solid form in small vials. All biodegradable material like agro waste, animal waste, kitchen waste and city waste decompose within 40 days. Waste decomposers can be applied for seed treatment, foliar spray and soil application also. Foliar spray with a waste decomposer controls all types of bacterial, fungal and viral diseases effectively in different crops. Soil application of waste decomposer at the rate of 1000 liters per acre changes the biological and physical properties of all types of soil in just six months, and helps to proliferate earthworms upto 4 lakh numbers in the soil.

### **TNAU Biomineralizer consortium**

TNAU biomineralizer is the consortium-based technology of bacteria and fungus recommended for composting all types of agro wastes. The technology primarily focuses on the efficient decomposition of paddy straw. Incorporation of paddy straw in the harvested field with a tractor mounted with half cage wheel and rotavator combined with the addition of biomineralizer (TNAU microbial consortia) along with 25 kg additional dose of N ha<sup>-1</sup> as basal favoured faster decomposition of paddy straw. In subsequent cropping of paddy, there had been the highest dry matter production, number of productive tillers, filled grains per panicle, 1000 grain weight, grain yield and straw yield. Incorporation of paddy straw with biomineralizer increases the crop growth, yield attributes, grain and straw yield due to higher utilization of nutrients in flowering and post-harvest soil nutrient status of rice and also could be recommended for getting higher productivity of black gram under rice fallow condition.



Efficient decomposition of sugarcane trash compost is often achieved by using effective microbes like *Aspergillus flavipes*, *Penicillium*, *Chrysogenum*, *Cochliobolus spicifer*, *Rhizopus oryzae* and *Trichoderma viride* that helps to convert sugarcane trashes into a well-decomposed compost in 60 days. *In-situ* composting of sugarcane trashes using a combination of rock phosphate: gypsum: urea in the ratio of 5:4:1 (named as SRS mixture) was added to narrow the C:N ratio. For the *in-situ* composting of sugarcane trashes, SRS mixture  $100 \text{ kg t}^{-1}$  followed by TNAU biomineralizer  $2 \text{ kg t}^{-1}$  may be applied immediately after the harvest of the canes.

### Conclusion

In the market, biofertilizers and bioinoculants are sold in various names, but consumers should be aware of the specificity, nature, purpose, method and time of application, adjuvants to be added for foliar application, shelf-life period of the product, dosage of the formulation to be applied and commercial prescription and delivery methods to be followed must be necessarily ascertained by the buyer. More awareness should be created to the farming community on microbial consortium based bioinoculants for encouraging organic farming across the country.



Fig 1. Bioformulations from Tamil Nadu Agricultural University, Coimbatore





**Fig 2. Liquid biofertilizers from Tamil Nadu Agricultural University, Coimbatore**



**Fig 3. Bio Potash from NBAIM, Mau, UP**

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# Gamma Irradiation in Insect Pest Management- A Promising Approach towards Food Security

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## Introduction

India holds a prominent position in global food grain production. The storage of agricultural commodities has been given utmost importance in our country. However, the damage caused by pests, both at the farm level and in storage facilities, is significant. According to the Food and Agriculture Organization (FAO), insects alone are responsible for causing 10-50 percent of the overall damage, primarily due to improper storage practices.

There is a continuous need to safeguard commodities from deterioration, particularly in terms of quality and quantity, during the storage process. The widespread and indiscriminate use of insecticides to control insect pests has resulted in the development of resistance in the targeted species. Additionally, this practice has led to various issues such as residual effects, environmental hazards, and threats to non-target organisms.

In our agricultural nation, globalization has facilitated the free and rapid exchange of goods between countries. Despite having strict quarantine regulations, we frequently encounter various introduced insect pests, which pose a significant threat to our nation's bio-security. To address this issue, fumigation has emerged as the most widely accepted method for phytosanitary treatment and pest control in our country. Methyl bromide (MBr), a highly effective broad-spectrum fumigant, has gained increasing popularity for pest control purposes. However, in 1992, Methyl bromide (MBr) was classified as an Ozone Depleting Substance (ODS) under the Montreal Protocol, an international agreement aimed at protecting the Earth's stratospheric ozone layer.

The use of Methyl bromide (MBr) was phased out for most phytosanitary purposes by the United States and around 60 other countries in 2005. Despite this, certain countries, including India, continue to use methyl bromide for quarantine and pre-shipment treatments due to the absence of effective alternatives. This has raised concerns about the escalating use of methyl bromide globally, as it poses a risk to ozone depletion.

Fortunately, radiation technology, particularly gamma irradiation, has gained significant importance in recent years as an alternative method for controlling insect pests, especially in stored products. This technology offers a viable solution without harming the environment or compromising the quality of the produce.

### **Regulatory status on the use of gamma irradiation**

Over 100 nations have embraced irradiation technology, and various irradiated food products are now accessible in the market. The following authorities have granted approval for the irradiation of foods:

- Codex Alimentarius
- Food Safety and Standards Authority of India (FSSAI)
- Food Standards Australia New Zealand
- American Medical Association
- Institute of Food Technologists
- International Atomic Energy Agency (IAEA)
- Food and Agriculture Organization (FAO)
- World Health Organization (WHO)

These reputable organizations have recognized and endorsed the use of irradiation for food safety purposes.

### **Gamma irradiation technique**

Gamma rays are a type of electromagnetic radiation characterized by their shorter wavelength and higher photon energy. The term "gamma ray" was coined by Ernest Rutherford in 1903. Irradiation is a process used to either sterilize target insects (such as fruit flies) by releasing sterile adults or to expose grains in agricultural commodities to eliminate stored product pests. This process requires controlled dose exposure and adherence to safety measures specified by national and international organizations, making it of utmost importance.



When gamma rays interact with organisms, they damage the structure of cell membranes or disrupt critical elements within cells. This, in turn, affects metabolic enzyme activity and often causes damage to Deoxyribose Nucleic Acid (DNA), which is essential for growth and replication of organisms. Radiation sources used for irradiation can include cobalt-60 or cesium-137 radioisotopes, as well as electrons generated from machine sources (e-beam), which are commonly used for insect sterilization. The absorbed dose, measured as the amount of radiation imparted per unit of mass of specified materials, is typically expressed in grays (Gy), where 1 gray is equivalent to 1 joule per kilogram.

### **Gamma irradiation in pest management**

Irradiation is widely employed in biological studies and agriculture to enhance crop production. It has proven to be effective against a broad range of insects and mites, without compromising the quality of the commodities, when used at standard dose levels. This technology is particularly valuable in combating quarantine pests like tephritidae (fruit flies), which pose a significant threat to horticultural crops. The Sterile Insect Technique (SIT), introduced by E. F. Knipling in 1937, utilizes irradiation as a generic treatment method. Gamma irradiation is currently the predominant approach for sterilizing mass-reared insects for SIT. The success of SIT depends on the production of high-quality sterile insects that are released into target wild populations, and the required sterility doses can vary across different species. Both CODEX and FAO recommend the use of gamma irradiation for grains, such as cereals and pulses, with a maximum dose of 1 kGy for disinfestation.

Existing literature suggests that gamma radiation doses ranging from 25 to 1200 Gy can effectively suppress various pests, including grain weevils, Mediterranean flour moths, Indian meal moths, cigarette beetles, medflies, onion flies, fall armyworms, tobacco budworms, and African cotton leafworms (Timbadiya *et al.*, 2018). Gamma radiation demonstrates greater efficiency in eliminating insect pests affecting stored grains and field crops. Notably, research indicates that irradiating last instar larvae of fall armyworms (*Spodoptera frugiperda*) with a dose of 200 Gy resulted in significantly reduced pupation rates and adult emergence. The emerged adults exhibited deformities, inability to fly, and mortality within approximately two hours (Arthur *et al.*, 2016).

In several countries, including India, invasive pests pose a significant threat to food security. Irradiation can provide valuable support in suppressing pest populations and addressing this critical challenge.



## **Advantages of gamma radiation in pest management**

Gamma irradiation offers several advantages in pest management:

1. **Effective Pest Control:** Gamma irradiation is highly effective in controlling and eliminating a wide range of pests, including insects, mites, and pathogens. It can target pests at various stages of their life cycles, such as eggs, larvae, pupae, and adults. This technology helps reduce pest populations and prevent infestations in agricultural crops, stored grains, and food processing facilities.
2. **Wide Spectrum of Activity:** Gamma irradiation has a broad spectrum of activity, making it effective against a wide range of pests. It can target both surface-dwelling and internal pests, including hidden pests that may be difficult to reach through other pest management methods.
3. **Resistance Management:** Unlike chemical pesticides, gamma irradiation does not lead to the development of pest resistance. Pests cannot build up a tolerance or immunity to radiation, making it a sustainable and long-term pest management solution.
4. **Quality Preservation:** Gamma irradiation does not compromise the quality, taste, or nutritional value of treated products. It helps maintain the freshness, appearance, and sensory attributes of agricultural commodities, ensuring that they meet market requirements and consumer expectations.
5. **Safety and Environmental Considerations:** Gamma irradiation is a safe method of pest management. It does not leave behind harmful residues in treated products, making it a preferred choice for food safety. It is also an environmentally friendly option as it does not contribute to pollution or environmental degradation.
6. **Compliance with Regulations:** Gamma irradiation is approved by international organizations, regulatory bodies, and food safety authorities such as the Codex Alimentarius, FAO, WHO, and national regulatory agencies. Its use in pest management aligns with stringent regulatory requirements for food safety and phytosanitary measures.
7. **Application Flexibility:** Gamma irradiation can be applied to various commodities, including fresh produce, grains, spices, nuts, seeds, and processed food products. It can be implemented at different stages of the supply chain, including pre-harvest, post-harvest, and during storage and transportation.
8. **Sterile Insect Technique (SIT):** Gamma irradiation is integral to the Sterile Insect Technique (SIT) for pest control. By sterilizing pests, such as fruit flies, and releasing them into the wild, it disrupts their reproductive cycle, reduces pest populations, and helps manage pest outbreaks effectively.





In summary, gamma irradiation provides an efficient, safe, and environmentally friendly approach to pest management. It offers effective pest control, helps preserve product quality, ensures compliance with regulations, and contributes to sustainable pest management practices.

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## Besides Providing Energy, Omega-3 and Omega-6 Fatty Acid Rich Oils Improve Nutrient Utilization and Growth performance of Heifers

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### Introduction

For rural Indians, raising livestock is a significant means of employment, income, and survival. Milk consumption is also steadily rising over time. Between their second and fifth lactation, dairy cows reach their genetic production potential. After that, disease incidence rises and milk production gradually declines. A sound approach to the rearing of dairy animals, including nutrition management and the replacement of older and unproductive cows through culling, plays a significant role for sustained year-round milk production and maximum output from the dairy farming sector. Dairy cows' growth, production, and reproduction depend heavily on supplementing with the right nutrients in the right quantities. Energy is the most important supplement for biological function, out of all the ones available. Ruminants typically consume cereals as an energy-rich concentrate. However, excessive cereal consumption causes acidosis, which impairs ruminal digestion. As a result, heifers with high energy densities benefit from oil supplements for better average daily gain. In addition to increasing the ratio's energy density, some oils and oilseeds also perform numerous important biological functions with ultimate improvement in utilization of nutrients and growth of heifers. This is because of the particular fatty acid composition of that oils.

Dairy animals need a lot of nutrients to grow and be productive, as well as to perform reproductive functions like the early onset of puberty and the ovarian cycle. In fast-growing heifers, prolonged depletion of body reserves can have a significant negative impact on growth, production, resumption of estrus, and conception rate. The PUFA in the supplemental fat have a positive impact



on the metabolism of carbohydrate, protein, and fat, as well as the growth and differentiation of cells by regulating gene expression in reproductive tissues, resulting in a noticeable improvement in production and fertility. Traditionally, supplemental lipid in the diet of dairy animals is included to increase dietary energy density. Due to the higher health benefits and improved reproduction efficiency of lower ratios, animal nutritionists have recently placed a greater emphasis on reducing the ratio of dietary  $\Omega$  -6 to  $\Omega$  -3 fatty acids in cattle diets. It is possible to incorporate a variety of polyunsaturated fatty acid-rich oil-feed ingredients as tabulated below, into the diet of dairy replacement heifers.

**Table 1. Major sources of unsaturated fatty acids for dairy animals (Thatcher and Staples, 2007)**

Sr. No.	Oil	C18:1 (%)	C18:2 ( $\Omega$ -6, %)	C18:3 ( $\Omega$ -3, %)
1.	Canola	64	19	8
2.	Palm	39	10	1
3.	Safflower	12	77	0
4.	Cottonseed	21	50	0
5.	Corn	25	60	1
6.	Sesame	42	45	0
7.	Soybean	24	53	7
8.	Sunflower	20	69	0
9.	Linseed/Flaxseed	19	14	58
10.	Fish oil	25	4	45

Results from various studies regarding the effect of the dietary polyunsaturated fatty acids (PUFA) on nutrient utilization in ruminants are inconsistent. It has been found that average postpartum dry matter intake (DMI) is not affected by dietary inclusion of roasted soybean and linseed as a source of  $\Omega$ -6 and  $\Omega$ -3, respectively, in the dietary regimen of heifer cows. Similarly, feeding whole flaxseed also has no negative effect on DMI and milk yield because of low release of VFA in the rumen fluid. Even greater postpartum dry matter intake and energy intake has been reported in animals fed extruded flaxseed. Some studies confirm that a diet containing a high proportion of saturated fatty acids causes reduced dry matter intake than one rich in unsaturated fatty acids. However, several other studies have found that decreased intake (DMI) results from abomasal infusion of unsaturated fatty acids or from feeding the cows increasing amounts of unsaturated fatty acids at the expense of saturated fatty acids. Animals fed PUFA rich oil either maintain or improve



their body condition score (BCS). As observed by Chilliard *et al.* (2009), BCS was not affected up to 40 days post-partum when cows were fed flaxseed as  $\Omega$ -3 fatty acid source as compared to control. Dietary supplementation of rumen protected fat sources rich in PUFA has also positive effects in the improvement of digestibility of ether extract and organic matter. If the PUFA fat is given in the form of rumen bypass fat, they increases the growth rate of the heifers. The bypass fat of PUFA with varying degree of unsaturation is available as calcium salts. Childs *et al.* (2008c) fed graded level of fish oil to crossbred heifers and reported that DMI and ADG was higher at 140g of fish oil compared to diet without fish oil. Fiorentini *et al.* (2013) found that adding soybean oil to the diets of crossbred heifers did not affect nutrient intakes and utilization. Although, PUFA supplemented in the appropriate amount in heifers' ration results in better growth and nutrient utilization in heifer, but, excess amount bears negative impacts also. Diets containing high fatty acid content ( $>7\%$ ), especially unsaturated fatty acids can adversely affect the ruminal fibre digestion, which in addition to being toxic to rumen microflora, adhere to the food particles and cause a physical barrier between microbes and feed particles preventing their action, consequently impairing the performance of growing animals. Rations containing below 6-7% of DMI as total fat (saturated plus unsaturated), have showed that  $\Omega$ -3 and  $\Omega$ -6 fatty acids supplementation in crossbred heifers resulted in no negative impacts on dry matter, organic matter, neutral detergent fibre and acid detergent fibre intakes and digestion.



## Maternal Dystocia In Buffalo: Incidence, Causes And Treatment

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### Introduction

Buffalos face a number of reproductive challenges that often lead to low productivity and reproductive capacity. Maternal dystocia is one of the common disorders among buffalos that disturb the normal process of birth. The process of giving birth is comparatively easy in animals with large pelvic areas. Buffaloes, even in comparison to cows, have a narrower and shorter cervix. Also, they have a small vagina with wider vulvar lips. Buffaloes usually have a gestation period of 305-340 days; however, the time period varies from species to species.

### What do you understand by Maternal Dystocia?



The term “Dystocia” is mainly used for a difficult or obstructed birth. It is of mainly three types; maternal, placental, and fetal. In Maternal Dystocia, the baby’s delivery gets blocked despite

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normal uterus contraction. In other words, it is delayed and tough calving and requires human support in most cases. Maternal dystocia also includes complications where the baby fails to get sufficient oxygen. Furthermore, it makes the mother vulnerable to several infections along with uterine rupture. The most common species that suffer from dystocia are bovines. They experience difficult birthing due to functional defects or physical hindrances.

### **Incidence of Maternal Dystocia**

The incident of maternal dystocia in buffalo, of course, varies across different regions of the world, including India. However, the incidence of uterine torsion, being one of the major causes of Maternal Dystocia, has been found to be higher in buffaloes. According to the study, the incidence is as high as 70%. In India and Pakistan, the cases of maternal dystocia have been seen more in dairy-type buffaloes.

Furthermore, the average incidence of cervical dystocia was reported to be around 5% in both buffaloes and cows. And in the case of pelvic deformities, it has been reported to be around 1% in buffaloes, according to Mishra, 2021.

### **What are the major causes?**

In buffaloes, maternal causes were found to be more prevalent (75%) than fetal causes (21%), and there are primarily two causes of Maternal Dystocia:

1. Birth canal obstruction
2. Failure of the expulsive forces

### **Birth canal obstruction**

Birth canal obstruction can occur for a variety of reasons, as detailed below:

- Pelvic abnormalities: Small-sized or tight pelvic regions can lead to maternal dystocia. Apart from this, any deformity in the pelvic region or vaginal hypoplasia can also cause dystocia-like issues. In many cases, buffaloes having pelvic fractures are subjected to breeding, which can also lead to a reduced pelvis and further lead to dystocia.

When small-sized cattle or buffaloes are crossbred with larger-sized breeds, there is a potential risk of obstructing larger-sized foetuses within the small-sized pelvis of the mother a less common factor contributing to a narrow pelvic opening is sacral luxation or displacement. In addition, twins and intra-pelvic haemorrhage are also mentioned as possible causes of dystocia; conversely, pelvic fractures and exostoses are considered infrequent causes of difficult labour in larger animals.



- Uterine Torsion: It is one of the chief disorders affecting buffalo, especially when the mother is nearing birth. Uterine torsion has been found to be responsible for more than 53% of cases of maternal dystocia. Basically, torsion is the uterus twisting on its axis. Whether uterine torsion occurs during pregnancy or parturition, complications of maternal dystocia are there in buffaloes as well as in cows. Uterine torsion, being the key reason for maternal dystocia, can lead to the foetus and the mother's death if left untreated. This way, the death rate highly affects dairy production and economic profit. However, the most typical therapy for buffaloes suffering from maternal dystocia is caesarean section.
- Vulva, Vagina, and Uterine Neoplasms: In the reproductive region, sometimes buffalo suffer from abnormal tissue growths called tumours. These tumours can result in maternal dystocia that hinders or obstructs the process of normal childbirth. The growth of neoplasms basically results in the obstruction of the birth canal. They occupy space in the reproductive region and narrow down the passage for the baby to pass during the time of delivery. However, the effect of these tumours varies according to the tumour size.

#### **Failure of the expulsive forces**

The circumstances in which normal uterine contractions occur during labour are not enough or inadequate to move the baby's delivery. This can result in maternal dystocia, which is defined as prolonged or obstructed labour. The incapacity of the expulsive forces can be caused by distinct variables, for example, primary & secondary uterine inertia, as well as uterine rupture. The primary and secondary uterine inertia has been discussed:

- Primary Uterine Inertia: It takes place when the uterus fails to contract with enough force rate to push it out through the delivery canal. This medical issue is common during the initial phases of labour and may be triggered by hormonal disorders, insufficient cervical dilation, or extreme tiredness. Primary uterine inertia can cause labour to be delayed or stopped.
- Secondary Uterine Inertia: It is commonly termed secondary uterine atony and actually is the lack of viable and frequent uterine contractions, which is usually followed by an episode of normal labour progression. This syndrome can develop right through the active phase of labour and is frequently connected with conditions like maternal weariness, water loss, drowsiness, or the existence of maternal or foetus well-being issues. Secondary uterine inertia can cause a stop in labour progression.



### What are the possible treatments of Maternal Dystocia?



Cases of maternal dystocia in buffalos can't be overlooked and should be treated on an urgent basis. It is crucial to carefully evaluate the patient's condition before taking any interventions. In buffaloes, the treatment involves both manual and medical interventions.

- **C-Section:** It is basically the ultimate solution to maternal dystocia when other treatments don't work out. This particular intervention involves an incision in the buffalo's uterus and abdomen, and this incision process is followed by the baby's delivery. Usually, a caesarean or C-section becomes necessary in serious conditions of maternal dystocia.
- **Medical management:** This is done to regulate and stimulate contractions in the uterus. This can include the use of Betnesol, Progyonon, Epidosin, and pragma for cervical dilation. The whole process may take 12-72 hours to complete. Basically, medical management is done to promote easy labour progression.
- **Manual effort and support:** If the medical management fails or doesn't seem much effective, manual techniques are practiced to assist the delivery process. To be specific, in manual support, repositioning and manipulation of the mother's baby is done to ease birthing and constriction.





- **Obstetric manipulations:** When the foetus is in an abnormal position during childbirth, it can impede the delivery process. Obstetric manipulations may include repositioning the calf or correcting malpresentation to facilitate a more comfortable birth. A twisted calf's neck or limb, for instance, can cause an obstruction in the birth canal.

In such cases, the experts may gently move the fetus to correct its position, properly aligning the neck or limbs to allow for a more straightforward delivery. Correcting malpresentation or repositioning the calf requires skill and expertise and is typically performed by obstetricians or experienced veterinary professionals.

### **To sum up**

When buffalo mothers experience dystocia during childbirth, it is often attributed to factors unique to them - such as uterine torsion or other potential issues that may arise. Despite this being common knowledge for most livestock owners & caretakers' alike - we must emphasize that adopting good care practices among buffalo herds goes a long way in reducing risks associated with foaling. By upholding proper husbandry methods coupled with timely medical interventions before giving birth - including adequate feeds rich in nutrients & minerals for both mother & calf. Routine check-ups- we maximize the chances of successful birthing processes while reducing the likelihood of dystocia incidents happening.

Furthermore, prompt diagnosis and intervention by experienced veterinary professionals are critical in the management of maternal dystocia in buffaloes. Diagnostic techniques and obstetric manipulations, such as repositioning the calf or correcting malpresentation, can aid in the resolution of complications and the facilitation of successful deliveries. To ensure successful outcomes, buffalo breeders and farmers should prioritize education and training in proper animal management practices. This includes gaining knowledge about the signs and risk factors associated with maternal dystocia, as well as implementing preventive measures to minimize its occurrence.

On the whole, maternal dystocia in buffaloes, often caused by uterine torsion and other maternal factors, can be effectively managed and minimized through the application of good managerial practices. By implementing proper nutrition, regular veterinary care, and prompt intervention, when necessary, breeders and farmers can enhance the well-being of their animals and promote successful births, reducing the risks associated with maternal dystocia.



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## Medias used in *In Vitro* Embryo Production (IVEP): A Review

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### Abstract

Assisted reproductive technologies (ARTs) can be any procedure, which involve the manipulation of reproductive cycles, gametes or embryos like Artificial Insemination, Multiple Ovulation Embryo Transfer (MOET), Semen sexing etc. *In Vitro* Embryo Production (IVEP) is one of the assisted reproductive technologies for faster propagation of superior germplasm in animals which includes Oocyte collection, *in vitro* maturation (IVM), *in vitro* fertilization (IVF) of oocytes with capacitated spermatozoa and *in vitro* culture (IVC) and Embryo transfer. For each step different medias are used in appropriate condition and concentration. Culture media are broadly divided into simple and complex. Simple media are usually bicarbonate-buffered systems and complex media contain, in addition to the basic components of simple media, amino acids, vitamins, purines, hormones, antioxidants, growth factors and other substances. It had been shown that monoculture medium system has the advantage of decreasing the number of manipulations and the length of time the embryo is out of the incubator when compared to sequential media for culture. Now a day, commercially available entire serum-free ready-to-use media are available, which suite for all the steps in IVEP. Long time effort of researchers has led to these successes in IVEP and still needs new research work in the field of IVEP for best outcomes.

### Introduction

*In Vitro* Embryo Production (IVEP) is one of the assisted reproductive technologies for faster propagation of superior germplasm in animals. IVEP involves, collection of oocytes from either slaughterhouse ovaries or live animals through ultrasound guided transvaginal aspiration, oocyte *in vitro* maturation (IVM), *in vitro* fertilization (IVF) of oocytes with capacitated spermatozoa and *in vitro* culture (IVC) of presumptive zygotes (Gordon, 2004). In IVM, good quality oocytes are selected and kept in maturation media for 20-24 hrs in CO<sub>2</sub> incubator at 38.5°C, 5% CO<sub>2</sub> and 90-95% relative humidity. After completion of IVM, oocytes are co-incubated with spermatozoa for up



to 16 to 18 hrs while undergoing IVF (Gordon, 2004). After IVF, the fertilized oocytes are submitted to IVC media for 6-7 days until they reach the blastocyst stage. In general, 20% to 40% of the cultured presumptive zygotes will reach the blastocyst stage. After reaching the blastocyst stage, embryo transfer is performed following similar procedures as with *in vivo* blastocysts, or embryos are cryopreserved.

### **Medias in IVEP**

For IVEP, different medias are used which have necessary elements for the development and maintenance of the oocytes, sperms, embryos and provides environment similar to the living body. Culture media are broadly divided into simple and complex.

Simple media are usually bicarbonate-buffered systems containing basic physiological saline with the addition of pyruvate, lactate and glucose; the main differences between the various forms of simple media lie in differences in their ion concentration and in the levels of the energy sources. The media are usually supplemented with serum or albumin with trace amounts of antibiotics (penicillin, streptomycin, gentamycin).

Complex media contain, in addition to the basic components of simple media, amino acids, vitamins, purines, hormones (FSH, LH, Prolactin, Growth hormone, Insulin, Estradiol, Melatonin etc.), antioxidants (glutathione, cysteamine,  $\alpha$ -tocopherol, L-ascorbic acid, etc.), growth factors (IGF-1, IGF-2, EGF, bFGH, TGF- $\alpha$  and TGF- $\beta_1$ ) and other substances, mainly in the concentrations found in serum. Some of the complex media, including; Tissue Culture Media- 199 (TCM-199), Tyrode's Albumin Lactate Pyruvate (TALP) stocks, Synthetic Oviductal Fluid (SOF), and Minimum Essential Media (MEM) which have been used for oocytes maturation, fertilization and embryo development in mammals. The IVF culture also should be useful in providing sperm the needed movement and adaptation, which eventually leads to its union with the ova and then the beginning of embryonic development.

### **Commercially available medias**

With the increasing implementation of IVEP of bovine embryos worldwide for commercial use, there is an increased focus on optimizing the yield of blastocysts. For this reason, in recent years, many companies have come up with commercially available medias having all essential components for oocyte, sperm and embryos development. These helps in performing all the procedures easily and with less contamination, in turn which helps in giving better results. Changes had been observed in the physiology and metabolism of the early developing bovine embryo, which gave increasing



attention towards the use of sequential media for culture, each medium reflecting the changing requirements during development. But recently, in human as well as in bovine IVF the monoculture medium system is gaining popularity. The monoculture medium is supplemented with all the required compounds to sustain embryo development to the blastocyst stage, and is based on letting the embryo choose the nutrients and components needed for an optimum development during the entire culture period. It has been suggested that monoculture medium system is as efficient as the sequential medium system. Knowing that the embryos worst enemy is the fluctuations, in particularly, of pH and temperature (Swain, 2010), a monoculture medium system has the advantage of decreasing the number of manipulations and the length of time the embryo is out of the incubator.

Refined serum-free culture conditions, based on BSA supplementation, have been developed allowing for improved fetal development and calving (George *et al.*, 2008), and in 2013 an entire serum-free ready-to-use media suite for all the steps, maturation, fertilization and culture, was made commercially available by IVF Bioscience, UK, combining synthetic serum replacements and BSA (Hyttel *et al.*, 2019). Nielsen *et al.* (2015) concluded that the developmental rates and gene expression of *in vitro*-produced bovine blastocysts were affected by the use of different culture media. Increased blastocyst rates, apparently superior embryo quality, and more abundant gene expression were achieved when blastocysts were cultured in Bo-IVC culture media (IVF Biosciences) compared with SOF. Pryor *et al.* (2016) showed from his research that ETB (EmbryoTrans Biotech) media was superior to control media for percent viable, HBL (hatching blastocysts), and combined HBL/expanded BL (51.9, 23.9, 45.8% vs. 29.2, 5.8, 20.5, respectively). Also concluded that ETB media produced more high-quality embryos than control media under varying conditions experienced by commercial IVF companies.

## Conclusion

In bovine IVEP, commercial 'ready to use' and 'serum-free' IVP media have contributed to a more stable production systems, as they reduce the batch-to-batch variability of a laboratory-made medium. Long time effort of researchers has led to this success in IVEP. Hence there is still need of new research work in the field IVEP for best outcomes.

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## Strategies For Reducing Age at First Calving in Dairy Animals

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### Introduction

Raising heifers is one of the most expensive components of the dairy farm operations as they are future of dairy farm. They are produced to replace the older and uneconomical females of the farm through culling. When genetic trends are positive, such replacements help harvest the benefits of genetic gain. Heifer production is most expensive part of the dairy farm operation as it requires more inputs for a longer period of time with no visible returns than any other farm operation. The major issue is the lower growth rate of calves during early months of their age and just after weaning which is either due to underfeeding or imbalanced feeding which results in higher age at puberty and thus higher age at first calving in heifers. Genetics also play its role as well. Age at puberty and calving is related with weight. Heifers can be bred when they have attained 60% of their adult body weight. Assuming an average adult weight of a buffalo as 550 kg, buffalo heifers can be bred when they have attained a weight of about 330 kg. Balanced feeding, improved management, using performance modifiers and better health care facilities can be helpful in reducing the age at first calving thereby reducing cost of heifer production. Mostly, dairy breeds of the tropics and sub-tropics are slow maturing and low milk producers, this inferiority is partly inherited and partly due to the malnutrition, management and environment to which they are exposed. In tropical climates buffaloes are preferred over cattle because of their better efficiency in utilization of nutrients from poor quality fibrous tropical feeds and relatively better disease resistance and adaptability to tropical climates.

## Management Of Age at First Calving (AFC)

Herds can minimize the variability in AFC by obtaining high pregnancy rates, but poor reproduction increases variability in AFC, although nutrition and growth rates may be adequate. Therefore, reducing the age of onset of puberty, early breeding, and thereby reducing age at first calving (AFC) is the obvious option to minimize expenses on these heifers. The AFC can be reduced by a combination of increasing pre-pubertal average daily gain and decreasing age at breeding or by reducing age at breeding alone.

- **Breeding season:** Buffalo is partially seasonal breeder. Acc. To a study, buffaloes calving in summer (June to August) had a shorter calving interval than those calving in other seasons. The longest calving interval was observed in buffaloes calving in winter (December-January). This means that the buffaloes that had calved just before the onset of their breeding season (October-November) had more chances of getting bred than those calving after passing their breeding season. This implies that the buffalo heifers attaining their proper weight just before their breeding season are more likely to get bred than those passing this period and thus may have lower age at puberty and consequently at calving than those attaining proper weight after this season. Based on this hypothesis, while raising the replacement buffalo heifers, efforts should be made to keep an eye on both critical weight of buffaloes for attaining the age at puberty and also the season in which this weight is attained. In this regard, adjustments in feeding regime may be required to get the critical weight of buffalo heifers just before their breeding season. Because once this breeding season is over then feeding for accelerated growth may not reduce the age at their puberty and then one has to wait for their next breeding season to see the puberty in the heifers.
- **Feeding:** In our country, calves are usually raised on fodders with limited amounts of concentrates low in protein and energy before and after weaning. This is one of the reasons for lower growth rate and delayed age at puberty in heifers. In buffalo calves, calf starter ration containing CP% and TDN%, 23-25% and 75% should be fed to achieve a higher daily growth rate. In our country, where there is no check on quality of concentrates for ruminants, offering a quality concentrate supplement with higher protein and energy may accelerate growth rate in the replacement calves with positive effects on reducing the age at puberty. Age at puberty can be reduced through additional concentrate feeding for a few months before the onset of puberty. Concentrates are supplemented with fodder to reduce the age at maturity.





- **Bio stimulation:** Bio stimulation (bull effect) can be defined as the stimulus provoked by the presence of males, which hastens onset of puberty, estrus and ovulation through genital stimulation, pheromones, or other external stimuli. Presence of a vasectomized bull has been reported to hasten the onset of puberty in heifers and also the early resumption of ovarian cyclicity following parturition.
- **Photoperiod:** Photoperiodism can be defined as the physiologic response of animals and plants to the variations of light and darkness. Seasonal changes in the photoperiod are a major determinant of reproductive activity. Management of photoperiod has influence on the attainment of puberty. So photoperiod is an effective noninvasive approach to improve performance of animals thereby achieving early puberty. Long day photoperiod (LDPP) i.e. 16-18 hrs light and 6-8 hrs dark during growth phase of animals is beneficial in attaining larger leaner animals at maturity with higher body weight gain.
- **Suckling vs hand feeding for calf health:** Direct suckled calves are healthier than weaned calves. The farmers should be encouraged to follow the indigenous practices that can enhance daily growth rate to get a healthier calf crop. It is also a need of hour to test whether weaning should or should not be practiced in Sahiwal cattle or Nili-Ravi buffalo as they have a very strong mother instinct to milk let down in the presence of calf which if weaned results into either abandoning of the lactation or milk let down through oxytocin.
- **Environment:** Performance of animals is a great challenge in tropical region due to high ambient temperature especially in summer month. Buffaloes are more susceptible to heat stress because of their dark colour and buffalo skin has one-sixth the density of sweat glands that cattle, so buffaloes dissipate heat poorly by sweating. Exposure of buffaloes to the hot conditions leads to depression in feed intake, efficiency and utilization, disturbances in metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. Such changes result in impairment of reproduction and production performances. The effect of heat stress is aggravated when heat stress is accompanied by high ambient humidity. Heifers reared under poor housing conditions reach puberty later than heifers reared under excellent housing conditions. Houses protecting buffaloes from direct heat and with proper ventilation favour faster growth in calves. Buffalo heifers allowed wallowing for more than 2 hours a day during summer months (April to September) attained puberty earlier at 30.45 months as compared to 42.55 months who



had given bathing once a day. Marked reduction in the age at puberty could be attributed to improved heat dissipation while bathing/wallowing

- **Bovine Somatotropin (bST):** Somatotropin is a hormone produced by the anterior pituitary, a small gland located at the base of the brain, and is transported by the blood to various body organs where it has its biological effects. It has been extensively used in ruminants for promoting growth and production. Injection of bST (25 µg/kg of body weight) in Holstein heifers increased daily body weight gain and weight at puberty by 10%, and 25 kg, respectively and reduced the age at puberty by up to 24 days. bST altered the intermediary metabolism in a manner that increased lean tissue and decreased fat deposition.
- **Ionophores:** Ionophores are antibiotics produced by a variety of actinomycetes. Feeding ionophores typically increases the efficiency of feed utilization in ruminant animals. Ionophores increase growth rate and decrease age at puberty in grazing heifers. Animals fed ionophores alone or in combination with anthelmintic leads to puberty at a younger age.

### **Conclusion**

Different approaches to raise the heifers on economical basis and reduce their age of puberty have been summarized above. In any particular situation, one has to keep in view the cost of inputs involved and returns thereof. Any system that is more feasible in a given set of environments should be applied judiciously. In our system, forage should be the main feed supplemented with concentrates and other performance modifiers to gain a faster growth rate for early puberty on cost effective basis.



## Spatial And Temporal Dimension of Agro Tourism In Post Independent India

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### Introduction

Agriculture is the primary sector that supports the Indian economy. Agriculture provides a livelihood for approximately 85 percent of the population in India, either directly or indirectly, and accounts for nearly 26 percent of the country's gross domestic product. More than 350 million tonnes of food grains are produced by 110 million farmers who live in 0.625 million villages across the country. These farmers feed the nation. Agriculture in India is considered a cultural tradition rather than a job or a business. The addition of new activities that generate income to those that already exist in agriculture would, as a result, almost certainly result in an increase in the contribution of agriculture to the national GDP. A number of significant efforts are being put in this direction, and one of the activities that is being considered is agritourism. Agro tourism has spawned an offshoot known as agritourism, which has enormous potential in India because of the country's vast agricultural landscape. Agritourism is one viable option for developing nations like India and other Asian economies that rely heavily on agriculture as their primary source of income. This is because agriculture is the primary occupation of the majority of the population in these nations. Agritourism is just one of the ways that these nations can diversify their sources of revenue.

A visit to a working farm or any agricultural, horticultural, or agribusiness operations for the purpose of enjoyment, education, or active participation in the activities of the farm or operation is an example of agricultural tourism. Agricultural tourism is a concept borrowed from the concept of "holidays." Agritourism, in its broadest sense, refers to the practise of luring tourists and vacationers



to a location or locations that are primarily utilised for agricultural purposes. Agritourism, on the other hand, is typically conducted on a modest scale, has a limited impact, and places an emphasis on education. The majority of Maharashtra's farms are run by their landowners, which means that there are virtually infinite opportunities for individuality and customization. There are numerous agritourism activities that can be carried out successfully with only a modest number of farm workers. For instance, farm tours, bed and breakfasts, tractor or bullock cart rides, grapes, mangoes, and other horticulture farms, by product farms, birds and animal zoos, and a great deal of other activities can be run with very little additional investment in labour.

People often refer to tourism as a tool for alleviating poverty, increasing employment opportunities, and fostering long-term human development. In 2017, the tourism industry was directly responsible for the creation of 21.5 million jobs. In addition to fostering national integration and international understanding, tourism also provides financial support for regional arts and crafts as well as cultural pursuits. 11.23 million people travelled to India from other countries in the year 2017, according to official statistics. Even though India holds only 1.75 percent of the global tour market, the country brings in Rs 32,000 crores thanks to its participation in this market. There are 120 million visits made by tourists from within their own country. In order to foster domestic tourism, the Government of India has prioritised the development of infrastructure, product development and diversification, the creation of eco-adventure sports, cultural presentations in expensive accommodations, the streamlining of facilitation procedures at airports, the development of human resources, the promotion of public awareness and participation, and the felicitation of private sector participation.

### **Need and Importance of Agri tourism**

It is believed that participating in agrotourism is the best way to learn about the traditional agricultural farming activities, which not only brings us much closer to mother nature but is also an extremely important component of living a life that is compatible with the environment on this planet. The cities of India are currently struggling with the problem of overcrowding as well as environment pollution. It is now commonly accepted wisdom that a break from the frenetic pace of life in urban areas can be had through the practise of agrotourism. Because of this, agrotourism, ecotourism, and rural tourism are quickly becoming some of the most important subsectors of the tourism industry in India. The vast majority of studies have conclusively demonstrated that agrotourism is of critical significance and is essential on the grounds that: One form of tourism that does not negatively impact



the environment is known as agrotourism. The farmers now have access to an additional source of income as a result of this. The rural way of life is elevated as a result, and new employment opportunities are made available at the community level. Those who live in cities can take advantage of this to get away from the hustle and bustle of city life. Those who live in the area benefit from an improved quality of life as a result. The village atmosphere, local cuisine, culture, and art are all things that can be experienced by tourists thanks to this. Rather than acting as a passive spectator, it anticipates that the tourist will actively participate in the activity, which helps to strengthen the connection between the guest and the host. It is environmentally friendly, which is very important in the current environmental scenario. It acquaints tourists with rural life and the roots of early civilization. In the current climate of the tourism industry in India, it possesses a significant amount of potential for growth. It is a less expensive gateway of tourism, and the cost of accommodation, food, travel, and recreation is very less in Agro-Tourism when compared to any other type of tourism.

It broadens the tourist base by increasing the scope of tourism due to the fact that it is cost effective. In the current global scenario, there is a significant appetite for it. It provides all opportunities to people of all ages, including children, young people, middle-aged people, and older people, and it does so at a cost that is more affordable for the entire family. It acquaints visitors with rural games, traditional clothing, festivals, and cuisine. It brings tourists closer to nature and offers them a variety of entertainment options to enjoy while they are there. It is a source of knowledge that can provide information about plants, animals, raw materials such as handicrafts and woods, the rural lifestyle, and the languages, culture, and tradition of the people who live there. This subset of the population may be able to have their inquisitiveness sated by participating in agrotourism, which typically centres on farming communities, rural settings, and agricultural pursuits. It is a way for tourists to consider agrotourism as a means of searching for peace and tranquilly in their vacations. It brings tourists very close to nature, and the fields, birds, animals, mountains, and bodies of water, as well as the villages, provide a completely different atmosphere to the urban population, allowing them to forget about the busy and hectic life they lead in the city. Guests are given the opportunity to travel to rural areas and spend time with their families as a result. It raises urban children's awareness of rural life and their knowledge of agricultural science, and it can be effectively used as an educational and training tool to educate urban tourists. It offers a wide variety of recreational opportunities to city dwellers by way of festivals and handicrafts.



## **Market for Agro tourism**

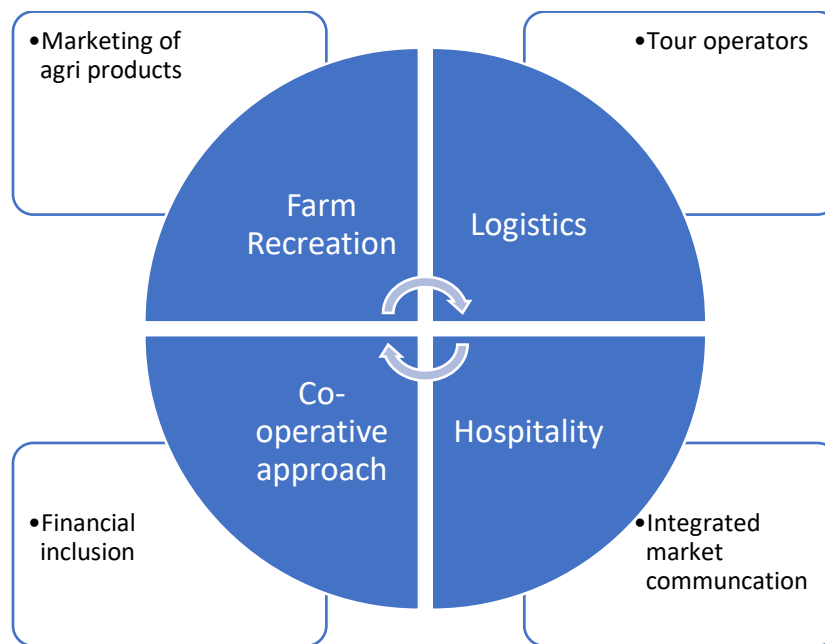
The agritourism market is segmented into activity, sales channel, and region. On the basis of activity, the market is categorized into on-farm sales, outdoor recreation, agritainment, educational tourism, accommodations, and others. By sales channel, it is segregated into travel agents and direct. Region wise, it is analysed across North America (the U.S., Canada, and Mexico), Europe (Germany, the UK, France, Russia, Italy, Spain, and rest of Europe), Asia-Pacific (China, Japan, Australia, India, South Korea, and rest of Asia-Pacific) and LAMEA (Latin America, the Middle East, and Africa)

The on-farm sales segment accounted for approximately one-third half of the global agritourism market share in 2019, and it is anticipated that it will maintain its share throughout the forecast period. On-farm sales are an essential component of agritourism centres, as well as a significant source of revenue. On-farm sales of agricultural products like cattle, wine, and grapes are bringing in a significant amount of revenue for cattle farmers, wine breweries, and grape growers. To boost their earnings from the products they grow themselves, some farmers have begun to operate their very own farm sales chain outlets. On-farm sales can include a wide variety of products, the specifics of which are determined by the farmer's production as well as the type of agritourism operation being conducted. Some examples of these products include grapes, apples, wine, goats, and rural antique show pieces. People are increasingly purchasing goods from on-farm sites because it is an incredible experience for them to pick farm products by hand, and also because the goods are fresh and have not been adulterated in any way. On top of that, people are realising that buying goods from on-farm sites saves them money.

In projects of this nature, where the farmers are the focus of everyone's attention and the activities that are related to them emerge from there, networking is an essential component. Because having a good road and connectivity to the farms is very important, the suppliers of fertilisers and seeds can also act as tour operators to attract visitors from urban areas. These visitors would spread the word about the benefits of visiting the farms. On the other hand, the hospitable nature of the locals would not only encourage more tourists to visit the area, but it would also pave the way for improved distribution of the regional arts and crafts. The majority of city dwellers who take a day trip out to the countryside in search of a change of scenery do so in the hopes of contributing to the local economy by purchasing locally made crafts, which they then take home with them as a memento of their time spent there. The tour operators, the villagers, and the tourists are all connected to one another in some way, making them all part of the integrated communications strategy. The villagers,



the majority of whom are farmers, can organise themselves into cooperatives to strengthen their faith in the viability of the agritourism model as an additional source of income and a more long-term way of life. Even when the monsoons are particularly harsh, the influx of tourists can help keep their income stable. Furthermore, when there are a significant number of villagers in an area, many banks are more likely to be willing to offer assistance in the form of loans and other value-added services. Children tourists will benefit from the final window of farm recreations and the opportunity to purchase fresh farm produce because they will be able to comprehend the entire process while also having fun with it. One of the most important aspects of sustainability is finding a solution to the problem of managing risks, which includes addressing issues such as delayed rainfall, pest epidemics, as well as death and loss of infrastructure. This is where the banks can come to the rescue.



**Opportunities for agritourism challenges and its networking**

**Conclusion**

Agritourism is a niche and emerging market segment of the tourism industry, and it has been gaining significant traction in urban areas. Urban areas have become increasingly interested in agritourism. This can largely be attributed to the rising popularity of farm stays as well as the growing interest among younger generations in rural life and agricultural pursuits. Due to the limited awareness that most people have regarding agritourism, ecotourism, and the other concepts that are closely related to it, travel agents in the agritourism industry play a very important role in the marketing and sale of agritourism packages. The majority of people may have participated in farm stays and activities, but they are unaware that these are examples of agritourism because this sector



of the tourism industry has not yet been fully explored. In addition to selling vacation packages, travel agents educate customers about the various aspects of agritourism, a factor that is expected to contribute significantly to the expansion of the market over the course of the period covered by the forecast.





## Exploring Pearl Millet in the Arena of Climate Change

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### *Abstract*

In the twenty-first century, achieving global food and nutritional security is difficult due to climate change and economic weaknesses. The dependence on three main food crops wheat, rice, and maize which account for 60% of all the plant-based calories consumed by humans threatens food security even more. Therefore, it is crucial to investigate additional native crops, often known as orphan crops, and discover alternative approaches to the problem of food security. In order to coordinate agricultural research across continents and increase the production of alternative nutritious crops and foods, the Food and Agriculture Organisation of the United Nations has declared 2023 as the International Year of Millets. One of the world's harshest warm-season cereal crops, pearl millet (*Pennisetum glaucum* (L.) R. Br.) is grown mostly in the semi-arid tropics of Asia and Africa for food, feed, fodder, and brewing. It is primarily grown for its high-quality, gluten-free grains with superior nutritious content. Pearl millet is a hardy plant that thrives in tough environments including low fertility, unpredictable rainfall, acidic and saline soils, and sweltering heat. The significance of pearl millet in climate change is highlighted in this article.

### **Introduction to Pearl Millet and its Importance**

Bajra, commonly referred to as pearl millet, is a grain crop that is widely farmed in Africa and Asia's dry and semi-arid regions. It is a highly prized crop because of all its advantages, especially considering climate change. Farmers in places prone to drought and heat stress may consider growing pearl millet since it is a climate-resilient crop that can thrive in environments with frequent dry spells [6][2]. Pearl millet is also a nourishing crop that is abundant in micronutrients like iron and zinc and can reduce malnutrition and covert hunger [3][6]. Pearl millet is a significant crop for farmers since it has a lot of potential to produce better and more reliable crop yields as a result of climate change. [5][3].

Pearl millet is an important crop in agriculture due to its resilience to climate change and its ability to grow in harsh environments. The extremely varied agro-morphological and adaptative characteristics of pearl millet landraces, such as flowering time and photoperiod, enable the crop to adjust to various environmental conditions. [6]. Furthermore, pearl millet is a hardy crop that requires less water than other cereal crops such as wheat and maize, making it an attractive option for farmers in water-scarce regions [9][3]. As a result, pearl millet has the potential to increase food security and improve the livelihoods of smallholder farmers in developing countries.

In conclusion, pearl millet is a nutritious and climate-resilient crop that has enormous potential for yielding higher and more stable crop yields under climate change. It is an important crop in agriculture due to its ability to grow in harsh environments and its resilience to drought and heat stresses. Pearl millet can also improve nutritional quality and combat malnutrition, making it a valuable crop for farmers in developing countries [6][5][3][1]. As climate change continues to affect agriculture, pearl millet can play a critical role in ensuring food security and improving the livelihoods of smallholder farmers.

### **Effects of Climate Change on Pearl Millet Cultivation**

Climate change has been observed to have a significant impact on pearl millet cultivation. Changes in temperature and rainfall patterns have led to a decrease in the productivity of pearl millet. Heatwaves and droughts, which are becoming more frequent due to climate change, have resulted in a decrease in pearl millet yield [2]. However, pearl millet is a climate-resilient crop that has the potential to increase yields and minimize the adverse effects of climate change [5].

Climate change-induced pests and diseases are also a significant threat to pearl millet cultivation. The downy mildew disease, which is caused by the fungus *Sclerospora graminicola*, is the most devastating disease affecting pearl millet crops [2]. The disease can cause significant yield losses, leading to economic losses for farmers. Furthermore, climate change has led to an increase in the incidence of pests and diseases in pearl millet crops, making it more challenging to cultivate the crop [2].

Despite the challenges posed by climate change, pearl millet remains an essential crop for farmers in arid and semi-arid regions. Pearl millet is a climate-resilient crop with enormous potential for yielding higher productivity under changing climatic conditions [3]. It can grow on poor sandy soils and is well suited for dry climates due to its ability to use moisture efficiently [9].



Additionally, pearl millet has drought and heat tolerance, making it an ideal crop for cultivation in areas prone to drought and heat stress [8]. As such, pearl millet is an essential crop that can help farmers adapt to the changing climate and ensure food security in the face of climate change [3].

### **Qualities of Pearl Millet that make it resilient to climate change**

Pearl millet is a climate-resilient crop that possesses qualities that make it well-suited to thrive in the face of climate change. One of the most notable qualities of pearl millet is its drought tolerance and water use efficiency. As a C4 grass, pearl millet is one of the most drought-tolerant cereals and is typically grown in areas with low annual rainfall [14]. Additionally, pearl millet is adapted to dry and arid conditions, making it a reliable crop in areas where drought is expected to intensify with climate change [5].

Another quality that makes pearl millet resilient to climate change is its heat tolerance and adaptability. Studies have shown that pearl millet has increased yields in dry and warmer sites due to its drought and heat tolerance traits [2]. The crop's high photosynthetic efficiency and excellent productivity in low-nutrient soil conditions make it less reliant on chemical fertilizers [5]. A 2017 study found that drought and heat tolerance in pearl millet increased yields under climate change in both arid and semi-arid tropical climates [8]. Furthermore, researchers have identified genetic variability in pearl millet that enables a better understanding of its heat tolerance and adaptation to changing climates [5].

Pearl millet also possesses pest and disease resistance, making it a hardy crop that can withstand harsh growing conditions. Disease management using resistant cultivars is a feasible way to manage pests and diseases in pearl millet production [19]. The crop's morphological characteristics, forage production, seed formation, and water-use efficiency have been monitored for ten years, and it has been found to be one of the most drought-resistant grains in commercial production [10]. In conclusion, pearl millet's climate-resilient qualities, combined with its high nutritional value, make it an ideal crop for improving nutritional quality and ensuring food security in regions vulnerable to climate change [6][3].

### **Conclusion**

Farmers in arid and semi-arid areas of Africa and Asia highly value the climate-resilient crop pearl millet. It is a sustainable crop that can grow in climates with frequent dry periods and is rich in micronutrients like iron and zinc. Pearl millet hence has the potential to boost food security and enhance the standard of living for smallholder farmers in underdeveloped nations. The



production of pearl millet has been extensively influenced by climate change, with reduced yields as a result of changes in temperature and rainfall patterns. However, because of its drought resistance, water use efficiency, superior yield in low-nutrient soil conditions, and great photosynthetic efficiency make it and heat tolerance, pearl millet continues to be a crucial crop for farmers in arid and semi-arid areas.

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the Agri Tourism Development Corporation (ATDC) located at Baramati in Maharashtra This paper will definitely serve as a platform for diverse stakeholders such as agrotourism practitioners, farmers, agripreneurs, policy makers, researchers, academicians to understand the concept of agrotourism, objectives, state policies, models of agrotourism, prospects and challenges.

The term “agritourism” is often used interchangeably with “agri-tourism,” “agrotourism,” “farm tourism,” “agricultural tourism,” or “agritainment.” Many young agrotourism practitioners and research scholars were invited to speak about their research and field experiences in the field of agrotourism. There are numerous hidden potentialities of agrotourism sector in our country and it is needed to underline about various prospects to farmers by taking several active examples of agrotourism models existing in our country.

### **Case 1: Shri Panduranga Taware- Pioneer of Agrotourism**

Shri Panduranga Taware who is fondly called the change maker in Agrotourism. He is the founder and managing director of Agrotourism Development Corporation Pvt. Ltd., Maharashtra and serves as a resource person for policy changes and developing agrotourism models across the country. His initiative on agrotourism policies and procedures across Maharashtra laid as a foundation stone across the country. He remarked that “It is interesting to observe that 43% of urban population did not have any relative left in village and around 97% of urban population wants to experience the rustic beauty of village life.” He discussed about the eligible components required for setting an agrotourism model, binding criteria and facilities needed to be provided at the unit under Maharashtra tourism policy. The need for a responsible, safe and sustainable agrotourism model was the crux of the presentation. Various government benefits and procedures for training and guidance were also emphasized. He also elaborated about the application process required for registration especially for the new practitioners. Besides the formal procedures and guidelines to be followed, he told that “an agrotourism unit just need a story to tell, a clean farm, clean villages, conservation of environment, culture and traditions and sustainable livelihood for rural folks.” The steps required for setting up agrotourism model for prospective farmers are delineated here.

*Step 1:* Mapping of resources by agricultural officers and prospective farmers

*Step 2:* Conducting a sensitization workshop including all resource persons in district

*Step 3:* Conducting a minimum three day residential programme with progressive farmers deriving direct contrived experiences



*Step 4:* Develop a work plan work plan according to guidelines and policies developed by state government.

### **Case -2: Cow tourism model- Back to Roots**

The next practitioner was Dr. Anuj Singhai (Founder, SajaiviCo India Pvt. Ltd.) who developed a cow tourism model. He is a social change maker working on a cow -centric approach for preserving native breeds- malwi and niwari in Bhopal, Madhya Pradesh. Besides offering a handful of services like cow tourism, cow therapy, panchagavya therapy, kitchen garden, pashu ayurveda, organic agricultural training, diverse alternate organic products have been sold at their unit. Cow tourism is basically an extended part of agrotourism, contributing to 10-12 units of vertical diversification.

Another popular practitioner was Dr. Kishore Indukuri (Founder & CEO, Sid's Farm, Hyderabad). From conducting school visits, arranging campsites and homestays, it provides the unique and relishing experience of milking the cows, milk testing facilities, visits to fodder grass farms and water harvesting systems. He is a pioneer in setting up the vision of a new benchmark in the milk industry with superior quality dairy products, a sustainable ecosystem and a thriving relation with farmers.

### **Case-3: Hachiko tourism**

Mr. Manoj Hadawale (Founder of Hachiko tourism) outlined about the tourism industry and contribution of agrotourism towards tourism sector of India. "Tourism for Inclusive growth" was the theme for World tourism Day (27 September, 2021) whereas "rural and community centric tourism" was the theme for National Tourism Day (25 January, 2022). Studies reveal that by 2028, annual growth of agrotourism sector will be 6.9% which will be 9.9% of total GDP. He gave a brief description about the tourism and hospitality industry in our country. In his words, "people have different reasons for setting up agrotourism and best part is to set your own trend rather than going with the trend". Various facets of tourism like niche tourism, post-covid tourism, experiential tourism, responsible tourism, tangible and intangible benefits and trends of tourism were discussed. Trends of agrotourism include farm recreation, hospitality, good communication and involvement of cooperatives. He mentioned that experiential tourism is the core of every tourism sector and cultural identity coupled with sustainable tourism enhances socio-economic development of nation.

### **Case-4: Farm of Happiness- The Rustic beauty of the Konkan**



Rahul Kulkarni (Director, Farm of Happiness, Ratnagiri), a vibrant agrotourism practitioner exposed his journey from advertising sector to being an agripreneur. The pillars of agrotourism revolved around what kind of agrotourism, why agrotourism and who caters to the need of agrotourism. Agrotourism offers tangible benefits such as growing profitable crops, selling value added products, and creating employment opportunities. Intangible benefits include establishing one's own identity, gaining respect in society and aesthetic value. Entertainment along with education gives added meaning to agrotourism. Responsible tourism must be given the main thrust and he is a visionary working towards better and better concept rather than bigger and bigger concept which makes him a unique agrotourism practitioner. He further added that lot of entertainment takes away the essence of agrotourism. Hence each aspect of agrotourism must be dealt with utmost caution.

Mr. Dattatray Shelke (Unique Path, Pune) is another agro-tourism practitioner. He discussed about the entrepreneurial development through agrotourism in rural India as well as strengths and weaknesses of the industry. The farm stay offers multiple services like camping, trekking, sightseeing, boating activities and serves as a venue for many social and corporate ceremonies. His vision of setting the farm was basically to motivate peer farmers to go beyond the traditional cultivation of crops.

#### **Case-5: Green Commandos-The paradise of East**

Another vibrant practitioner was Mr. Samir Bordoloi (Founder, Food Forest, Sonapur). He initiated 'Green Commandos' who are the green tribe of people who believed in the principle of local food, local people and local economy. They in turn conserve, consume and commercialize local food wisdom so that one can protect farming profession. He stressed about the importance of 'compassionate farming' and importance of going back to the roots of our tradition. He persistently envisions the world where naturally organic farming and sustainable agricultural practices are the basic ruling factors. Through the Green Commandos, Mr. Samir is building a farming movement that substantially lowers input costs and reduces ecological damage by employing natural farming techniques. This is being done by reducing jhum cultivation, using existing land, that has already been burnt. Through the use of bamboo forests and groves, the Green Commandos are helping to create natural carbon sinks, reducing the impact of global warming. The adverse impact of monocropping is being reversed by creating edible food forests, using existing forest land, rather than destroying and replanting. Summing up, he is the pioneer of the social agripreneurs and local





food advocates- the green commandos and urges to practice low-cost ecological farming and constantly look forward for an inclusive, sustainable and equitable farming.

### **Case 6: Deva Giri Farm, Kolhapur**

Yet another practitioner from Maharashtra was Mr. Sukhadev Giri (Deva Giri Farm, Kolhapur). He briefed about the activities and services provided at his natural resort. It gives an exotic experience of rural ambience and pleasure of exploring field activities as well. Environmental sustainability should be the sole criteria of agrotourism. He added that there is no dire need of infrastructure nor huge investment for agrotourism, instead passion towards agriculture is just sufficient to explore this venture.

### **Conclusion**

The concept of agrotourism which is still in its infancy stage in our country. Different dimensions of agrotourism have to be learnt through case studies and potential agrotourism practitioners from across the nation. These insights will definitely serve as a starting point of hosting several workshops in near future for mobilizing extension workers to develop further core competencies in relatively new area of agrotourism.



## Dry flower technology: An amelioration in floriculture industry

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### Introduction

Dry flower technology, also known as dried flower preservation or dried flower arrangements, is a process that involves preserving fresh flowers by removing their moisture content. This technique allows flowers to maintain their natural beauty and structure for an extended period, even after they have been cut from the mother plant. The art of drying flowers dates back centuries and has been practiced in various cultures around the world. Initially, drying of flowers was primarily done to preserve their fragrance, but over time, it has been evolved into a decorative and creative craft. Dried flowers can be combined with other materials, such as foliage, herbs, or ribbons, to create visually appealing compositions. The final arrangements can be displayed in vases, shadow boxes, or framed as art work. Major dry flower producing areas in India are Tamil Nadu, Karnataka, Maharashtra, West Bengal and Andhra Pradesh. These states produce dried rose, marigold, jasmine, tube rose, gerbera, chrysanthemum and shola flowers for domestic and international markets.

### Advantages of dry flower technology

- ✓ Longevity: dried flowers can retain their beauty and shape for months or even years.
- ✓ Low Maintenance: preserved flowers do not need any special care.

- ✓ Versatility: Preserved flowers can be easily manipulated into various shapes and forms, allowing for unique and customizable designs. They can be arranged into bouquets, wreaths, centerpieces, or used in art and craft projects.
- ✓ Color Retention: Dried flowers maintain their original colors even after the drying process.
- ✓ Sustainability: Dry flower technology promotes sustainability by reducing the need for fresh cut flowers.
- ✓ Preservation of Sentimental Value: Dried flowers hold sentimental value and can serve as keepsakes for special occasions or memorable moments.

### Steps involved in production of dry flowers

- A. Selection of Flowers: The process begins with carefully selecting flowers at their peak freshness. Flowers with vibrant colors, intact petals, and minimal signs of damage are preferred for drying.
- B. Harvesting: The flowers are typically harvested early in the morning. This helps in preserving the flower's natural colors and shape.
- C. Conditioning: After harvesting, the flowers are conditioned by removing excess foliage, thorns, and leaves. The stems are trimmed to the desired length, and any damaged or wilted petals are removed.
- D. Drying Methods: Some common drying methods include

1. **Air Drying:** This is one of the oldest and simplest methods of drying flowers. The flowers are gathered into small bunches and hung upside down in a warm, dark, and well-ventilated area. Good air circulation helps in the gradual evaporation of moisture from the flowers, preserving their natural shape. Ex: Gerbera, Marigold, Statice, *etc.*



2. **Pressing:** Pressing involves flattening the flowers between heavy objects, such as books, paper, or specialized flower presses. The flowers are carefully arranged and pressed for a certain period until all the moisture is removed. Pressed flowers are often used for crafts, art, or as decorative elements in cards and bookmarks. Ex: Hibiscus, Pentas, Ixoras, *etc.*
3. **Silica Gel Drying:** Silica gel is a desiccant that effectively absorbs moisture. Flowers are buried in



a container filled with silica gel crystals or beads. The container is sealed, and the flowers are left for a few days to allow the silica gel to draw out the moisture from the flowers. Silica gel can preserve the flowers' shape, color, and delicate details effectively. Ex: *Strelitzia reginae*, *Hemerocallis fulva*, *Antirrhinum majus*, etc.



1. **Microwave oven drying:** Microwave oven drying works by producing an electronic microwave that releases the moisture from organic substances by agitating the water molecule. It is a quick strategy, and the product quality is better in terms of colour and shape maintenance. Ex: Golden Rod, Gypsophilla, Carnation, etc.
  2. **Hot air oven drying:** In this method, the plant material is kept at controlled temperature conditions for a specified time. The flowers are slotted through holes in a wire mesh rack in the oven. Time and temperature combinations vary from plant to plant and mainly depend upon the compactness and thickness of flowers Ex: *Callistephus chinensis*, *Dendrobium sp.*, etc.
  6. **Skeletonization:** involves removing the moisture content from the flower petals, leaving behind only the intricate veins or "skeleton" of the flower. This technique creates delicate and transparent floral structures that can be used for various decorative purposes. Ex: Maple Leaves, Delphinium, Orchids, etc.
- E. **Preservation Time:** The drying time varies depending on the method used and the type of flower. Air drying can take a few weeks to a month, while hot air oven and desiccant drying methods are faster and can take a few days to a week.
- F. **Preservation of Color:** To maintain the natural colors of the flowers during the drying process, it is important to dry them in a dark or dimly lit area. Exposure to sunlight or bright light can cause the colors to fade.
- G. **Preservation of Shape and Structure:** Proper handling during the drying process is crucial to preserve the shape and structure of the flowers. Care must be taken to prevent petals from overlapping or sticking together, which can result in distorted shapes.
- H. **Treatment and Finishing:** Once the flowers are completely dry, they can be treated with floral sprays or sealants to enhance their appearance, preserve their colors, and provide extra protection.



These treatments can help prolong the longevity of the dried flowers.

- I. Arranging and Displaying: Dried flowers can be arranged into various forms, including bouquets, wreaths, wall hangings, and centerpieces. They can be combined with other materials, such as foliage, herbs, or ribbons, to create visually appealing compositions. Dried flower arrangements can be displayed in vases, shadow boxes, or framed as artwork.



- J. Preservation and Storage: Proper storage is essential to maintain the quality of dried flowers. They should be kept in a dry and cool area.

### Conclusion

Dry flower technology is a captivating and artistic method of preserving flowers, enabling individuals to cherish the beauty of blossoms for an extended period. Whether used in home decor, special occasions, or personal mementos, dried flower arrangements offer a unique and enduring way to appreciate the elegance and charm of nature's floral creations.



## Role of Biofertilizer Inoculants in Crop Productivity and Soil Fertility

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### Bio-inoculants

Bio-inoculants are living organisms containing strains of specific bacteria, fungi, or algae which: - take nitrogen from the air and make it available to plants- reducing the need for nitrogen fertilizer. - make inorganic phosphate and micronutrients soluble and available to plants.

Soil microorganisms play an important role in the plant growth and development by various means viz. nitrogen fixation, phosphate solubilization, phytohormone production etc. Therefore, bio-inoculants for agriculture purpose i.e., bio-fertilizers could be a better alternative to chemical fertilizers for agricultural as well as environmental sustainability (Ritika and Uptal, 2014).

### Commercialization of microbial inoculants

The commercialization of microbial inoculants popularly known as biofertilizers started in late seventies in India and currently they are being manufactured on a large scale. The journey of mass production of inoculants that started with Rhizobium, has now been diversified and various types of inoculants are commercially produced and utilized for nutrient mobilization and plant protection which play a key role in crop productivity. However, despite good potentiality of biofertilizer usage, the actual utilization is very low at about 2% of its potential. One of the initially introduced high potential biofertilizer, Rhizobium is losing the steam and is being gradually replaced by other inoculants such as phosphate solubilizing bacteria (PSB) (Jehangir *et al.*, 2017). With growing demand, many new inoculants are being launched and manufactured by the industry. Potash

mobilizers, zinc solubilizers and consortia of biofertilizers are latest additions to the existing pool of biofertilizers manufactured in India.

### **Production of Biofertilizers in India**

As per the latest compilation on mass production, India has about 225 biofertilizer production units with an installed production capacity of about 98,000 MT per annum. Against this, the actual production during the year 2010-11 was 37,997 MT. Out of various types of biofertilizers, PSB biofertilizers accounted for nearly 50% of total production and use. Remaining installed capacity is being used for the production of other microbial inoculants, mainly *Trichoderma*, *Pseudomonas fluorescens*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Verticillium*, being traded as biopesticides. Share of biofertilizers to biopesticides in total production.

### **Types of Microbial Inoculants (Biofertilizers) in Market**

*Rhizobium* was the first microbial inoculant, which was introduced as biofertilizer during early seventies with the introduction of soybean into the country. *Azotobacter* and *Azospirillum* were added to the list in mid- nineties. Phosphate solubilizing biofertilizer (PSB) was introduced in late nineties. During the same period, few more inoculants were added such as *Acetobacter* (or *Gluconacetobacter*), Potash mobilizer (*Frateuria aurantia* and *Bacillus* sp.), Zinc solubilizers and lately consortia of microorganisms comprising a mixture of *Azotobacter*, *Azospirillum*, PSB and *Pseudomonas fluorescens* (Satyaprakash, 2017). Mono cultures although continue to dominate the market but mixed cultures are picking up fast and may surpass the single strain inoculants in next 5 to 7 years (Jnawali, 23015).

### **Methods of application of biofertilizer inoculants:**

#### **Rhizobium**

Seed treatment has been found to be the suitable method of *Rhizobium* inoculation. Some adhesive is used to make proper contact between seeds and inoculants (bacteria). About 900 g soil base culture is sufficient to inoculate the seeds for an area of one hectare in case of legumes. A 10% jaggery (gur) solution is used as sticker for *Rhizobium* cells to seed. First, the solution is spread over the seeds and mixed to build up a thin coat over the seeds. After ascertaining the proper coating of slurry over the seeds, the inoculant is sprinkled over the seeds and the content is again mixed thoroughly. Then the content is dried in the shade by spreading thinly on a polythene sheet for at least overnight.

#### **Azotobacter**



Field experiments carried out on *Azotobacter* indicated that this is suitable when inoculated with seeds or seedlings of crop plants like onion, aubergine, tomato and cabbage under different agro-climatic conditions. *Azotobacter* inoculation curtails the requirement for nitrogenous fertilizers by 10 to 20% under normal field conditions.

### **Azospirillum**

*Azospirillum* inoculation helps to improve the vegetative growth of the plants, cutting back on nitrogenous fertilizers by 25–30%. So far, only four species of *Azospirillum* have been identified. They are *A. lipoferum*, *A. brasilense*, *A. amazonense* and *A. iraquense*. In Indian soils, *A. brasilense* and *A. oferum* are very common.

### **Acetobacter**

Under field conditions, the yield of sugarcane increases after *Acetobacter* inoculation. Productions of auxins and antibiotic type substances have also been observed after its application.

### **Blue-green algae**

The blue-green algae inoculum is applied after transplantation of rice crops in the main field. The inoculum required is 10 kg/ha. For higher nitrogen fixation, 3 to 4 t/ha of farmyard manure and 200 kg/ha of superphosphate are applied.

### **Azolla**

*Azolla* is applied to the main field as a green manure crop and as a dual crop. As a green manure crop, *Azolla* is allowed to grow on the flooded fields for 2 to 3 weeks before transplanting. Later, water is drained and *Azolla* is incorporated by ploughing in. As a dual crop, 1000 to 5000 kg/ha of *Azolla* is applied to the soil one week after transplanting. When a thick mat forms, it is incorporated by trampling. The leftover *Azolla* develops again and is trampled in as a second crop. For better growth of *Azolla*, 25 to 50 kg/ha of superphosphate is applied and standing water of 5 to 10 cm is maintained continuously in the rice fields.

### **Frankia**

*Frankia* inoculation enhances the growth, nodulation, nitrogenase activity of nodules and nodule dry weight of *Casuarina* and *Alnus* plants.

### **Biofertilizer inoculants in Abroad**

In Brazil, Argentina and in other South American countries, successful results have been achieved with the re-inoculation of soybean, i.e., the yearly inoculation even in soils presenting well-established compatible rhizobial population from previous inoculations. This practice led to the





commercialization of over 70 million doses of inoculants for soybean in Brazil in the last crop season. Estimates in Brazil are that re-inoculation increases soybean grain yield by 8% in average and by 6.8% to 14% in Argentina. In the USA, re-inoculation is traditionally not recommended, based on results from a former study showing that rhizobial populations as low as 10 cells/g would inhibit the nodule formation by inoculant strains. However, mean yield increases due to inoculation considering areas of traditional soybean cropping have been recently estimated at 1.67%, but could probably be higher if high N-fertilizer levels were not applied to the crops comprising the soybean agricultural systems. Amazingly, even the most recent studies on the quantification of soybean BNF in the USA take into consideration a large number of sites, soil fertility, and application of mineral N, but not the re-inoculation component. Certainly, the annual re-inoculation is responsible for the high contribution of BNF to the soybean N nutrition in Brazil, with values as high as 94% of the aboveground N accumulation, while in the USA these values range from 23 to 65%.

The acidic, saline, and low organic matter of the SSA soils, the average soybean yield is usually well below the world average. Therefore, in addition to the soybean genetic breeding, further studies have been carried out aiming at increasing yields. For example, in Ethiopia, searched for acid-tolerant rhizobia as strategy to increase soybean performance. A local isolate was able to improve soybean yield, indicating that search for indigenous or naturalized elite isolates might represent an interesting strategy to be adopted in other African countries. Impressive yield increases have also been observed by combining application of P-fertilizer and rhizobial inoculant in Nigeria, and along with other studies suggest that P is probably the main limiting factor to the BNF in Africa (Rahimi, 2014)

## Conclusion

Biofertilizers can help solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realize the useful aspects of biofertilizers and implement its application to modern agricultural practices. However, the lack of awareness regarding improved protocols of biofertilizer applications to the field is one of the few reasons. Biofertilizers lead to soil enrichment and are suitable with long-term sustainability. Further, they pose no danger to the environment and can be substituted with chemical fertilizers. The application of bio-fertilizers can minimize the use of chemical fertilizers, decreasing environmental hazards, enhance soil structure and promote agriculture. They play a key role in maintaining long term soil fertility and sustainability by fixing insoluble P in the soil into forms available to plants,



thus increasing their effectiveness and availability. Biofertilizers are the alternative sources to meet the nutrient requirement of crops. In Biofertilizers, beneficial bacteria are Azotobacter, Azospirillum, Rhizobium, Mycorrhizae which are very essential in crop production. Biofertilizer can also make plant resistant to unfavorable environmental stresses.

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