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# High Throughput Cell Culture

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Cell culture is an essential tool in biological science, clinical science, and biotechnology. For the past two decades, a major focus of cell culture technology was been on developing high-throughput cell-based assays capable of providing valuable information on potential drug targets as well as advancing cell biology. There is tremendous interest in the pharmaceutical industry for improved high-throughput cell-based screening platforms to expedite target validation as well as for use in preclinical trials. The demand for mammalian cells in pharmaceutical screening and cell biology research is constantly increasing. The evaluation of drug candidates was based on their biological effect on cell cultures in high-throughput screening (HTS). Basic research relies on numerous cell lines and primary cells, which constitute a tool for the elucidation of basic mechanisms of cell proliferation, differentiation and function.

**What is high throughput cell culture?**

High-throughput system involving use of robotics, data processing and control software, liquid handling devices and sensitive detectors for the culturing of *in-vitro* cells without any operator intervention. It also involves automation of the whole system. Automation is an important element in high throughput system. Integrated robot system consisting of one or more robots transports assay-micro plates from station to station for sample and reagent addition, mixing, incubation, and finally readout or detection and cell culture flasks for media change and for sub culturing of cells. An HTS system can usually prepare, incubate, and analyze many plates simultaneously, further speeding the data-collection process. Currently existing HTS robots can test up to 1, 00,000 compounds per day. Whereas ultra high throughput screening (uHTS)can able to screen nearly more than 1, 00,000 compounds per day.

**Robotics**

Robotics is based on the fully integrated robotic systems in which all the works are carried out by robots. Robotic arm looks for moving of plates between workstations and collection of different workstations which is controlled by software.

**Softwares**

Software are based on common language and processes. Best example is the CellGEM™ (Cell Growth Expansion and Maintenance) software package guides users through all the cell culturing processes and maintenance actions. Main feature of this software is it gives a complete effective overview of the cell culture history. It also maintains consumable status report and incubator inventory to report all the scheduled tasks for maintenance and production.

**Automated cell culture systems**

Various high-throughput cell culture systems were developed based on the laboratory requirements. Some of the available automated cell culture systems are:

Hamilton is acompletely automated system for culture of primary cells, cell lines and embryonic stem cells. Manual processes like plate agitation are perfectly mimicked by analogous robotic movements. The system is controlled by a standard computer and it provides high-quality cells in large numbers.

**SelecT™**is the leading automated cell culture system for multiple cell-lines and assay-ready plate production.It improves the quality and availability of cellular reagents and fully automates manual cell culture methods. **Cello™**is an automated system for mammalian cell culture in plates, and enables efficient selection of optimal clones. It can able to culture multiple clones in parallel, from seeding through expansion and sub-cloning.

**Piccolo™** is also a fully automated system for the rapid optimization of recombinant protein production in microbial or insect cells.The system has been designed in the way that it is flexible to vary and control a wide range of protein culture parameters independently and conduct experiments in parallel.

**Cellmate** is an established system that fully automates all the processes needed to culture cells in roller bottles and T-flasks. It takes cell culture processing from the lab bench to high volume production without process change.

**Applications**

- Mainly involved in generation of stable cell line which are used in the field of drug discovery, production of therapeutic proteins and in stem cell therapy.
- Cell culture and assay assembly for absorption, distribution, metabolism and elimination (ADME) testing and in permeability testing assays.
- High throughput screening is also used in the development of cell therapies, gene therapies and veterinary viral vaccines, etc.,

## CONCLUSION

Introduction of the high-throughput and automated based systems has revolutionized the field of cell culture system in terms of mass production and analysis of multiple assays at the same time. These platforms were also found to have high reproducibility compared with the conventional experiments. In future the automation of such systems without the help of human intervention will reduced the error rate and efficient use of the available resources.

# MicroRNA:

# A Therapeutic Potential Asfuture Drug

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A novel mechanism of action, the ability to function as master regulators of the genome and an apparent lack of adverse events in normal tissue make microRNA a promising technology for current and future therapeutic development.It was only 10 years ago that the first human microRNA (miRNA) was discovered, and yet a miRNA-based therapeutic has already entered Phase 2 clinical trials. This rapid progress from discovery to development reflects the importance of miRNAs as critical regulators in human disease, and holds the promise of yielding a new class of therapeutics that could represent an attractive addition to the current drug pipeline of Big Pharma.

**What are micro RNAs?**

MicroRNAs (miRNAs) are 15-22 nucleotide, short, non-coding RNAs that have emerged as critical regulators of gene expression – affecting a multitude of biological processes including cell proliferation, differentiation, survival and motility. They are conserved from plants to man and are encoded by their respective genes. miRNA genes are defined in separate gene loci, or alternatively can be found within introns and exons of other genes.Micro RNAs interact with mRNAs and either block protein translation or lead to protein degradation. In normal or healthy state Micro RNAs act as the delicate switch and fine tuner of gene expression. miRNAs play an integral role in numerous biological pathways and dysregulation of micro RNAs can negatively impact normal gene expression and play a role in initiation, progression and maintenance of disease conditions such as cancer, cardiovascular diseases, metabolic diseases, fibrosis and immune inflammatory diseases.

**Biogenesis**

miRNAs are transcribed from the genome as primary transcripts and processed further to produce mature and functional miRNAs. The majority of microRNA genes are transcribed by RNA polymerase II and occasionally by RNA polymerase III into a hairpin shaped structure primary microRNA (pri-microRNAs) from which the mature microRNA is processed. The pri-

microRNA transcripts are similar to mRNA with polyadenylated tail, contain a 5' cap structure that undergoes splicing. In the nucleus pri-microRNA are processed into precursor microRNA (pre-microRNA) by a complex that includes Drosha and double stranded RNA binding domain protein DGCR8, which creates a 3' overhang. The pre-microRNAs are transported to the cytoplasm by Exportin 5, where they are further processed by complex that includes Dicer and TRBP/Loquacious. This ribonucleoprotein complex cleaves the hairpin loop from the pre-microRNA to from a double-stranded complex containing the microRNA and microRNA* (star) sequence. The star strand is the passenger strand that is degraded. During strand selection strand that is thermodynamically less stable at 5' end the mature microRNA guide strand along with the Argonaute protein 2 and the ribonucleoprotein complex is known as RISC (RNA-induced silencing complex). However, in some cases microRNAs are derived from both arms of the microRNA precursor.

The RISC complex scans the cellular mRNA to locate the microRNA's target sequences to hybridize with microRNA. Binding can be through either perfect or imperfect complementarity to conserved sequences within the target untranslated region (UTR) or complementarity within the ORF, resulting in mRNA cleavage and degradation of the mRNA by Argonaute 2 (Ago2), a catalytic endonuclease component of RISC. Both mechanisms ultimately result in downregulation of protein expression from target genes. Some miRNAs target the 5'UTR of the mRNA with partial complementarity and result in degradation of the mRNA. Although several mechanisms of regulation exist, 3'UTR targeting appears to occur most frequently and most effectively followed by endogenous ORF targeting, and finally, 5'UTR targeting. Depending on the complementarity between microRNA sequence and target mRNA the negative regulatory effect can range from weak repression of protein translation to complete cleavage of the mRNA.

**Pathology**

Potential mechanisms that link miRNAs to diseases are mainly associated withgenomic alteration of miRNA. Rearrangement of miRNA genes leads to change in miRNA expression levels due to mechanisms like chromosome deletion, amplification and translocation of genes. Similarly any Single Nucleotide Polymorphism of miRNA or miRNA targets leads to change in the miRNA target spectrum.Alteration in the microRNA biogenesis processes also causes change in the expression of miRNA levels in the normal cells leading to diseased conditions.

**Cancer and microRNA**

Canceris a group of diseases characterised by uncontrolled cell division leading to growth of abnormal tissue. Cancer is caused due to mutation in the DNA. Two groups of genes that are mainly responsible for regulation of cancer conditions are oncogenes and tumour suppressor genes. The function of oncogenes is to allow the cell to divide where as the tumour suppressor genes are involved in apoptosis and cell cycle arrest. Any mutation in anyone of these genes

leads to cancer condition. In cancer conditions oncogenes are activated and produce oncogenic proteins which make a normal cell into cancerous cell. Another group of genes tumour suppressor genes are deactivated which makes oncogenic genes to be expressed more and leads to cancer condition.

These cancer conditions can be treated by miRNA targeted therapy in two ways either by activating the tumour suppressor genes or by deactivating the oncogenes. Tumour suppressor genes can be activated by using miRNA antagonists which binds to the miRNA suppressing the expression of tumour suppressor genes. Oncogenes can be deactivated by miRNA mimics (replacement therapy) which leads to decreased expression of oncogenes (oncogenic proteins) and suppress the tumour formation.

**MicroRNA as future drug**

The rapid progress from discovery to development reflects the importance of miRNAs as critical regulators in disease conditions and holds the promise of yielding a new class of therapeutics that could represent an attractive addition to the current drug pipeline. miRNAbased therapy is considered as a double-edged sword. On the one hand, modulation of a single miRNA offers the opportunity to target multiple genes and regulatory networks simultaneously. However, for the same reason, caution and careful design are needed to prevent unwanted off-target effects.

**Therapeutic miRNAmodalities**

miRNA antagonists and miRNA mimics are the two approaches widely being used for developing miRNA-based therapeutics.

**miRNAantagonists**

miRNA antagonists are generated to inhibit endogenous miRNAs that show a gain-of-function in diseased tissues. This is similar to other inhibitory therapeutics that target a single gene product such as small molecule inhibitors and short interfering RNAs (siRNAs). Introduction of a highly chemically-modified miRNA passenger strand (anti-miR or antagomiR) binds with high affinity to the active miRNA strand. Binding is irreversible, so the new miRNA duplex is unable to be processed by RISC and/or degraded. Antagonist may also non-specifically bind to other RNAs, which could result in unwanted side effects.

**miRNAmimics**

miRNA mimics are used to restore a loss of function. It is also known as 'miRNA replacement therapy', aims to re-introduce miRNAs into diseased cells that are normally expressed in healthy cells. The re-introduction of these miRNAs leads to re-activation of pathways that are required for normal cellular processes. Mimics of tumour suppressor miRNAs introduced into cells which inturn stimulated anti-oncogenic pathways, apoptosis and ultimately lead to an eradication of tumour cells.

miRNA mimics can be delivered systemically using technologies that are also used for therapeutic siRNAs. Therefore, the application of miRNA mimics will face less of a delivery hurdle. Besides, miRNA mimics are expected to be highly specific and well tolerated in normal

tissues. The delivery of mimic did not show any adverse immunological response as no elevation of cytokines or liver and kidney enzymes was observed, suggesting that the therapy is well tolerated.

## CONCLUSION

As single miRNA may target 100 of genes, the signaling pathway of each miRNA needs to be recognized before we try to infer the disease condition and standard procedure for determining the "molecular signature" of a diseased tissue has to be developed for diagnosis of disease conditions. Development of more efficient delivery and regulated tissue-specific or differentiation-dependent expression of miRNA are critical issues for gene expression studies and gene therapy. Combination of miRNAs and their dosage for a particular disease condition needs to be established. In future increasing list of miRNAs and their role in various diseased conditions will surely overcome these obstacles and will lead to the development of miRNA based therapeutic used to cure specific diseases without any side-effects.

# Management Approaches to Ameliorate Heat Stress in Buffaloes

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Heat stress is an imbalance between heat production within the body and its dissipation from the body. Heat stress is the conditions are normally associated with a decline of production performances as the determine the activations of thermo-regulation mechanism in order to avoid hyperthermia and maintain the vital functions of the animals.Buffalo is more sensitive to heat stress due to thick black skin colour that absorb more solar radiations.Sparse hair coat which have inadequate insulation in buffalo from high temperatures.The water buffalo has only 1/10$^{th}$ the number of sweat glands per unit area of skin compared to zebu cattle and must rely on wallowing or wetting to the skin during heat conditions to reduce the heat load. The prominent signs of heat stress in buffalo include decreased feed intake, increased reddening of hide, protruded tongue, panting, obvious blood shot eyes, very hot to touch and increased rectal temperature. Increased incidence of certain health problems during the summer months may be manifested as increased occurrences of mastitis, retained placentas, metritis, and ketosis. It can also elevate cortisol levels. Buffaloes have poor heat tolerance capacity compared to other domestic ruminants.Air temperature (13-18 ˚C), RH (55-65%) and wind velocity (5-8 km/h) are the optimum conditions for buffaloes as suggested by Payne (1990).

Thermo-neutral zone: Environmental temperature at which animal body is at equilibrium, neither tends to heat gain or loss heat.Temperature range of thermo-neutral zone of buffalo is 100C- 360C.The buffalo adjusts to the temperatures within the zone through different responses requiring little energy. It can show postural changes where it changes its body shape or moves and exposes different areas to the sun/shade, and through radiation, convection and conduction, heat exchange occurbelow the thermal neutral zone there is the zone of LCT (lower critical temperature) Is -2ºCand above there is the zone of UCT (upper critical temperature) is 48ºC. The organism reaches the LCT when the (ambient temp.) decreases. When an organism reaches this stage the metabolic rate increases significantly and thermo genesis increases the Tb (body temp.) If the Ta continues to decrease far below the LCT hypothermia occurs. When the reaches too far out of the UCT the rate heat gain and heat production become higher than the rate of heat dissipation

(heat loss through evaporative cooling), resulting in hyperthermia.

## Assessment of heat stress level

The most practical and easy to determine index for assessment accurately the potential of an environment to induce heat stress in farm animals. In this respect, a measurement was developed, including degree of temperature and relative humidity (RH%), which are the most closely involved with heat balance. Such measurement was termed as temperature–humidity index (THI). THI offers a method of combining two of the more important and easily measured weather factors into a possible measure to compare temperature and humidity data and animal response at different locations. The THI is defined by equations.When temperature is measured in Fahrenheit, the equation applied is as followsTHI = db°F – {(0.55 – 0.55 RH) (db°F – 58)}, where db°F = dry bulb temperature in Fahrenheit and RH = relative humidity (RH %) / 100. The obtained values indicate the following: < 72 = absence of heat stress, 72 to < 74 = moderate heat stress, 74 to < 78 = severe heat stress and 78 and more = very severe heat stress.

**Adaptive changes in response to heat**

The environmentalfactors associated with heat stress which affect the physiological systems governing thermal regulation and the maintenance of positive heat loss, are primarily ambient temperature, relative humidity (RH%) and radiant energy. In tropical and sub-tropical areas, heat stress is the major constraint on animal productivity The effect of heat stress is aggravated when heat stress is accompanied with high ambient humidity (Marai*et al.,*2007). In response to heat-stress, various physiological and behavioural changes occur in the buffalo which are increased pulse rate, respiration rate, rectal temperature, peripheral blood flowi.e vasodilation and sweating reduced feed intake, decreased activity and buffalo seek shady and airy places. Rectal temperature has been considered an indicator of heat storage in animals body and an important measure of physiological status in animals (West *et al*., 1999). The rectal temperature and skin temperature have been fluctuate much more in buffaloes than in tropical cattle under increased ambient temperature.

**Effect of heat stress on buffalo**

Under the hot climate conditions in the tropical and sub-tropical areas, exposure to elevated ambient temperature is the major constraint on animal productivity and depression in feed consumption is the most important reaction (Marai*et al*., 2007). Rectal temperature (RT) and respiration rate (RR) are the most sensitive indices of heat tolerance among the physiological reactions.The increase in ambient temperature from spring to summer (29 to 31°C) resulted in an increase in RT from 37.8 to 38.0°C and RR from 20.5 to 22.4 breaths/min in buffalo heifers, and from 37.9 to 39.7°C and from 23.4 to 41.0 breaths/min, respectively, in lactating buffalo cows (Kamal*et al.,* 1969).Their RR increased by 5–6 times, their tongue protruded and salivary activity increased.

The experimental studies showed that acute heat expo-sure (33–43°C, 40–60% RH) of young (aged 6 months) and old Egyptian buffalo calves (aged 12 months)

induced more significant increases in RT (3.4 and 3.2%) and RR (495 and 335%, respectively) than in the control. The chronic heat exposure of 6 and 12 month old buffalo calves was accompanied with increases (Pb0.01) in RT (4.1 and 3.0%), RR (528 and 318%) and evaporative water loss (69.4 and 51.2%, respectively) (Nessim, 2004).Respiration rate was the indicator of heat stress in hot environment and gave significant correlations with circulating corticosteroids concentration.Normal respiration rate is approximately 10-30 breaths/minute.

**Strategies to ameliorate heat stress**

To reduce the heat stress in buffaloes require multi-displinary approach. It should include structural design of the shade, environmental control and nutritional management. Good management practices is important to alleviate heat stress and maintain high production levels in lactating buffaloes under hot environment conditions

**1. Shade**

**Natural shade:** Shade is the basic method to protecting the animals from direct solar radiationin day-time during summer. The most effective source of shadeprovide are tree and plants. They provide not only protection from sunlight, but also create cooling effect through the evaporation of moisture from their leaves, Shade has a beneficial effect on the physiological response of buffaloes to heat as the body temperature, heart rate and respiration rate all are decreased when shade is provided during summer. The mango tree (Mangiferaindica) provided the best shade with the least radiatiant heat load, the pinus which present high heat loads that worst type shade.

**Artificial shade:**Artificial shadeprovided by housing of animal. The longer side of the dairy barn should have an east-west orientation. That reduces the amount of direct shining on the side walls. White painting ofthe roof may increase the level of sunlight reflection, thus reducing the amount of absorbed solar energy. Side curtain are preventing sunlight from entering the house. Efficiently designed sheds can help lesser the thermal stress thereby increasing feed intake, milk production and reproductive efficiency.

**2. Modification of the micro-environment**

**Air movement:** Air movement becomes more important during hot-humid climate for providing cooling and comfort to the animal. Apart from shifting animal to shaded airy place, fans or dairy fans and different types of coolers can also be installed for making the place airy. Air movement increases the rate of heat loss from animal's body surface, only as long as the air temperature is lower than the animal's skin temperature.

**Evaporative Cooling:**Various cooling system are applicable for the animal in covered shed. An evaporative cooling system which uses water mist with fan, sprinkler, mister, fogger, repeated bathing and wallowing of animals.

**Wallowing:** Wallowing is the cheapest and least laborious device to beat the heat in summer. Buffalo are made to wallow in clusters in ponds,rivers,tanks or other water bodies. Cooling by wallowing was more efficient in reducing body temperature at all points of measurement, reduces stress and restores the physiological responses to

normal values without affecting quality of milk and health of animal. Further, wallowing was more economical due to limited recurring expenditure and increase in milk production in comparison to shower.

**3. Nutritional management**

Reduced dry matter intake with greater availability of key nutrients and to compensate for dietary heat increment while avoiding nutrient excesses. The energy requirements of lactating buffaloes also increase under high temperature conditions, but this increase is apparently caused primarily by the increase in metabolic energy.

**Water intake:**Water is the most important nutrient for buffalo during hot climate. Water intake is closely related to dry matter intake and milk yield, but regardless of the rate of increase, it is important that abundant water must be available at all times under hot conditions. Hot weather, declining dry matter intake and high lactation demand requires increased dietary mineral concentration. Sharp increases in the secretion of potassium through sweat occur during hot climatic conditions Alterations in mineral metabolism also affect the electrolyte status of buffalo during hot weather. Water intake in buffaloes increased 13.5% with increasing environmental temperature and the ratio of water intake/food consumption also increased.Mullick (1964) found that water intake increased 36–40% in buffaloes and 75% in cattle from winter to summer. Water consumption was 31.6 and 46.5 L/day for buffaloes, while the corresponding values for cattle were 25.9 and 29.6 L/day in winter and summer, respectively.

**Night Grazing:** Buffaloes kept in a shed maintain rapid heartbeat during the night. However, when the animals are allowed out into a pasture at night, these physiological responses decrease immediately. This is the result, both of a reduction in radiation heat from the surrounding buffaloes, as well as increased heat loss from the animal itself.

**Feeding High-Energy Diets**: Low-fibre, high fermentable carbohydrate diets lower dietary heat increment compared to high fibre diets. Although the metabolic energy of dairy buffaloes increases in a hot environment, heat stress depresses feed intake. For this reason, it is important to increase the energy content of the diet of dairy buffaloes, in order to maintain their energy intake under hot conditions. The heat increment, which is an internal heat stressor in hot environments, is lower in highly metabolizable diets. So it is imperative to use fatty feeds, or calcium salts of fatty acids, as the means of improving energy supply for buffaloes in summer. Buffaloes fed on such diets have higher milk yield, and a lower body temperature and respiration rate.

**Feeding by-Pass Protein:** Dietary protein degradability is also critical under heat stress conditions. It is well known that excessive protein intake increases heat production and decreases reproductive performance. However, the protein requirement of buffalo increases and dry matter intake decreases in a hot environment, consequently, the protein supplied to lactating buffaloes during summer is not always sufficient. By using fish meal, which is a by-pass protein, the milk yield and protein content of buffalo

milk increases but the ruminal ammonia production decreases.

**Vitamins :**Both vitamin C and vitamin E have antioxidant properties. Antioxidant vitamins have proved to protect the biological membranes against the damage of ROS and the role of vitamin E as an inhibitor –"chain blocker"- of lipid peroxidation has been well established (Seyrek*et al.,*2004). The effect of vitamin E was studied on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. Vitamin C along with electrolyte supplementation was found to ameliorate the heat stress in buffaloes.

**Minerals and trace elements :**Zinc and other trace elements like Cu and Cr act as typical antioxidants as they work indirectly. Zinc is a catalytic cofactor for Cu/Zn SOD and catalyzesdismutation of superoxide anion, producing molecular oxygen and $H_2O_2$, the latter product is usually metabolized by GPx and CAT.West *et al*., (1999) reported that Na and K status of the body stay normal during heat stress when supplementation with electrolyte.

**CONCLUSION**

Heat stress is a cause of great concern among livestock owners in tropical countries. Cooling by wallowing was more efficient in reducing body temperature at all points of measurement, reduces stress and restores the physiological responses to normal values without affecting quality of milk and health of animal. Further, wallowing was more economical due to limited recurring expenditure and increase in milk production in comparison to shower. Dietary supplementation of salts and exogeneous antioxidants should be tried to cope up with heat stress.

**REFERENCES**

Kamal, T.H. and Seif, S.M. 1969. Changes in total body water and dry body weight with age and body weight in Friesians and water buffaloes. *Journal of Dairy Science*, 52: 1650-1656.

Marai, I.F.M., Daader, A.M., Abdel-Samee, A.M. and Ibrahim, H. 1997b. Lactating Friesian and Holstein cows as affected by heat stress and combination of amelioration techniques under Egyptian conditions. Proceedings of the International Conference on Animal, Poultry, rabbits and Fish Production and Health, Cairo, Egypt.

Marai, I. F. M., El-Darawany, A. A., Fadiel, A., and Abdel-Hafez, M. A. M. 2007. Physiological traits as affected by heat stress in sheep – a review. Small Ruminant Research, 71: 1-12.

Mullick, D.N. 1964. A study on the metabolism of food nutrients in cattle and buffaloes under climatic stress.*Arid Zone Research,* 14: 137.

Nessim, M.G., 2004. Heat-induced biological changes as heat tolerance indices related to growth performance in buffaloes. Ph.D. Thesis, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Payne, W.J.A., 1990. Cattle and buffalo meat production in the tropic.Intermediate Tropical Agriculture Series.Longman Sci. and Tech.

Seyrek K., KarginKiral F. and Bildik A. (2004).Chronic ethanol induced oxidative alterations in the rat tissues and protective effect of vitamin E. *Ind. Vet. J.* **81**: 1102-1104.

West, J.W. 1999. Nutritional strategies for managing the heat-stressed dairy cow. American Society of Animal Science and American Dairy Science Association, 2: 21-35.

# Standard operating procedure:

## A tool to improve herd performance of commercial dairy farms

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### Abstract

India is the top most milk producing nation in the world. Commercial dairy farming is a fast upcoming business in India. Dairies are the complex process where multiple activities are taken by number of workers to achieve same goal of milk production. A standard operating procedure usually describes the steps that people should use to complete the process. Thus, on a dairy farm cleaning the bulk tank is a process. Measuring, loading, and mixing feed ingredients for a total mixed ration are example of a process. Process control involves standardizing a procedure so that all workers are expected to complete it in the same way.We know from both experience and research that variation is harmful to cows. Variation in performance leads to reduced milk production, poor milk quality, imbalances in feeding programs, reduced reproductive performance, and a host of other problems that ultimately diminish dairy farm productivity and profitability.Standard operating procedure is a written step by step set of instruction on how to complete a particular task, just like a cookbook recipe. It means to remove variation in work performance caused by people completing the same work processes in different ways. Dairies are a perfect place for adoption of standard operating procedures because multiple employees often share responsibility for a single task, and because variation in these tasks (milking, feeding, bedding, health and reproductive management) can have detrimental consequences for herd performance (Stup*et al*., 2006). As in India many entrepreneurs are moving towards commercial dairy farming. Those who don't have any knowledge/background of dairy science and animal husbandry want to set up dairy farm as a business; these SOPs will help them as a guide or reference book to fulfil the basic need of dairy farm management.

**INTRODUCTION**

Dairy farms are manufacturing operations. They take raw materials (feedstuffs, water, air), process like materials using their assets (cows, equipment, labor), and sell a product (milk) that is more valuable than the original raw materials. In this respect, dairy farms are value-adding businesses just like any other manufacturing operation. Therefore, just like many other successful manufacturing businesses, dairies can make dramatic and lasting improvements to their productivity and profitability by applying a pro-active, quality-driven management approach such as total quality management, Six Sigma, or ISO 9001.The central idea of quality management is to improve inputs and manufacturing processes to the greatest extent possible so that defects in the finished products do not occur. In dairy terms, examples of defects could include the poor quality forages caused by equipment breakdown, mastitis cases caused by improper sanitation, production loss caused by errors in feed formulation, or even excess employee turnover caused by poor supervisory practices. Management, workers, and external advisors or suppliers work together in a quality management system as they strive to improve the farm's processes. Milking, feeding, breeding, calf care and all the other daily work that ultimately leads to producing milk are the dairy farm's processes. This is where standard operating procedures (SOPs) enter in picture. SOPs define exactly how the farm's processes should be completed. Because management, workers, and advisors all help create and manage SOPs, they serve as a focal point where all can contribute to continuous improvement of work processes. Standard operating procedure is a written step by step set of instructions on how to complete a particular task, just like a cookbook recipe. It means to remove variation in work performance caused by people completing the same work processes in different ways. It is seen that variation in the performance of work processes is a very important problem for dairy farms. Dairies are a perfect place for adoption of standard operating procedures because multiple employees often share responsibility for a single task, and because variation in these tasks (milking, feeding, bedding, health and reproductive management) can have detrimental consequences for herd performance (Stup*et al*., 2006)

**What is a SOP?**

A Standard Operating Procedure (SOP) is simply a written step-by-step set of instructions on how to complete a task. It is just like a cookbook recipe, and gives an employee a detailed description of how to handle a specific task within their job. Here various writers defined standard operating procedure in different way. For example, the U.S. Environmental Protection Agency and European Medicines Agency defined it as, "a set of written and detailed instructions that document a routine or repetitive activity followed by an organization to achieve uniformity of the performance of a specific function". SOP describes a set of steps that a person or group of people must perform to complete a job by removing variation (Stup, 2002). It

is a living document that details the way an operator should perform a given function (Treville, 2012). In short, SOP is a document that clearly defines who does what, where, how and why?

**Why Use SOPs?**

- Dairy cattle thrive on consistency. If tasks are performed correctlyand consistently, cow performance will be optimized.
- SOPs help workers to do their job correctly. They provide guidance for an employee who isfaced with a situation that requires action. They help eliminate confusion an indecision.
- Awritten protocol puts all employees on the same page and helps a team approach togetting tasks done correctly and consistently.
- A well-written SOP makes job training easier. It also helps when someone has to do a job thatthey don't usually perform. Even small farms with a single owner/worker can benefit fromhaving written protocols so that if someone has to step into that person's shoes in an emergencythere are guidelines to follow.
- Data entry can be easier when SOPs are used. Protocols can be incorporated into almost allcomputerized dairy records systems, helping to reduce mistakes in data entry and records.

**What Parts of the Dairy Should Have SOPs?**

Every discipline on the dairy (milking, feeding, bedding, herd health and reproduction, maternity Management, replacement herd health, etc.) Should have a written protocol that clearly describes What is to be done, how it is to be done and why it is important that it be done that way (Sumrall,2011). the areas that are likely to benefit the most from use of SOPs is a good place to implement.

**Sample Basic Milking Procedure**

**Dun Milking Dairy: Parlor SOP # 1**

**Effective Date: March 21st, 2012**

**Developed By: Parlor staff**

1. Wear gloves at all times when working in the parlor, for hygiene and mastitis control
2. Strip and dip the first 4 cows
   a. Strip 3-4 squirts of milk out of each teat to check for abnormal milk/mastitis. If abnormal milk is foundrefer to parlor SOP #5, "Dealing with cows with abnormal milk"
   b. Dip the skin of every teat with the teat dip cup
   c. Teats from blind or dead quarters as indicated by a leg band do not need to be stripped
3. Return to the first cow to wipe teats
   a. Wipe each teat completely and carefully with a clean towel to remove all teat dip
   b. Teats should be clean and dry after wiping; use more than one towel if needed
4. Hang the milking unit on the first cow
   a. Press the on button on the control panel to turn on the unit
   b. Attach the milking unit to the teats
   c. Adjust unit and hoses so that the unit hangs level from front to back
5. Repeat steps 3 and 4 for the remaining 3 cows

6. Repeat procedures for the next group of 4 cows on that side of the parlor (cows 5-8), and so on, until all 12 units onthe side are attached

7. When this line is all milking, start on the other side, repeating previous steps

a. Your goal should be to have a constant rhythm from one side to the other throughout the milking, rather than having both sides waiting to be milked at the same time

8. When all units on a side have detached, postdip all the skin on every teat with the teat dip cup and release cows

9. Liner slips/squawks should be corrected as soon as possible by adjusting the machine

10. Rinse gloves frequently and avoid milk contact on gloves. Change gloves if they are visibly dirty, milk contaminated, or after stripping a cow with mastitis.

**The influence of milking routine on performance for Wisconsin freestall Farms (n=101)**

| Variable | Cows per hour per operator | Monthly rate of clinical mastitis (%) |
|---|---|---|
| **Written milking routine** | | |
| Yes | **46.9** | 5 |
| No | 35.6 | 7.1 |
| **Training frequency** | | |
| Never | 33.6 | 9.6 |
| At hiring | 41.6 | 4.8 |
| Frequently | **49.4** | 5.8 |
| **Complete milking routine** | | |
| Yes | **40.8** | 5.5 |
| No | 35.3 | **10.3** |
| **Forestrip** | | |
| Yes | **40.9** | 5.8 |
| No | 32.9 | 9.4 |

(Rodrigues, *et al*. 2005)

Study based on 101 freestall farms in Wisconsin with an average herd size of 377 cows found the following findings.

Written routines, with frequent training sessions, that include a complete set of milking procedures provide two big advantages to dairy producers:

1)They will likely milk more cows per hour - increasing parlor throughput.

2)They will likely decrease their rate of clinical mastitis - improving the overall milk quality of the herd

**CONCLUSION**

Standard operating procedure help to commercial dairy farms in terms of increase labor utilization efficiency, production performance and health performance of dairy animals.

**REFERENCES**

Rasmussen, M.D., E.S. Frimer, Z. Horvath, and N.E. Jensen. 1990. Comparison of a standardized and variable milking routine. J. Dairy Sci. 73:3472.

Stup R. Standard Operating Procedures: Managing the Human Variables, Pennsylvania State University, University Park, Pennsylvania, National Mastitis Council Regional Meeting Proceedings.2002.Accessed at http://www.nmconline. org/articles/SOP.pdf. April 7, 2012.

Sumrall DP. Managing people in today's production dairy environment. Chapter 23 in: Risco CA & Melendez-Retamal P (eds). 2011. Dairy Production Medicine, Wiley-Blackwell.

Treville S, Antonakis J, &Edelson N. Can Standard Operating Procedures be Motivating? Reconciling Process

Variability Issues and Behavioural Outcomes; Total Quality Management, VOL. 16, NO. 2, 231–241.2005. Accessed at http://www.hec.unil.ch/jantonakis/ SOP%20motivation.pdf. April 17, 2012.

United States Environmental Protection Agency (EPA). Guidance for Preparing Standard Operating Procedures (SOPs); EPA/600/B-07/001. 2007. Accessed at http://www.epa.gov/quality/qs-docs/g6-final.pdf. April 07, 2012.

# Importance of Neonatal behaviour of calves in pair-bonding

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**Abstract**

*The neonatal behaviour has an important influence on the survival of the newborn calves. Early weaning or separation of newly born calf from its mother induces behavioural changes in the calf. The understanding of neonatal behaviour in calves will enhance the welfare of the offspring and thereby improve the reproductive efficiency of the dairy cows and buffaloes.*

## INTRODUCTION

The neonatal bond to the mother is a major ethological phenomenon. The primary function of behavior is to enable an animal to adjust to changes in the environment. The vitality of neonatal behaviour is very closely correlated with survival prospects. It is very essential for the mammalian newborn to make a bonded relationship with the mother and to nurse from her quickly and successfully. Neonatal behaviour can form a strong maternal bond. The expression of maternal behaviours like licking and nursing protects neonates from potential predators (Grandinson, 2005).

## PAIR BONDING

The calf's recognition and attraction to its mother begins soon after birth which is characterized by being exclusive and not transferable in mammals is referred as pair bonding (P Jensen, 2001). The average distance between cow and calf increases over the first few hours after birth (Edwards, 1983) as the cow begins feeding. Under natural conditions, the survival of the newborn depends on the establishment of a strong and lasting social bond with the dam (Enríquez *et al*., 2011).

**Stages in neonatal behaviour:**

During the formation of the neonatal-maternal bond there are different stages of neonatal behaviour. These behaviours allow the neonate to bond with her mother for protection and to get nourishment.

They are:

i. Co- ordinating recumbency
ii. Elevation
iii. Ambulation
iv. Orientation
v. Trophic initiation

**Co-ordinating recumbency:**

*Fig.1: New born calf*

This is a postnatal recumbent stage and it facilitates grooming by the dam over the dorsal areas of the neonate. The neonate lies in extension, raising the head and neck. There is erection and mobility of the ears.

**ELEVATION:**

In this stage the neonate attempts to rise to an erect stance. More than one attempt is made to establish upright equilibrium. The new born calf makes pronounced extension and muscular tension of the forelimbs.

**AMBULATION:**

The neonate in this stage starts attempting to walk by making four steps walking. Unsteadiness is observed which is a prominent feature because of the presence of fetal hoof pads. This neonatal mobility stimulates the maternal drive.

**ORIENTATION:**

Myopia is a constant neonatal condition. In this stage nosing of the dam and orientation towards the dam is facilitated by discrete postural and positional adjustments on the parts of the dam (Faser, 1990).

**Trophic initiation:**

This stage of neonatal behaviour has the following sub stages

1. Thigmotaxis
2. Mammary gland localisation
3. Tactile characteristics
4. Lactiferous reflex
5. Suckling reflex

**Mammary gland localisation or teat seeking behaviour.**

Within a few hours after parturition, the buffalo calves begin to show hunger by exploring the mother's body with muzzle and tongue until a teat is located, the calf readily mouths and sucks any protuberance on the mother's body (Hafez, 1992).

**Guidance of Calf finding teats (Fraser and Broom (1990)**

1. The pendulous shape of the udder;
2. The udder tilting (movement of udder), and
3. Thigmotaxis effect

**Thigmotaxis effect:**

This is the slightly high temperature between thighs. The mother helps the calf find a teat by positioning her body and this includes rotating her body, abduction of the hind legs or moving forward bringing the udder closer to the calf, and finally licking, nuzzling and nudging the calf.

**Suckling Behaviour**

Suckling behaviour begins 2-5 hours after birth.. The calf vigorously butts the mother's udder with its head while suckling. Teat sucking by the calf is most intense soon after it stands up. Vision, olfactory and vocal senses are involved cow-calf identification. The mother helps in suckling by positioning her body (Hafez, 2000).

**Neonatal kinetic behaviour / standing behaviour:**

The newborn calf stand within an hour. The nursing calf assumes a crouched stance this udder bunting behaviour stimulates milk let down. Newborn calves suckle 5-8 times a day for about 5-10 minutes. Calves plays and exhibits in a variety ways

**Neonatal sensory development and exploratory behaviour:**

Precocial neonates are exposed to sensory stimulation. Investigation is a major part of young animal's behaviour. Mother's behaviour can influence the neonate's behaviour. The earliest expriences received by young ruminants

are tactile and olfactory followed by auditory and visual signals. Calves are born with sound sense organs systems.

**Neonatal nursing progress:**

There is a progression of reflex movements. Orientation is the first important part of searching behaviour. The calves have a strong tendency to put their noses under the dams' abdomens and then to push their muzzles as high as possible. The calf usually begins to suck on one of the teats on the near side of the udder.

## CONCLUSION

Neonatal behaviour helps to study ethological phenomenon. Suckling

behaviour is most important for the survival of neonates. Nursing behaviour provides an important field of investigation in understanding the importance of pair bonding. It stimulates and increases the maternal instincts. Maternal behaviour and neonatal behaviour is essential for the welfare of the calves as well as reproductive efficiency of the mother.

## REFERENCES

Daniel Enríquez, Maria J Hötzel and Rodolfo.(2011). Ungerfeld.Minimising the stress of weaning of beef calves: a review Enríquez et al. Acta Veterinaria Scandinavica, 53:28

Edwards, S.A. (1983).The behavior of dairy-cows and their newborn calves in individual or group housing. Appl. Anim. Ethol. 10, 191–198.

Fraser, A.F. and Broom, D.M. (1990). Farm Animal Behaviour and Welfare. 3rd edition, Bailliere Tindall, London, PP. 156- 161.

Grandinson, K. (2005). Genetic background of maternal behaviour and its relation to offspring survival. Livest. Prod. Sci. 93, 43–50.

Hafez, E.S.E and B. (2000). Reproduction in farm animals 7th edition. Lippincott, William and Wilkins.

Hafez, E.S.E.(1992): Reproduction in Farm Animals. 6th edition, Lea & Febiger press, Philadelphia.

# Drinking Water Quality in Rural India: Issues and Approaches

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Rural India has more than 700 million people residing in about 1.42 million habitations spread over 15 diverse ecological regions. Meeting the drinking water needs of such a large population can be a daunting task. The non-uniformity in level of awareness, socio-economic development, education, poverty, practices and rituals and water availability add to the complexity of the task. Despite an estimated total of Rs. 1,105 billion spend on providing safe drinking water since the First Five Year Plan was launched in 1951, lack of safe drinking water continues to be a major hurdle and a national economic burden. Around 37.7 million Indians are affected by waterborne diseases annually, 1.5 million children are estimated to die of diarrhoea alone and 73 million working days are lost due to waterborne disease each year. The resulting economic burden is estimated at $ 600 million a year. While 'traditional diseases' such as diarrhoea continue to take a heavy toll, 66 million Indians are at risk due to excessive fluoride and 10 million due to excessive arsenic in groundwater. In all, 1, 95,813 habitations in the country are affected by poor water quality. It is clear that the large investments have not yielded comparable improvements in health and other socio-economic indicators.

**Water Resources and Utilisation**

- India has 16 per cent of the world's population and 4 per cent of its fresh water resources.
- Estimates indicate that surface and ground water availability is around 1, 869 billion cubic meters (BCM). Of this, 40 per cent is not available for use due to geological and topographical reasons.
- Around 4, 000 BCM of fresh water is available due to precipitation in the form of rain and snow, most of which returns to the seas via rivers.
- 92 per cent ground water extracted is used for agricultural sector and 5 per cent and 3 per cent respectively for industrial and domestic sector.
- 89 percent of surface water use is for agricultural sector and 2 per cent and 9 per cent respectively for industrial and domestic sector.

**Rural Water Supply**

The provision of clean drinking water has been given priority in the constitution of India, with Article 47 conferring the duty of providing clean drinking water and improving public health standards to the State. Rural water supply (RWS) programmes in India can be divided into several distinct phases.

***Early Independence (1947-1969)***

**1949**: The Environment Hygiene Committee (1949) recommends the provision of safe water supply to cover 90 per cent of India's population in a time frame of 40 years.

**1950**: The constitution of India confers ownership of all water resources to the government, specifying it as a state subject, giving citizens the right to portable water.

**1969**: National Rural Drinking Water Supply programme launched with technical support from UNICEF and Rs. 254.90 crore is spent during this phase, with 1.2 million bore wells being dug and 17, 000 piped water supply schemes being provided.

***Transition from technology to policy (1969-1989)***

**1972-73**: Introduction of the Accelerated Rural Water Supply Programme (ARWSP) by the Government of India to assist states and union territories to accelerate the pace of coverage of drinking water supply.

**1981**: India as a party to the International Drinking Water Supply and Sanitation Decade (1981-90) declaration sets up a national level apex committee to define policies to achieve the goal of providing sate water to all villages.

**1986**: The National Drinking Water Mission (NWDM) is formed.

**1987**: Drafting of the first National Water Policy by the Ministry of Water Resources.

***Restructuring phase (1989-1999)***

**1991**: NDWM is renamed the Rajiv Gandhi National Drinking Water Mission (RGNDWM).

**1994**: The 73rd Constitutional Amendment assigns panchayat raj institutions (PRI's) the responsibility of providing drinking water.

**1999**: For ensuring sustainability of the system's steps were initiated to institutionalise community participation in the implementation of rural drinking water supply schemes through sector reform.

**1999**: Total sanitation campaign as a part of reform principles initiated in 1999 to ensure sanitation facilities in rural areas with broader goal to eradicate the practice of open defecation. As part of the programme, a nominal subsidy in the form of incentive is given to rural poor households for construction of toilets. TSC gives strong emphasis on information, education and communication, capacity building and hygiene education for effective behaviour change with involvement of PRIs, CBOs, and NGOs.

***Consolidation phase (2000 onwards)***

**2002**: Nationwide scaling up of sector reform in the form of Swajaldhara.

**2002**: The National Water Policy is revised, according priority to serving villages that did not have adequate sources of safe water and to improve the level of service for villages classified as only partially covered.

**2004**: All drinking water programmes are brought under the umbrella of the RGNDWM.

**2005**: The government of India launches the Bharat Nirmal programme for overall development of rural areas by strengthening hosing, roads, electricity, telephone, irrigation and drinking water infrastructure.

**2007**: Pattern of funding under Swajaldhara Scheme changes from previous 90:10 central-community share to 50:50 centre-state share. Community contribution is now optional.

**2014**: Government launches Swachh Bharat Abhiyan to make India clean by 2019.

**Water Quality: Cause for Alarm**

While accessing drinking water continues to be a problem, assuring that it is safe is a challenge by itself. Water quality problems are caused by pollution and over exploitation. The rapid pace of industrialisation and greater emphasis on agricultural combined with financial and technological constraints and non enforcements of law have led to generation of large quantities of waste and pollution. The problem is sometimes aggravated due to non uniform distribution of rainfall. Individual practices also play an important role in determining the quality of water.

Water quality is affected by both point and non point sources of pollution. These include sewage discharge, discharge from industries, run-off from agricultural fields and urban run-off. Water quality is also affected by floods and droughts and also can arise from lack of awareness and education among users. The need for user involvement in maintaining water quality and looking at other aspects like hygiene, environment sanitation, storage and disposal are critical elements to maintain the quality of water resources.

**Towards Cleaner Water**

Providing safe drinking water to all in rural areas is a challenging task. Given the diversity of the country and its people, solutions have to be diverse. One has to look at an approach that seeks the

participation of users through interventions engaging the communities with various government schemes and policies. Citizens should be made aware of the demand for clean drinking water as a right. Such an integrated approach would incorporate collaborative efforts of various sectors involving the government, civil society and nneedless to say the people. Role of Government include (i) supporting awareness drives. (ii) Testing and remedial action. (iii) Capacity building of communities. (iv) Inter-agency coordination. (v) Making the service provider accountable. Role of Civil Society and communities includes (i) Awareness (ii) Accountability (iii) Community based water quality monitoring and (iv) Maintenance.

**Concluding Remarks**

In India investments in community water supply and sanitation project have increased steadily from the 1st plan to the 11th plan. However, the health benefits in terms of reduction in water borne diseases have not been commensurate with the investments made. Though health sector is bearing the burden of water and sanitation related infectious diseases, presently it does not have adequate institution or expertise for monitoring and surveillance of community water supply programmes in the country.

India has witnessed significant improvement in rural water supply with increasing coverage of areas and a large volume of financial resources made available. A series of schemes are aimed at improving the supply of drinking water for rural habitations and now for monitoring and ensuring quality. The past few years have seen greater emphasis on water quality monitoring and surveillance with specific allocation being made under Central grants. There has been great focus on setting up and upgrading laboratories at the state and district levels, and on water monitoring through field testing kits. One of the greatest challenges has been the convergence of various departments associated with water: water and sanitation programme have operated largely in isolation from programmes in health and education. A wider approach is needed where water and sanitation issues are looked at with the aim of reducing disease, improving hygiene, improving educational levels and reducing poverty.

There can be little doubt that water is basic necessity for the survival of humans. There is interplay of various factors that govern access and utilisation of water resources and in light of the increasing demand for water it becomes important to look for holistic and people-centred approaches for water management. Clearly, drinking water is too fundamental and serious an issue to be left to one institute alone. It needs the combined initiative and action of all, if at all we are serous in socio-economic development. Safe drinking water can be assured, provided we set our mind to address it.

***Table: States affected by various water quality problems***

| Parameter | Maximum permissible limit | Health impact | Affected states |
|---|---|---|---|
| Fluoride | 1.5 mg/L | Immediate symptoms include digestive disorders, skin diseases, and dental flurosis.<br>Fluoride in larger quantities taken over a period of 10-20 years results in crippling and skeletal flurosis which is severe bone damage. | Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Haryana, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan Tamil Nadu, Tripura, Uttar Pradesh , West Bengal |
| Arsenic | 0.05 mg/L | Immediate symptoms of acute poisoning typically include vomiting, oesophageal and abdominal pain and bloody 'rice water' diarrhoea.<br>Long term exposure to arsenic causes cancer of the skin, lungs, urinary bladder and kidney. There can also be skin changes such as lesions, pigmentation changes and thickening. | Assam, Bihar, Chhattisgarh, Jharkhand, Tripura, West Bengal, Uttar Pradesh |
| Iron | 1 mg/L | A dose of 1500 mg/L has a poisoning effect on a child as it can damage blood tissues.<br>Digestive disorders, skin diseases and dental problems. | Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Jharkhand, Jammu & Kashmir, Karnataka, Kerala, Manipur, Meghalaya, Mizoram, Madhya Pradesh, Maharashtra, Nagaland, Orissa, Punjab, Rajasthan, Sikkim, Tripura, Tamil Nadu, Uttar Pradesh |
| Nitrate | 100 mg/L | Causes Blue Baby Disease where the skin of infants becomes blue due to decreased efficiency of haemoglobin to combine with oxygen. It may also increase risk of cancer | Bihar, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh. |
| Salinity | 2000 mg/L | Objectionable taste to water.<br>May affect osmotic flow and movement of fluids. | Orissa, Punjab, Tamil Nadu, Rajasthan, Uttar Pradesh, West Bengal, Pondicherry. |
| Pesticides | Absent | Weakened immunity, abnormal multiplication of cells leading to tumour formation. They contain chlorides that cause reproductive and endocrinal damage. | |
| Persistent Organic Pollutants | None | High blood pressure, hormonal dysfunction and growth retardation. | Delhi, Himanchal Pradesh, Jharkhand, West Bengal |

# Therapeutic Antibodies

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An antibody (Ab), is basically an immunoglobulin or protein having a large Y-shape like structure. These are produced by B-cells present in circulation functioning as an important component of immune system involved in identifying and neutralizing foreign objects. Antigen is the unique part of the foreign object which is recognised by the tip of antibody. Antigens have epitope while antibodies have paratope , these fit together like lock and key and evokes immune response. Therapeutic antibodies differ from normal antibodies as these are man-made antibodies that are specifically designed for certain diseases or conditions and binds to specific proteins on the surface of cell. These are usually used to treat cancer and autoimmune disease.

The discovery of antibodies began with the development of serum therapy as an effective treatment against diphtheria and tetanus for which Emil adolf Von Behring received nobel prize in physiology. Later on Gerald Edelman and Rodney Porter got the nobel prize for discovery of chemical structure of antibody but advancement towards therapeutic antibodies began with the production of monoclonal antibodies. Monoclonal antibodies are the antibodies which are secreted from a single B cells and therefore have identical paratopes and recognise same antigen. As they were the first antibodies to be produced by human intervention they formed the first set of therapeutic antibodies. Yet as these antibodies were produced from mouse B cells they sometimes acted as an antigen for humans and evoked immune response thus their use was limited. With the advent of science and technology it was possible to form better therapeutic antibodies with lesser antigenecity. The modification in basic structure of antibodies like glycosylition of Fc region, engineering or tailoring the carbohydrates etc lead to enhancement in functioning of antibodies. Thus to gain a proper understanding of new generation therapeutics it was mandatory to explore various mechanism of action of antibodies and develop strategies for engineering them.

**Need for new generation therapeutic antibodies:**

As mentioned earlier the basic drawback of monoclonal antibodies was its high antigenecity thus in order to overcome the problem engineering of therapeutic anticodies was explored but with advancement in these studies not only the

basic problem was resolved but also there was adttition of various other attributes. These includes Bi specific antibodies which can target two antigen at same time, target specific drug delivery, antibodies activating other immune response component suct as cytotoxic T cells and many others. The limitations of previous generation therapeutic antibodies such as limited tissue penetration, unavailability to reach systemic circulation, or limted antigen targeting were nullified using engineered therapeutics. The current generation have higher affinity, enhanced functioning, multiple targets for a specific disease, greater safety, improved delivery to system, increased bioavailability, oral intake, ocular delivery and sustained release of required dosage over a period of time.

**Classification of therapeutic antibodies:**

These can be broadly classified based on 1) level of modification, 2) On the basis source of antibodies and 3) development with time. Based on the level of modification these are further divided into a)Murine b) chimeric c) Humanized. Murine source antibodies have excellent affinities and specificities. These are generated using conventional hydrioma technology. Clinical efficacy of these antibodies are compromised by HAMA(human anti murine antibody) response, which lead to allergic or immune complex herpersensitivities. Chimeric antibodies are combination of the human constant regions with the intact rodent variable regions. Affinity and specificity unchanged but these also cause human antichimeric antibody response (30% murine resource). Humanized antibodies contains only the CDRs of the rodent variable region grafted onto human variable region framework. These are obtained by genetic engineering in order to increase its similarity to antibodies produced naturally in humans, thereby its immunogenicity is drastically decreased. A common humanization method is known as CDR grafting which involves introducing the CDRs from a non-human antibody of interest into a framework acceptor sequence of a human germline V gene that is closely related to the antibody of interest. The suffix -zumab is used to identify humanized antibodies. These are also known as 'Me better' antibody.

On the basis of source of antibodies these can be derived from a) Transgenic plant b) Transgenic animals c) Phage display library. Certain transgenic plant such as tobacco variants are designed with gene inserts of antibodies such that the produce it in their saps or extracts such as IgA while transgenic mice are used to make humanized IgG. In phage display libraries certain regions of gene are made to be expressed on filamentous bacteriophage and these are used to screen for effective antibodies.

Finally based on the time evolved development of antibodies, these are classified as first generation, second generation, third generation and recently fourth generation antibodies. For instance Rituximab a first generation chimeric monoclonal antibody used to treat Non-Hodgkin's lymphoma evolved to 2nd generation Ofatumumab acting on same disease but at different epitope and having

different mechanism of action and finally to 3rd generation Ofatumumab with even better specificity and efficacy. These development are need based and generally target overall efficiency of the drug.

**MECHANISM OF ACTION**

The antibodies exert their effect through various modes which defines their mechanism of action. Improvisation in the basic mechanism of action is one of the main areas of focus for generation of better antibodies. The action can be classified as Cytokine and growth factor blockade (ligand blockadge), receptor blockade, receptor down regulation, depletion, signalling induction and effector function modulation.

*Cytokine and growth factor blockadge:* In these kinds of action either the entire antibody or the fragment of antibodies are involved which target the cytokine or factor related to the disease such that the downregulation or decreased concentration of the harmful cytokine makes them unavailable to reach the target site in a concentration required to cause their effect. For instance Tumor necrosis factor (TNF) involved in many inflammatory diseases such as Crohn's disease is downregulated by infliximab binds both solubleand membrane-associated tumour necrosis factor (TNF). Other antibodies in this class act as TNF antagonist, these are the most successful class of drugs for treating many other inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, ankylosing spondylitis and plaque psoriasis.

*Receptor blockade and receptor modulation:* Therapeutic antibodies can also block ligand–receptor interactions by targeting the receptor. These include antibodies that target the Il-6 (interleukin receptor-6) such as tocilizumab, αl integrin such as efalizumab and many more. Targeting of receptors adds a secondary level of mechanistic activity as in addition to blocking the ligand binding there is also downregulation of the cell surface expression of the targeted receptors. Thereby there is overall drastic effect on the activity of the harmful ligand. There is a drawback in using such kind of antibodies as antibody-induced internalization of the target results in antigen-induced clearance of the therapeutic antibody and decreases its serum half-life resulting in short term effect of antibodies requiring repeated dosing.

*Depleting and signalling antibodies:* Another class of therapeutic antibodies depletes antigen-bearing cells by binding to cell surface antigens — for example, CD20, CD22 and CD52. Rituximab, a chimeric CD20-specific antibody, was approved in 2005 for the treatment of patients with rheumatoid arthritis who had inadequate responses to TNF blockade. These kinds of antibodies evoke cytokine dependent cytotoxicity or phagocytosis or other effector function through their constant regions. The main concern of using such kind of antibodies as opposed to their corresponding soluble ligand targeting drug is a greater potential for inducing an immunogenic response.

*Signalling induction and effector function modulation***:** Therapeutic antibodies that

bind to cell surface antigens can transmit intracellular signals required for clinical efficacy and/or contribute to adverse events. The first FDA-approved therapeutic antibody for the treatment of acute allograft rejection in renal transplantation, muromonab-CD3 acted through this mechanism but its constan region stimulated T cell proliferation and cytokine production resulting in undesirable mitogenic effect. Their second generation product teplizumab was produced to overcome this drawback.

**Next-generation therapeutic antibodies:**

Strategies for new antibody generation can be broadly classified as the endowment of antibodies with new capabilities or modification of existing antibody properties. The generation of bispecific antibodies i.e. antibodies that can target two related diseases/ two different targets of same disease, at the same time and modulation of Fc-mediated functions (constant region of antibodies) comes under these classifications. These kinds of antibodies seem promising for the generation of autoimmune and inflammatory diseases. Besides these antibody fragments are being widely used for engineering antibody properties and they are becoming important therapeutic agent. Modulation of effector functions (Antibody Dependent Cell Cytotoxity, Antibody Dependent Cell Phagocytosis and Cytokine Dependent Cytotoxity) and pharmacokinetic half-life can be achived through modulating or modifying Fc-mediated activities such as tailoring Fc glycosylation/carbohydrate addition. Optimization of antigen-binding domains provide another better option for antibody improvement. Antibody –drug conjugate and bispecific antibodies have provided momentum for endowing antibodies with new activities.

**Limitations, strengths, and future opportunities of therapeutic antibodies**

The several limitations of therapeutic antibody includes limited clinical application as surface or extracellular targets are mainly influenced or acted upon by the antibodies. Secondly they are highly expensive as they need to be administered in large doses and are costly to produce even at large scale thus potentially benefited patient decreases drastically. Thirdly most of the therapeutic antibodies cannot be orally administered which makes it difficult to use. Fourthly due to their large size there is limited penetration of central nervous system owing to inefficient penetration of blood brain barrier.

There are many strengths of therapeutic antibody which makes them highly influential in disease treatment. There are several well established and applicable methods for antibody optimization and generation. These are well tolerated by patients and they have high success rates as compared to other drugs. Due to increasing new technologies it is becoming relatively easy to design antibodies with modified or new properties to enhance their clinical potential. Besides these there is a burst in the knowledge of antibody drug development facilities and many broad experiences are facilitating the generation of new antibodies.

*Future opportunities:* Antibodies with greater efficacy, higher potency or affinity, greater safety, greater selectivity for targets forms the future of therapeutic antibodies. Moreover there is need for antibodies with enhanced effector functions such as complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) particularly in treating autoimmune and inflammatory diseases. Antibodies with shorter half life and those that have redundant roles in the same pathway or those which targets multiple disease mediators from distinct signaling pathways (bispecific antibodies) will be more favored over other therapeutics in future. Activation of undesirable effector functions as observed in certain antibodies needs to be worked upon. Next generation antibodies should have lower or less frequent dosing will reduce the cost of administration. Drugs that can cross blood brain barrier and are thus available to ocular and central nervous systems are the need of the time. Furthermore focus on antibodies that have increased bioavailability, oral administration and sustained availability will resolve many pressing limitations of present generation of therapeutics.

**CONCLUDING REMARKS**

The lessons learnt from the first and second generation antibodies have provided the foundation for the discovery in their functioning. and development of future therapeutic antibodies. The development of therapeutic antibodies has evolved over the past decade into a mainstay of therapeutic options for patients with cancer, autoimmune and inflammatory diseases. The insights obtained from the development of therapeutic antibodies complemented by newer antibody engineering technologies are delivering next generation of therapeutic antibodies with promise for greater clinical efficacy, ease of administration and safety. The understanding of the basic mechanism of therapeutic antibody like *ligand blockade*, *receptor blockade and receptor down regulation, cell depletion and signalling antibodies* enabled greater understanding and lead to the strategies for improvement of next generation. Strategies for new antibody generation has been broadly classified as modification of existing antibody properties or the endowment of antibodies with new capabilities this includes modification of these antibodies in terms of modulation of interactions by using protein and glycosylation strategies, *optimization of* Fc-mediated antibody functions, effector function minimisation or enhancement, engineering the Fc protein sequence or tailoring the Fc carbohydrates, pharmacokinetic half life modification and optimization of antigen binding domains. These have lead to the foundation of third and fourth generation antibodies in terms of overall betterment

# Effect of water temperature on water intake, feed intake, production and health of dairy animals

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Water is the most important dietary essential nutrient for dairy cattle and greatest component of milk (approximately 87%). Requirement of water is influenced by physiological stage, milk yield, dry matter intake, body weight, composition of diet and environmental factors. Water content reduction by 10% reduces the milk yield (Herrero, 1998).Dairy cows are in the comfort zone when environmental temperatures are between 5 and 25 ºC (Roenfeldt, 1998).Under heat stress conditions water needs are increased by 1.2-2 times.High specific heat of water promotes heat dissipation.

Water provided to animals may have different temperature depending upon the source which may affect productivity of animals Water intake can be influenced by its temperature and further it can affect feed intake and milk production. Most mammals prefer drinking water with a temperature near body temperature (Szlyk et al., 1989).

**Palatability and preference**

Drinking water temperature between 20 and 28 ºC is the most accepted by cattle (Lanham et al., 1986).Cows drank an average of 97.2% ambient water with a range of 87.4 to 100%; cows prefer to drink only warm water if given the choice. Milam et al. (1986) and Wilks et al. (1990) did not find any significant differences in water intake, but noted animal preference for water at a higher temperature. Beck et al. (2000) who observed that 94% of the animals preferred water at 24 ºC, instead of 17 ºC.Dairy cattle prefer more water at ambient temperature (NRC. 2001).Ruminants prefer to drink warmer water (30 vs. 12 to 14°C) even when environmental temperatures are quite warm (>25°C; Beede, 2006).

**Water intake**

Huuskonen et al. (2011) observed a linear response of water intake to increase in drinking water temperature from near freezing to about body temperature in Holstein cows. Lofgreen *et al*. (1975) recorded that animals consumed a lesser quantity of water at 18 ºC compared to 31ºC. According to Wilks et al. (1990) Cows consume 7.7% more water at 10.6 ºC than at 27 ºC. While Savage et al., (2006) observed that animals preferred to drink more water at 30 °C than at 20 °C in warm climatic conditions.Osborne et al. (2002) found that in the four annual seasons dairy cows drank between 3.40 to 5.95% more water at temperatures between 30 and 33 °C than water between 7 and 15 °C.

**Dry matter intake**

More feed and improved body weight gain was observed in cows given 18 °C water as compared to 31°C. Cows offered 10°C water showed .47% of body weight increase in mean DM intake over control cows 28°C (Milam et al 1986). Cows offer cooler water (10.6°C) consumes more feed (3%) than the water at 27°C (Wilks et al 1990). There is increase in body weight of beef cattle given 18.3 °C drinking water compared with 31.2°C water in the summer (Ittner et al 1951). However, Osborne et al (2002) found that feed intake increased by 4.5% when cattle were offered heated water (30 to 33°C vs. 7 to 15°C) during summer (mean daily temperature was 21°C). Gohler (2014) observed non-significant effect of water temperature on dry matter intake.

**Milk yield**

Warm water increases the milk production in winter with the assumption that cows consume warmer than ambient cold water in winter (Grzegorzak et al. 1976).In a hot environment, cooling of the water is of primary interest, whereas for high-yielding dairy cows in a cold environment it may be advantageous to warm the drinking water. Coldest water (3°C) caused a decrease in milk yield without affecting the dry matter intake (Andersson, 1985). It was hypothesized that feed energy required to warm water may have depressed the milk production.There is increase of 2.4 kg/cow/d milk yield followed dry matter intake for the cows given chilled water (Milam et al 1986). Baker et al (1988) observed no effect of temperature of drinking water on the milk yield. Milk yield was increased 4.8% for the cows consuming chilled water (Wilks et al., 1990). Beck et al. (2000) did not find any differences in milk production between cows that consumed water at 17 and 24 ºC but indicated increase in butyric fat production in those animals that consumed fresh water

**Tropical regions**

Chilled drinking water is effective as it absorbs body heat which help cow in maintaining its body temperature. The coldest water (10°C) reduced body temperature (.75°C) more than 28°C water (.46°C) (Stermer et al).However 10º C temperatures was only 32% effective in reducing body temperature. However, Baker et al (1988) found no differences in respiratory rates and rectal temperature

Even though cows drank less cooled water (4.7 L/cow/d), heat load decreased by 706 kcal/ cow/d(Ittner et al). 10°C drinking water reduced respiration rates and provided greater cooling effect than 28°C water in lactating Holstein cows in summer (Lanham et al 1986). Physiological improvements were observed for 2 hours when animal subjected to heat stress environmental conditions when given colder water ((Purwanto et al., 1996). Water refreshing capacity facilitates heat dissipation and helps to decrease metabolic load (Beck et al., 2000).

**Temperate regions**

In thermoneutral conditions (mean temperature was 15.3°C, and the range was 10 to 24°C) water consumption was lower when cow offered with 24°C water than for 3, 10 or 17°C water (Andersson, 1985). Cunningham and coworkers investigated

effect of drinking water when the average ambient temperaturewas 3 °C and found that lactating dairy cattle prefer warm drinking water when the ambient temperature is low.In the research carried out by Gohler et al., (2014) in crossbred dairy cattle at high altitude temperate Himalayas (mean temperature 6°C), 8.3% and 10.07% high milk yield were observed when given water at temperature 35–40°C than at 15–20°C and 10.25°C respectively. They concluded that it might be due to conserved energy and enhanced water intake when hot water is given to cows. Dry matter intake was not affected in this experiment.

**Economics**

The economic advantage of chilled drinking water can be gained by increase milk yield. There is no economic advantage by providing chilled drinking water to lactating Holstein's cattle in ambient temperatures above the thermal neutral zone (Baker et al., 1986).Generally the water in ground water doesn't heat up enough to affect intake of animal. Purwanto et al (1996) suggested that most economical methodof reducing heat stress in lactating dairy cattle would be by using chilled deep well wateroffered in restricted amounts during feeding periods.

**CONCLUSION**

Temperature affects the quality directly by changing palatability and acceptance by animal. Water temperature between 15-27°C is most acceptable to dairy cattle and temperature of more than this should be chilled. Cow prefers to consume water close to ambient temperature than chilling water when the ambient temperature is above thermoneutral zone. Chilled water is beneficial in reducing heat load in summers but the cooling effect is temporary and it should be cost effective so as to decrease heat load

**REFERENCES**

Andersson, M. 1985. Effects of drinking water temperatures on water intake and milk yield of tied-up dairy cows. Livest. Prod. Sci. 12:329–338.

Beck, J., D. Katschke, and H. Steingass. 2000. Heated drinking water for dairy cows. AgrartehnischeForschung 6(4):97-101

Beede, D. K. 2006. Evaluation of water quality and nutrition for dairy cattle. Page 129–154 in Proc. High Plains Dairy Conf., Albuquerque, NM. Accessed Feb. 8, 2012. http://www.highplainsdairy.org/2006/Beede.pdf.

Cunningham MD, Martz FA, Merilan P: 1964, Effect of drinking water temperature upon ruminant digestion, intraruminal temperature, and water consumption of nonlactatingdairy cows. Joumal of Dairy Science 47: 382-385.

Herrero, M.A. 1998. El agua y suimportanciaen la producciónganadera. Rev. Georgica 6:67-87. Universidad de Zaragoza, EscuelaPolitécnica de Huesca, España.

Huuskonen A, Tuomisto L and Kauppinen R. 2011.Effect of drinking water temperature on water intake and performance of dairy calves. Journal of Dairy Science 94: 2475–80.

Ittner, N. R., C. F. Kelly, and H. R. Guilbert. 1951. Water consumption of Hereford and Brahman cattle and the effect of

cooled drinking water in a hot climate. J. Anim. Sci. 10:742.

Lanham, J. K., C. E. Coppock, K. Z. Milam, J. M. LaBore, D. H. Nave, R. A. Stermer, and C. F. Brasington. 1986. Effects of drinking water tem- perature on physiological responses of lactating Holstein cows in summer. J. Dairy Sci. 69:1004

Lofgreen, G.P., R.L. Givens, S.R. Morrison, and T.E. Bond. 1975. Effect of drinking water temperature on beef cattle performance. J. Anim. Sci. 40:223-229.

Milam, K. Z., C. E. Coppock. J. W. West, J. K. Lanham. D. H. Nave, and 1. M. Labore. 1986. Effects ofdrinking water temperature on production responses in lactating Holstein cows in swnmer. J. Dairy Sci. 69:1013.

NRC. 2001. Nutrient requirements of dairy cattle. 7th revised ed. p. 178-183.National Research Council, Natl. Acad. Press, Washington D.C., USA.

Osborne, V.R., R.R. Hacker, and B.W. McBride. 2002. Effects of heated drinking water on the production responses of lactating Holstein and Jersey cows. Can. J. Anim. Sci. 82: 267-273.

PunvantoBP, Harada M, Yamamato S: 1996. Effect of drinking water temperature on heat balance and thermoregulatory responses in dairy heifers. Australian Journal of Agricultural Research 47 (4): 505-512

Roenfeldt, S. 1998. You can't afford to ignore heat stress. Dairy Manage. 35(5):6-12.

Savage, D., J. Nohlan, I. Godwin, A. Aoetpah, T. Nguyen, N. Baillie, et al. 2006. Importance of drinking water temperature for managing heat stress in sheep. Available at http://www.agric.wa.gov.au/objtwr/imported_assets/content/aap/heat_stress_savage.pdf(accessed September 2009)

Stermer R A, C F Brasington , CE Cappock J. K. Lanham, and K. Z. Milam. 1986. Effect of Drinking Water Temperature on Heat Stress of Dairy Cows . J. Dairy Sci. 69:546

Szlyk, P. C., I. V. Sils, R. P. Franesconi, R. W. Hubbard, and L. E. Armstrong. 1989. Effects of temperature and flavouring on voluntary dehydration in men. Physiol. Behav. 45:639–647.

Wilks, D.L., C.E. Coppock, J.K. Lanham, K.N. Brooks, C.C. Baker, W.L. Bryson, et al. 1990. Responses of lactating Holstein cows to chilled drinking water in high ambient temperatures. J. Dairy Sci. 73:1091-1099.

# Uses Hormones and Drugs in Therapeutic Management of Infertility and Uterine Infections

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The choice of hormones and drugs acting on reproductive system are important as therapeutic agents during reproductive disorders like anoestrus, metritis, pyometra, repeat breeder, retention of fetal membranes, delayed puberty, ovarian disorders etc. The administration of specific chemotherapeutic agents is of vital importance in treating reproductive disorders in dairy animals. The dosage, route, frequency and indications of chemotherapeutic agent acting on reproductive system are mentioned in tubular forms as below.

| Hormone | Pharmacological action | Indications | Dosage and route in cow and buffalo |
|---|---|---|---|
| Buserelin - GnRH analogue | It stimulates follicular development, estrus and ovulation by enhancing release of FSH and LH | Anoestrus, Delayed ovulation | 2.5 mL , IM,IV |
| | | True anoestrus, Follicular cyst | 5 mL , IM,IV |
| Gonadorelin - GnRH analogue | It stimulates follicular development, estrus and ovulation by enhancing release of FSH and LH | Cystic ovarian disease | 100 mcg IM, IV |
| | | Delayed ovulation, Repeat breeder, Improvement of post partum fertility | 250 mcg IM, IV |

| | | | |
|---|---|---|---|
| Follicle stimulating hormone | Induces folliculogenesis | Superovulation in ETT | 50mg BID for 4 days |
| Luteinizing hormone | Pituitary gonadotrophin LH induces maturation, estrogen production and finally ovulation | Anovulation, Delayed ovulation, Cystic ovaries, Repeat breeding | 25 mg, IV |
| Humen chorionic gonadotrophin | It mimics the effect of pituitary LH casing ovulation. Promotes the formation & maintenance of CL | Repeat breeding<br>Cystic ovaries<br>Delayed ovulation<br>Fetal resorption<br>Early abortion | 1500-3000 IU, IM<br>1500- 3000 IU, IM<br>1500- 3000 IU, IM repeat on day 8, if CL is poorly developed<br>1500- 3000 IU, IM / week for 4 weeks as IM<br>1500- 3000 IU, IM / week for 4 weeks as IM |
| Pregnant mare serum gonadotrophin/eCG | It has FSH like action, it induce follicular growth | Superovulation in ETT | 1500- 3000 IU, IM |
| Hydroxy Progesterone caproate | It act on the endometrium inhibiting myometrial activity and thus quieting uterus | Pre partum uterine/ vaginal prolapse<br>Post partum propalse | 500 mg. on alternate days for 3 occasions IM<br>500 mg for 3 days followed by 500 mg/ week for 3week, IM |
| | | | 500 mg at the |

| | | | |
|---|---|---|---|
| | | Habitual pronounced heat | beginning of estrus, IM |
| Estrogesns (Estradiol valerate) | Cause uterine contraction, cervical relaxation and positive feedback on surge centre | Induction of estrus | 1.5 mL on day1& 1.5mL after 3 days |
| | | | 3mL |
| | | Induction of parturition | 3mL |
| | | Pyometra | 3mL |
| | | RFM | 20-30 IU, IM,IV |
| Oxytocin | It has specific effect on uterine muscles and myoepithelial cells of mammary glands. | To speed up uterine involution Control of uterine bleeding(Cesarean section) | 2mL IM/SC or 5mL IM. |
| Prostaglandin | Potent luteolytic action which brings about regression of CL, Induce fertile heat following luteolysis.It enhances cervical dilatation and also has selective spasmogenic action. | Luteal cyst, Estrus synchronization protocol, Mummified fetus, pyometra, induction of parturition. | 40-50 mg I/M |
| Valethamate | Anti-cholinergic. It is quarternary ammonium compound with peripheral actions | Hard cervix in dystocia. Inadequate cervical dilation in parturition. To prevent cervical and vaginal tear in | |

| | | | |
|---|---|---|---|
| | similar to those of atropine enabling cervical dilation | dystocia. | |

**Intrauterine preparations**

| | | |
|---|---|---|
| Cephalexin 7.5% w/w | Metritis, Pyometra and Cervicities. | 20g/ 50mL distilled water I/U |
| Cephalexin 1.5g and Serratiopeptidase 10g/4g | Vaginitis, Retained fetal membrane | Reconstitute with 60mL of sterile water and infuse intrauterine. |
| Ciprofloxacin 40 mg/mL | Vaginitis, Retained fetal membrane | 5-10 mg/kg b.wt for 3-5 days In RB 12-24 mL post AI. |
| Ciprofloxacin 125 mg + Tinidazole 150 mg per 5mL | | 30-60 mL I/U |
| Levofloxacin 20 mg per mL | | 30 mL I/U |
| Metronidazole 1g + Furazolidone 0.2 g | | 2-4 boli I/U |
| Metronidazole 1g, Nitrofurazone 60 g, urea 5g | | 2 boli I/U affected horn |
| Oxyteracycline hcl 500 mg | Metritis, Pyometra and Cervicities &Vaginitis | 2-4 boli I/U |
| Oxyteracycline hcl 50 mg/mL | Retention fetal membrane | 10-30mL diluted with equal quantity of sterile water |
| Povidone iodine 200mg/tab | Retention fetal membrane | 2-4 boli I/U |
| Povidone iodine 1% W/V | | 18mL I/U |
| Povidone iodine + Metronidazole | | 30-60mL I/U |
| Sulphamethoxazole 0.5g +Trimethoprim 0.1g | | 2-4 boli/ horn I/U |
| | | |

**Parenteral antibiotics**

| | | |
|---|---|---|
| Amoxicillin +Cloxacillin | Metritis +Pyometra | 6-10mg per kg b.wt IM, IV daily for 3-5 days |
| Ceftriaxon | Endometritis, Metritis, Pyometra | 5-10mg per kg b.wt IM, IV daily for 3-5 days |
| Ceftriaxon +Tazobactum | Metritis | 5-10mg per kg b.wt IM, IV daily for 3-5 days |
| Enrofloxacin | Endometritis | 1mL/20kg kg b.wt IM, IV |

| | | |
|---|---|---|
| 100 mg per mL | | daily for 3-5 days |
| Lincomycin | Metritis | 1mL/30kg kg b.wt IM,S/C or slow IV |
| Oxytetracyclin-LA 200mg per mL | Enzootic abortion Brucellosis | 1mL/10kg kg b.wt deep IM |

**Indigenous preparations**

| | | |
|---|---|---|
| Exapar Bolus | RFM, Delayed uterine involution, Uterine cleanser | 2-4 boli BID |
| Exapar liquid | RFM, Delayed uterine involution, Uterine cleanser | Initial dose 100mL followed by 50 mL BID |
| Exenta liquid | RFM, Delayed uterine involution, Uterine atony, Uterine infections, Repeat breeder | 100mL BID/TID<br>In RFM administer double dose immediately after parturition followed by normal dose |
| Exenta Powder | RFM, Delayed uterine involution, Uterine tonic, Ecbolic | 20 g BID |
| Involution bolus | RFM, Delayed uterine involution, Uterine tonic, Cleansing agent | 1 bolus BID |
| Prolapse- In tab | Prolapse of vagina, uterus and rectum | 4-6 boli daily orally |
| Replanta powder | To promote uterine involution, Uterine cleanser. Retention of fetal membranes | Initial dose 100g followed by 50-60 g QID |
| Uterine liquid | RFM, Uro-genital infections. Repeat breeder. Abortion Flushing of lochial discharge | 100-125 mL oral |

# Bacterial Wilt: A Threat to Tomato Production

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**Abstract**

*Tomato is one of the most popular vegetables in the world. Tomato yields are low due to poor fruit setting caused by the high temperatures, as well as many severe disease problems. Among diseases, bacterial wilt caused by Ralstonia solanacearum (Smith) is one of the most economically important and devastating disease of tomato crop. Other than chemical fumigants, there is no commercial pesticide available for control of bacterial wilt. The main control strategy has been the use of resistant varieties. However, the stability of bacterial wilt resistance in tomato is highly affected by pathogen density, pathogen strains, temperature, soil moisture, and presence of root knot nematode. The objective of this article is to provide information to farmers and extension specialists on how to use management strategy for control of tomato bacterial wilt.*

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown fruit vegetable in the world. India ranks second in the area as well as in production of Tomato. China is the largest tomato producing country in the world, followed by India and USA. In India, the area under tomato cultivation was 880 thousand hectare with production of 18227 thousand MT and productivity of 20.7 MT/ha (Anonymous, 2014). Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi (formerly *Pseudomonas solanacearum)* is one of the most economically important and devastating disease of tomato crop. The disease was first reported from Asia and South America (Smith, 1896). In India bacterial wilt of tomato was first reported in Solan area of Himachal Pradesh (Gupta *et.al*., 1998). *R. solanacearum* (Smith) is a serious soil borne pathogen of solanaceous vegetable crops grown during summer, rainy and winter seasons.

**Distribution and host range**

*R. solanacearum* is a highly heterogeneous bacterial pathogen that causes severe wilting of many important plants (Smith *et al*., 1995). Bacterial disease was widely distributed in tropical, subtropical and warm temperate regions of the world with a host range of 44 plant families (Hayward, 1991). *R. solanacearum* cause severe

wilting in numerous plant including eggplant, pepper, peanut, tomato and tobacco (race1); banana, heliconia (race 2); potato and geranium (race 3); ginger (race 4) and mulberry (race 5) (Hayward and Hartman, 1994).

**Dissemination**

*R. solanacearum* is a soil-borne disease which is mainly disseminated through soil and enters roots through wounds or natural openings. It multiplies after infection and moves up through the vascular system, and finally blocks water transportation, which causes wilting. The pathogen is released into soil from infected plants, and neighboring plants can be infected via root contacts. It can also enter plants through pruning wounds. The pathogen can be disseminated into a clean field through contaminated water sources, symptomless yet contaminated seedlings, as well as humans or machinery carrying infested soils.

**Symptomatology of the disease**

***Figure 2 Typical symptoms of bacterial wilt***

Disease develops rapidly in warm weather, especially after heavy rain or flooding. The most characteristics symptoms of the disease is yellowing and wilting, which may be followed by necrosis and death. The vascular bundles of the infected plants showed brown discoloration. Recently wilted plants are green this is a distinct symptom compared to other vascular diseases like *Fusarium* wilt, which develop yellowing of leaves.

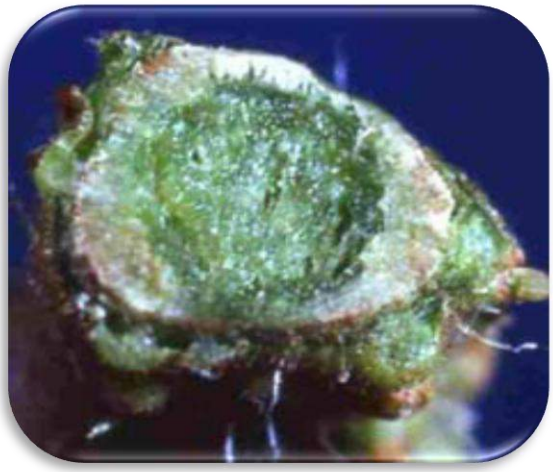

***Figure 1 Vascular browning***

**Diagnosis of the disease (Ooze test)**

The disease can be diagnonize by applying ooze test, which was attempted by placing wilted tomato plant cut stem pieces in clean glass container with water. Let it stands for few minutes. If fine milky ooze exuded in the water making it turbid, which was the indication of the presence of the bacterium.

**Phenotypic characteristics of the bacterium**

The appearance of colonies of *R. solanacearum* can be observed on TZC (Tetrazolium chloride agar) medium after 48 to 72 hrs of incubation at 28˚C. Colonies of virulent or pathogenic strains of *R. solanacearum* appeared smooth, fluidal, irregular, colored with pink centers of growth and somewhat translucent under transmitted light. Virulent colonies after 48

hrs of growth could be differentiated from avirulent and non-pathogenic type colonies on the basis of colony texture, pink pigmentation and fluidity (Kelman, 1954; Javeria and Kumar, 2014).

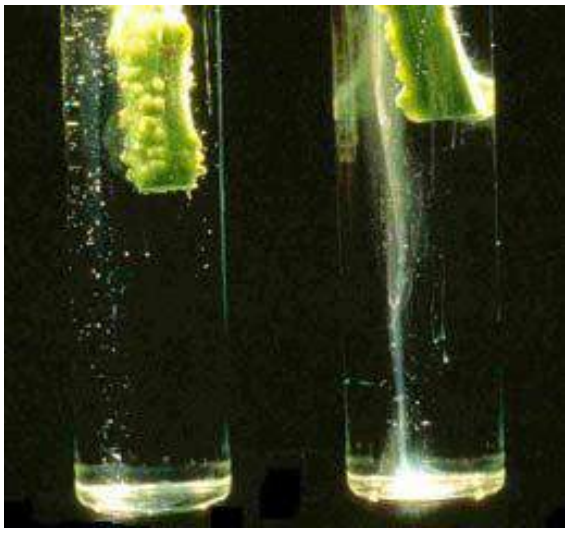

***Figure 3 bacteria ooze from infected stem (right) but not from a healthy stem (left)***

**Control measures:-**

**1. Choose a clean field**

- Field selection is a key step in managing this soil-borne disease.
- Regular rotation with paddy rice and other non-host plants reduce the disease incidence.
- Flat topography and good drainage.
- Free from water that flows within fields having the disease.

**2. Suppress the pathogen in infected fields**

- *R. solanacearum* once introduced into a field are difficult to eradicate but they can be suppressed.
- Fumigation with chemicals like methyl bromide is often used to control bacterial wilt disease.

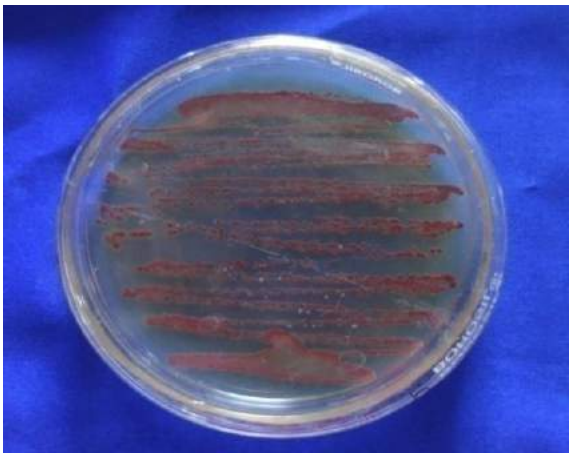

**Figure 4 Avirulent colonies of *R. solanacearum* on TZC media**

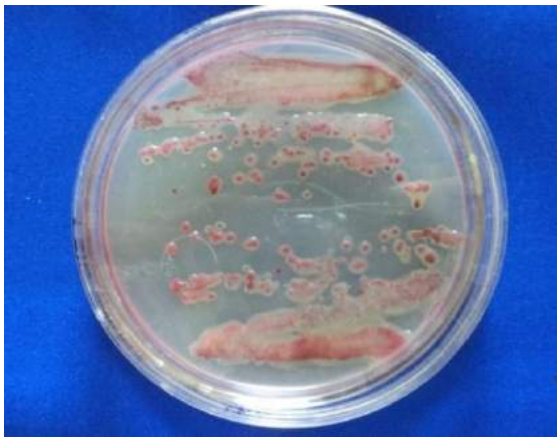

**Figure 5 Virulent colonies of *R. solanacearum* on TZC media**

- Flooding the field for 1 to 3 weeks will reduce the incidence of bacterial wilt and other soil borne diseases and nematodes.
- Growing *Brassica* manure like Indian mustard (*Brassica juncea*) and incorporating the plants into soil at flowering stage can also suppress the *R. solanacearum*.
- Urea and lime are also reported to suppress bacterial wilt in the field.
- Soil amendments also reported to suppress bacterial wilt.

**3. Use resistant varieties and clean seedlings**

- Planting a variety with resistance to bacterial wilt the simplest way to control the disease.
- Examples of resistant or tolerant varieties include 'Arthaloka' in Indonesia, 'Delta' in Thailand, and 'Taichung AVRDC 4' in Taiwan.
- Use healthy and disease-free seedlings.

**4. Prevent the spread of disease in the field**

- Remove and destroy infected plants
- Reduce irrigation frequency and water amounts
- Drain the field quickly after rain
- Isolate diseased spots
- Disinfect pruning tools

**Conclusions**

Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi is one of the most destructive diseases of tomato (*Solanum lycopersicum*), causing accountable losses of about 10-90 per cent. The pathogen has wide host range and race specific. It is a soil borne motile bacteria which is disseminated through soil and enters roots through wounds or natural openings. Drooping of leaves, yellowing followed by necrosis and death are symptoms of infected plants. Virulent colonies after 48 hrs of growth could be differentiated from avirulent and non-pathogenic type colonies on the basis of colony texture, pink pigmentation and fluidity. Use of resistant varieties is the simplest way to control the disease. Crop rotation, fumigation with chemicals and flooding also suppress the bacterial wilt.

**REFERENCES**

Anonymous (2014). Indian Horticultural Databse (NHB) Ministry of Agriculture, Govt. of India.

Gupta, S. K., Dohroo, N. P. and Shyam, K. R. (1998). Occurance of bacterial wilt of tomato in Himachal Pradesh. *Pl. Dis. Res.,* **13**: 174.

Hayward, A. C. (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathol.,* **29**: 65-67.

Hayward, A. C. and Hartman, G. L. (1994). Bacterial wilt: The disease causative agent, *Pseudomonas solanacearum*, CAB International Wallingford, United kingdom, pp.259.

Javeria, S. and Kumar, M. (2014). Cultural and biochemical properties of *Ralstonia solanacearum* causing bacterial wilt in potato. *Current Discovery*, **3** (1): 11-15.

Kelman, A. (1954). The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathol.*, **44**: 693–695.

Smith, E. F. (1896). A bacterial diseases of the tomato, potato, eggplant and Irish potato (*Bacillus solanacearum* nov sp.) U. S. Dept. Agric. Div. Veg. Physiol, Path. **12**: 1-28.

Smith, J. J., Offord, L. C., Holderness, M. and Saddler, G. S. (1995). Genetic diversity of *Burkholderia solanacearum* (synonym *Pseudomonas solanacearum*) race 3 in Kenya. *Applied Environ. Microbiol.,* **61**: 4263-4268.

# Mass Media and Its Role in Enhancing Livelihood Support Of Livestock Farmers

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Livestock is an integral part of Indian economy and plays a multifaceted role in providing livelihood support to about 1.3 billion rural population especially to those who are resource poor, small, medium and landless labourers. Livestock sector contributes 42 percent of the total value of output from agriculture (Central Statistical Organization report, 2013) whereas its contribution in agricultural and national GDP is 27.25 and 4.11 percent respectively (BAHS, 2014-15). Although empirical evidences indicates India to be largest producer of milk and livestock to provide additional source of income along with nutrient cover to huge segment of country populace but even after 68 years of independence we are yet confronting parcel of crevices amongst the demand and accessibility of milk , meat and eggs. This gap is attributed due to the lack of awareness about improved package of practices and unawareness of new scientific technology available for farmers. The scientific methods being done in research stations seldom reach to the grass root level. Furthermore, it is impractical for the researchers and millions of stakeholders to meet on customary premises to educate and learn things for themselves. Thus the need of mass media arises to bridge the gap between the researchers and the rural population.

Mass media operate in all fields of life. Through the mass media large and widely dispersed audience can be communicated within short interval of time. It conveys noteworthy changes in agriculture and aids in expanding productivity and livelihoods. Its major objective is to communicate feasible farm technologies in such a manner to attract the attention of farmers to help them understand and remember the message and ultimately facilitate them to take appropriate decision. It provides rural women with information on improved home making, on supporting their male counterpart on improved farming and to encourage them to participate them in decision making for the purpose of scientific farming. There are different types of mass media namely

1. Electronic media
2. Printed media
3. Folk media

## ELECTRONIC MEDIA

There are three traditional media which includes radio, television, internet and few hybrids like smart phones, electronic displays etc. Among them radio , television and mobiles assumes the most essential part in disseminating

information about improved animal husbandry practices. Radio serves to be the speediest, least expensive and intense mass media having reached up to the unreached especially due to its minimal cost and capacity to be available at any time, at any place cutting the barrier of illiteracy. Similarly television is used as a medium of education, rural upliftment and community development. It has proven to be an important audio visual aid in diffusion of innovation and adoption of improved farming practices among rural masses. The list of recent radio programmes includes "MAN KI BAAT", KISANVANI, and FARM AND HOME PROGRAMMES etc. All India Radio observes 15th February as Radio Kisan Diwas over all the stations mounting special programmes on the occasions. Farmers, who are benefited by the information generated through these programmes on AIR, share their experience with fellow farmers in local dialect. Health and welfare programmes are broadcasted on a regular basis in AIR. The topics are related to maternal care, child survival, medical terminancy of pregnancy, post natal care, drug abuse, child care, girl child, dispropionate sex ratio etc.

In the last year the government has brought about new schemes through the farmers by launching mobile apps known as Crop Insurance mobile app ,Agri market mobile app, Pusa Krishi mobile app .They were launched by honourable prime minister and agriculture minister in Krishi Unnati Mela in March 2016 with an aim of getting information launched by pusa,weather,crop insurance etc.


## PRINTED MEDIA

Printed media are those communication techniques that rely principally on combination of printed words and pictures. They include newspaper, farm magazines, journals, bulletins etc. The words which are spoken are soon forgotten but printed material remains for a time being. Agriculture and rural development on a Newspaper serves as a good medium of communication in times of crisis and urgent situation. Most of the Indian languages daily newspaper devotes a page or a part of it in agriculture and animal husbandry section. The literate section of farmers can take advantage through this medium. In a same way farm publications are used by all types of extension functionaries, input dealers, bank personnel and media personnel. The educated masses can greatly be benefitted through printed media. The list includes KRISHI KHETI,

KRISHI VIGYAN, KRISHI JAGRAN,KISAN KI AWAZ,LEISAINDIA etc. They cover articles about new scientific technology to bring the technology from research stations to the farm level.


**FOLK MEDIA**

Folk media is the communication system embedded in the culture which existed before the arrival of mass media and still exist as a vital mode of communication. The folk media consists of folk's songs, folk dance, folk painting, storytelling, folk theatre, puppet show, ballad and mime. The use of folk media was done for the purpose of entertainment, mutual participation at the social level, religious activities etc. These media tends to be personal, familiar and more credible source of communication because of their familiar formats and contents also the colloquial dialogue contribute towards rapport building and clarity in communication. Off lately it has turned to be an educational device through which people communicate knowledge and help in conveying real message of technology, crop cultivation, home making etc where it can be enjoyed by large group of people even without high educational standards.

According to Sashidhan and Sharma (2006), Information communication technology.(ICT) tools have the potential to change the economy of livestock, agriculture and artisan in India. In addition the mass media can possibly aid in rural development. It can provide attention on different development programmes, mobilize people and give them opportunity to express their reactions. They can inform people about needs and problems, innovations and results. Consumption of mass media has to be regarded as one of the indices of development. As of now various government schemes are currently being run in our country known as Digital India programmes. These programmes were launched in 2014 to change the face of India digitally and electronically through which the government had new initiatives to make more services available to the masses. The government is also working on the concept of digital villages where the rural areas would be given telemedicine's facilities, virtual classes' etc. It also motivates people in a community to adopt a new practice by showing its result. This aids in building up the confidence of farmers and motivate them to adopt the practices under field condition.

In respect with the knowledge gained through information media regarding animal husbandry practices ,it has been found that livestock farmers shows

increase in knowledge regarding feeding, breeding, disease control and managemental practices. The livestock farmers are also medium adopters with regard to these practices. The media has a huge impact on the society in shaping the public opinion of the masses. Social impact includes impact on family system, change in food habit, education, health and sanitation, leadership etc. It is due to the awareness created regularly by mass media programmes that importance is being given to family planning, there is increase in awareness of marriage alliances from distant places , precautionary measures are being taken to keep the children disease free etc. The programmes being broadcasted in Krishi Darshan and farm magazine provides information on timely announcement of packages of practices in agriculture and timely veterinary information which has resulted in increase in farmer's income etc.

The present age has been rightly termed as an "information age". People want adequate and authentic information as early as possible. The mass media, namely, newspaper, radio and television try to satisfy this important need of the people craving for information and in today's age mass media serves to be the most exciting means of communication ever device by man.

## REFERENCES

Arya, S., Singh, D .K. and Singh, S. (2012).Extent of Knowledge of Change Agents and Correlation Analysis of Variables with their Communication Skill. *Indian Research Journal of Extension Education.* Vol.12 (2):21-24

Chapke, R.R. and Bhagat, R. (2005). "Traditional folk media in rural Maharashtra". *Indian Journal of Extension Education.* Vol. 41, No.1&2: 43-47

Eshetu, S. (2008). "Utilization pattern of methods and media by extension personnel in Dire Dawa Administrative Council, Ethiopia". *Rajasthan Journal of Extension Education*. Vol. (16): 1-8.

Kubkomawa, H.I. and Salihu, J.M. (2010). Role of Information and Communication Technology in the Nigerian Livestock Industry. *International Journal of Sustainable Agriculture*. Vol. 2(3): 51-54

Meenambigai, M. and Seetharaman, R.N. (2004). "Cable Television Viewing Behaviour Farmers and Farm Women". *Indian Journal of Extension Education*. Vol. XXXX (1 & 2) pp. 49-51.

# Sweet Sorghum: An alternative energy crop for biofuel, food and high biomass

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India is one of the fastest growing economies in the world and energy security is critical for its socio-economic development. India's energy security would remain vulnerable until alternative fuels to substitute/supplement petro-based fuels are developed based on indigenously produced renewable feedstocks. Biofuels also called agrofuels,are environment friendly fuels and their utilization would address global concerns about containment of carbon emissions. The Government of India (GOI) approved the National Policy on Biofuels on December 24, 2009. The policy encourages use of renewable energy resources as alternate fuel to supplement transport fuels and had proposed an indicative target to replace 20 % of petroleum fuel consumption with biofuels (bioethanol and biodiesel) by end of 12th Five-Year Plan (2017). It is estimated that by end of 2017, India would require more than 6.3 billion litres of ethanol to meet its ambitious target of 20 % EBP (Ethanol Blending Program).

Crop plants are one of the best sources of renewable energy which can be used as feedstock for biofuel production. The first generation biofuel crops viz., corn and sugarcane are presently used for fuel production. However, as these crops are majorly grown for food, their use for fuel production will not meet the current energy demands. Hence, the second generation biofuel resources (biomass crop plants like sweet sorghum, other herbaceous grasses and fast growing forest trees) are on their way to meet the energy requirements.

Sweet sorghum [*Sorghum bicolor* (L.)Moench], a $C_4$Graminaceous crop which has sugar-rich stalksis being widelyconsidered to be suitable biofuel feedstock that addresses food-versus-fuel issue favourably to a tropicalcountry like India as sugarcane is grown primarily forsugar while corn is used in food and poultry industry(Zhang *et al.*, 2010). It is

the only crop that provides grain and stem that can be used for sugar, alcohol, syrup, jaggery, fodder, fuel, bedding, roofing and fencing. It has been reported that pulps of sweet sorghum lines can be used for the manufacture of fine quality writing and printing paper as well as corrugated and solid particleboard. Sweet sorghum, similar to grain sorghum except for its juice-rich sweet stalk, is considered to be a potential bioethanol feedstock. It is a multi-purpose crop grown for food, fuel, fodder & fiber (FFFFs).

Some sweet sorghum lines attain juice yields of 78 % of total plant biomass, containing 15–23 % soluble fermentable sugar (Srinivasarao*et al*., 2009). It is an "opportunity crop" for resource-poor farmers. The grain contains high levels of iron and zinc (more than 70 ppm and 50 ppm, respectively), offering the potential for reducing micronutrient malnutrition. The sweet sorghum crop can be established from seed (as compared to setts in case of sugarcane) and is more water use efficient than sugarcane.

**Composition of sweet sorghum-**

| Parameter | Quantity |
|---|---|
| Fermentable sugars | 13% |
| Fiber | 15% |
| Water | 68% |
| Dissolved non-sugars | 3.5% |
| Ash | 0.5% |
| pH | 6.8% |

**Sweet sorghum is currently being looked for the productionof bioethanol because-**

1. It contains large amounts of sugars in its stalk that are directly fermentable,
2. It not only tolerates drought but also grows in colder regions of the temperate zones and hence has wider growing areas.
3. The whole plant (grain, stalk juice and lignocellulosic biomass) can be used for fuel ethanol production.
4. The bagasse from sweet sorghum has a higher biological value than from sugarcane when used as a forage for animals. The bagasse can be dried and burned to fuel ethanol distillation. These residues can also be used for animal feed, paper or fuel pellets.
5. The simplicity of ethanol production from sweet sorghum could lend itself to on-farm or small-cooperative efforts at fuel-making as it doesn't require the long fermentation and cooking time needed to process corn ethanol.

Therefore, it is suggested to plant sweet sorghum for biofuel production in hot and dry countries to solve problems such as increasing the octane of gasoline and to reduce greenhouse gases and gasoline imports (Almodares and Hadi 2009).

The ICRISAT has made the first attempt in India to evaluate and identify useful high biomass producing sweet sorghum germplasm from world collections. The first sweet sorghum hybrid released in India is CSH 22SS.

All India Coordinated Sorghum Improvement Project by the National Research Centre for Sorghum at Hyderabad has released the first sweet sorghum variety, SSV 84 in 1992.

**Candidate traits of sweet sorghum as biofuel feedstock-**

| As crop | As ethanol source | As bagasse | As raw material for industrial products |
|---|---|---|---|
| Short duration (3–4 months) | Amenable to eco-friendly processing | High biological value | Cost-effective source of pulp for paper making |
| $C_4$dryland crop | Less sulphur in ethanol | Rich in micronutrients | Dry ice, acetic acid, fuel oil and methane can be produced from the co-products of fermentation |
| Good tolerance of biotic and abiotic constraints | High octane rating | Used as feed, for power co-generation or biocompost | Butanol, lactic acid, acetic acid and beverages can be manufactured |
| Meets fodder and food needs | Automobile friendly (up to 25 % of ethanol-petrol mixture without engine modification) | Good for silage making | |

Source- Reddy *et al*., 2010 and Srinivasarao*et al*., 2009

SSV 84 has average yielding ability of 40.4 t/ha green cane, 1.38 t grain, and 12-13% sucrose. It is able to produce 15% fermentables at 50-60% recovery and can yield up to 2000 l/ha ethanol.

The introduction of thebrown midrib (bmr) trait in sweet sorghums would result ina dual-purpose bioenergy crop that supplies fermentablesugars from the stem juice, and from the enzymatic saccharificationof the bagasse that remains after the juice. Rusni Distillery was the first sweet sorghum distillery established in the year 2007 near Sangareddy, Medak district of Andhra Pradesh, India. It generated 99.4 % of fuel ethanol with a total capacity of 40 kilo liters per day (KLPD). It is the world's first sweet sorghum- based ethanol production distillery.

**Some sweet sorghum varieties are:-**

SPV 422, NTJ2, SPV 1411, ICSR 93034, ICSV 93046 and ICSV 700.

**Conversion of Sweet Sorghum Bagasse(Lignocellulosic Feedstock) to Ethanol-**

Lignocellulosic biomass consists of three components:lignin, cellulose and hemicellulose. Cellulose and hemicelluloses arethe sources for bioethanol production in the lignocellulosicbiomass. Chemical, physico-chemical and biological methods ofpretreatment which remove lignin/hemicelluloses, decreasethe crystallinity of cellulose

and increase surface area ofthe material for easy access to the enzymes in the subsequentsteps were developed. Several methods of pre-treatment are reported and alsostudied for pre-treatment of sweet sorghum feed stock whichinclude the use of very high gravity technology, steampre-treatment, ammonia fiber expansion (AFEX)- pre-treatment, and $H_2SO_3$-steam-pretreatment, *etc*.

**Enzymatic Hydrolysis-**Cellulases are a mixture of three different enzymes, endoglucanase(1,4-b-D-glucan glucanohydrolase), exoglucanase(1,4-b-D-glucan cellobiohydrolase), and cellobiase(b-glucosidase), that act synergistically to hydrolyze cellulose.Endoglucanase randomly attacks b-(1–4) glycosidicbonds of cellulose to create free chain-ends, exoglucanasereleases cellobiose from the free chain-ends, and b-glucosidasedegrades cellobiose into glucose. Very limited work was reported in sweet sorghumthrough this process.

**Fermentation**

Fermentation of feedstocks to bioethanol is carried out bymicroorganisms, both prokaryotes (bacteria) and eukaryoticyeasts and fungi. The most commonly used organismfor ethanol production is the yeast *Saccharomyces cerevisiae*which is known to generate high ethanol yields apartfrom being more resistant to fermentation inhibitors.

There are two common categories in fermentation:-

1.Separate hydrolysis and fermentation (SHF) where, enzymatichydrolysis and fermentation are carried out separately.

2. Simultaneous saccharification and fermentation(SSF) where hydrolysis is done in presence of fermentativemicrobes.

SSF has advantages over SHF in that productinhibition is avoided as the sugars released in the hydrolysisare immediately utilized by the fermentative microbespresent.

**Bio ethanol production from sweet sorghum-**

**Sweet sorghum juice to ethanol-**

Sugars in sweet sorghum stalk include sucrose, glucose and fructose. Depending on the sugar profile in juice, sweet sorghum stalk is processed to produce jaggery (low reducing sugars), alcohol, syrup (high reducing sugars) and high fructose syrup (no relation to sugar ratio).

**Technical challenges of using sweet sorghum for biofuels are-**

- A short harvest period for highest sugar content.
- Fast sugar degradation during storage
- Preserving the juice with low cost against bacterial contamination,
- Limited availability of genotypes suited to different agro-climatic conditions with all built-in resistances for biotic and abiotic stresses
- Photoperiod sensitivity and non-availability of required quantity of feedstock suited to off-season crushing in sugar industries.
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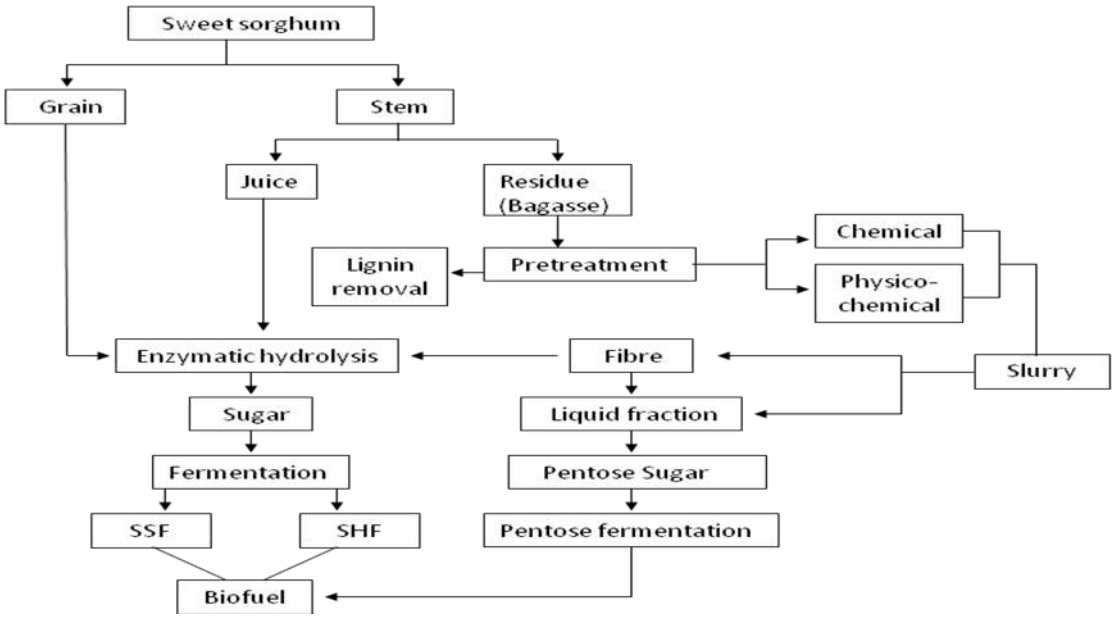


The main technological constraint is the integration of process steps i.e. simultaneous saccharificationand fermentation (SSF) and Simultaneous saccharification, fermentation and product recovery (SSFR)

**Way-Forward-(Sweet sorghum juice to ethanol)-**

1. Scaling up of sweet sorghum cultivation in liaison with biofuel distilleries and entrepreneurs.
2. Develop improved crop management techniques including pre-and post harvest and processing methods.
3. Biotechnology–developing transgenics for stem borer resistance and
4. Rapid sugar accumulation immediately after flowering.

**CONCLUSION**

Sweet sorghum is a newly introduced promising crop for the production of bioethanol. Research is on to develop promising cultivars for higher yield and juice content. So are pilot projects linking farmers to the bioethanol industry. Hence, funding support for ongoing research on sweet sorghum and its promotion are critical. Identifying institutional mechanisms through PPPs and funding support by national and international funding agencies to promote such biofuel crops will go a long way in promoting alternative feedstocks.

**REFERENCES**

Almodares, A. and Hadi,M.R. 2009. Production of bioethanol fromsweet sorghum: A review. *African Journal of AgriculturalResearch***4**(9): 772–780.

Reddy, P.S., Reddy, B.V.S. and SrinivasaRao,P. 2010. Genetic analysis of traits contributing to stalk sugar yield in sorghum. *Cereal Research Communications***39**(3): 453–464.

Srinivasarao, P., Rao, S.S.,Seetharama, N.,Umakanth, A.V.,Sanjana ReddyP., Reddy, B.V.S. and Gowda,C.L.L. 2009. Sweet sorghum for biofuel and strategies for its improvement.

Information Bulletin No. 77, *International Crops Research Institute for the Semi-Arid Tropics*, Patancheru 502324, Andhra Pradesh, India. p 80.ISBN 978-92-9066-518-2.

Zhang, C., Xie, G.,Li,S., Ge, L. and He,T. 2010. The productive potentials of sweet sorghum ethanol in China. *Applied Energy***87**: 2360–2368.

# Role Of Ethylene In Maturity And Ripening Of Horticultural Crops

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**ABSTRACT**

*Ethylene regulates many aspects of the plant life cycle, including seed germination, root initiation, flower development, fruit ripening, senescence, and responses to biotic and abiotic stresses. Ethylene is synthesized in certain fruits and vegetables at certain stages of maturity and development, when it reaches a high enough concentration, it triggers the ripening process and more ethylene is produced and the process of ripening is accelerated. Compositional changes like softening and toughening during ripening causes post harvest losses in fruits and vegetables which is undesirable. Ethylene is also used artificially to ripen, enhance colour, taste and quality in horticultural crops at commercial level. Ripening during the later stages is usually considered as the beginning of senescence.*

## INTRODUCTION

Horticulture plays a significant role in Indian Agriculture. It contributes 30% GDP from 11.73 % of its arable land area. India is the second largest producer of both fruits and vegetables in the world (88.97 Mt and 162.89 MT respectively). According to Chadha (2009) India loses about 35-45% of the harvested fruits and vegetables during handling, storage, transportation etc; ethylene production is one of the major cause of such losses making product degrade quickly and susceptible to various fungal infections. Ethylene is a colorless gas, naturally occurring organic compound which readily diffuses from tissue. It is produced from methionine. The chemical proof that plant tissues naturally produce ethylene was provided in 1934 by Gane in an experiment involving the collection of gas emitted by ripening apples. Later it was demonstrated that a strong increase in ethylene production was associated with peak in respiration during ripening. Finally, scientists considered ethylene as a plant hormone produced by plants that is active at very low concentrations. $C_2H_4$ synthesis is inhibited by $C_2H_4$ itself in vegetative and immature

reproductive tissue whereas, its synthesis is promoted (autocatalytic) by $C_2H_4$ itself in mature reproductive climacteric tissue. It is effective at ppm and ppb concentrations; expressed in brix and requires $O_2$ to be synthesized, and $O_2$ and low levels of $CO_2$ to be active (Pech *et al.*, 1992).

**ETHYLENE BIOSYNTHESIS**

The pathway of ethylene synthesis is well established in higher plants. The amino acid methionine (MET) is the starting point for synthesis. It is converted to S-adenosyl methionine (SAM) by the addition of adenine, and SAM is then converted to 1-amino-cyclopropane carboxylic acid (ACC) by the enzyme ACC synthase. In the final step, ACC is oxidized by the enzyme ACC oxidase (ACO) to form C2H4. This oxidation reaction requires the presence of oxygen, and low levels of carbon dioxide to activate ACC oxidase. The rise in ACO activity precedes ACS activity in pre-climacteric fruit in response to ethylene, indicating that ACO activity is important for controlling ethylene production. The first step in catalytic ethylene biosynthesis is the *de novo* synthesis of ACO1, the ethylene produced induces ACS gene expression, which in turn produces more ACC. ACS accumulates in roots and migrates to their working place i.e. shoots through the process of transpiration (Bradford & Yand, 1980) (Figure1). Ultimately it is clear that ACC synthase (ACS) and ACC oxidase (ACO) regulate the ethylene synthesis (Kende, 1993).

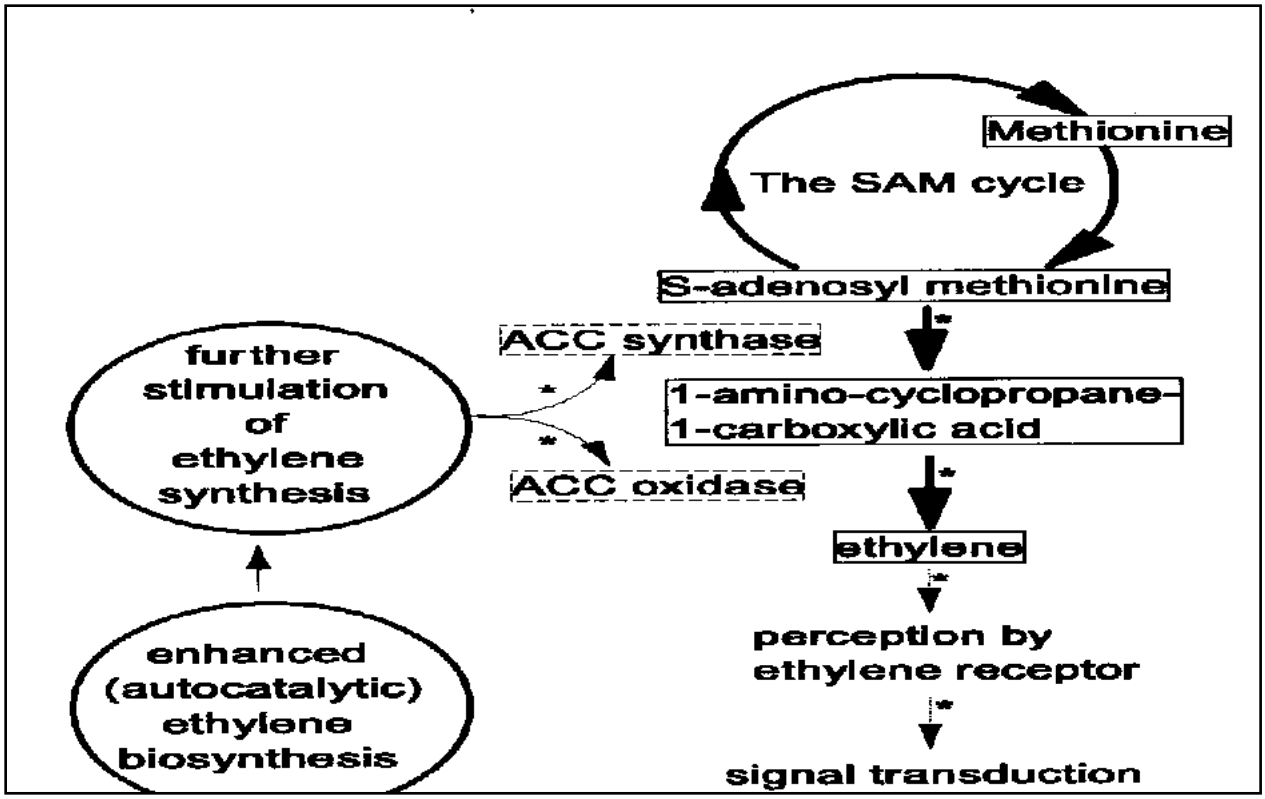


**Figure1. Mechanism of biosynthesis of ethylene (Bradford and Yand, 1980)**

**ETHYLENE AND MATURITY**

It is the stage of full development of tissue of fruit and vegetables only after which it will ripen normally. During the process of maturation the fruit receives a regular supply of food material from the plant. When mature, the abscission or corky layer which forms at the stem end stops this inflow. Afterwards, the fruit depend on its own reserves, carbohydrates are dehydrated and sugars accumulate until the sugar acid ratio form. In addition to this, typical flavour and characteristic colour also develops. The stage of maturity at the time of picking influence the storage life and quality of fruit, when picked immature like mango develop white patches or air pockets during ripening and lacking in normal brix to acid ratio or sugar acid ratio, taste and flavour on the other hand if the fruits are harvested over mature or full ripe they are easy susceptible to microbial and physiological spoilage and their storage life is considerably reduce (Table 1). Such fruits persists numerous problems during handling, storage and transportation. Therefore, it is necessary to pick the fruits or vegetables at correct stage of maturity to facilitate proper ripening, distant transportation and maximum storage life.

Vegetables are harvested at harvest maturity stage, which will allow it to be at its peak condition when it reaches the consumer, it should be at a maturity that allows the produce to develop an acceptable flavor or appearance, it should be at a size required by the market, and should have an adequate shelf life. Time taken from pollination to horticultural maturity under warm condition, skin colour, shape, size and flavour and abscission and firmness are used to assess the maturity of the produce (Table 2). However in tomato the fruit are harvested at breaker or turning stage having 10% and 25% lycopene for distant transportation (Figure 2). The different stages of maturity are as follows:

a) **Horticultural maturity:** It is a developmental stage of the fruit on the tree, which will result in a satisfactory product after harvest.
b) **Physiological maturity:** It refers to the stage in the development of the fruits and vegetables when maximum growth and maturation has occurred. It is usually associated with full ripening in the fruits. The Physiological mature stage is followed by senescence.
**c) Commercial maturity:** It is the state of fruit required by a market. It commonly bears little relation to physiological maturity and may occur at any stage during development.
d) **Harvest Maturity:** It may be defined in terms of physiological maturity and horticultural maturity, it is a stage, which will allow fruits / vegetables at its peak condition when it reaches to the consumers and develop acceptable flavour or appearance and having adequate shelf life.

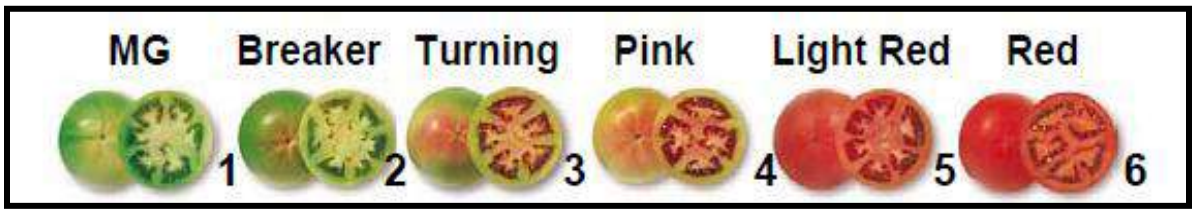


**Figure2. Different stages of maturity in tomato (MG stands for mature green).**

Many changes in the fruits take place during the development and maturation:

a) Color changes like development of carotenoids (yellow and orange color) in fruits, red colour in tomato due to lycopene; orange color in carrot due to beta-carotene etc.
b) Changes in carbohydrates include starch to sugar conversion (undesirable in potatoes; desirable in fruits), sugar to starch conversion (undesirable in peas and sweet corn; desirable in potatoes); conversion of starch and sugars to carbon dioxide and water through respiration.
c) Breakdown of pectin and other polysaccharides resulting into softening of the fruits and a consequent increase in susceptibility to mechanical injuries.
d) Increased lignin content is responsible for toughening of asparagus spears and root vegetables. Changes in organic acids, proteins, amino acids, and lipids can influence flavor and quality.
e) Loss in vitamin content, especially ascorbic acid (vitamin C) is detrimental to nutritional quality however, production of flavour volatiles associated with ripening of fruits is very important to their eating quality.
f) Sprouting of potatoes, onions, garlic and root crops greatly reduces their food value and accelerates deterioration.
g) Rooting of onions and root crops is also undesirable.
h) Asparagus spears continue to grow after harvest; elongation and curvature (if the spears are held horizontal) are accompanied by increased toughness and decreased palatability.
i) Seed germination inside fruits such as tomatoes, peppers and lemons is undesirable.

**CLIMACTERIC & NON CLIMACTERIC FRUITS**

Fruits with different ripening mechanisms can be divided into two groups. Climacteric fruits are defined as fruits that enter 'climacteric phase' after harvest *i.e*. they continue to ripen even after harvesting due to peak in respiration and a concomitant burst of ethylene. Ripe fruits are soft and delicate and generally cannot withstand rigours of transport and repeated handling. Such fruits are harvested hard and green or fully mature and are ripened near consumption areas. Small dose of ethylene is used to induce ripening process under controlled conditions of temperature and humidity eg. melons, tomatoes, apple, apricot etc.

**Table1. Criteria of maturity/ maturity indices of various fruits**

| S.no. | Fruits | Maturity indices |
|---|---|---|
| 1. | Mango | Colour, shoulder development, size, days from fruit set & specific gravity |
| 2. | Banana | Colour, drying of leaves of the plant, brittleness of floral ends, angularity of the fruit, pulp/peel ratio & starch content |
| 3. | Citrus | Colour change, sugar/acid ratio, TSS & size |
| 4. | Grapes | Peel colour, easy separation of berries, TSS (12-18) |
| 5. | Apple | Colour, size, days from full bloom & firmness |
| 6. | Papaya | Yellow patch or streaks & jellyness of seed |
| 7. | Cherries | Specific gravity |
| 8. | Pear | Starch contents, sugar/ acid ratio, size |
| 9. | Persimmon | Astringency (Tannin content) |
| 10. | Pomegranate | Sugar/acid ratio |
| 11. | Lemon | Juice content |
| 12. | Lettuce | Solidity |
| 13. | Peas | Tenderness & mean heat unit during fruit development |
| 14. | Muskmelon | Development of abscission layer & full slip stage (easily separate from vine) |
| 15. | Watermelon | By tapping (dull sound) & specific gravity |
| 16. | Cauliflower & broccoli | Compactness of curd and bud clusters |
| 17. | Asparagus | Toughness |
| 18. | Tomato | Colour change, cuticle formation & internal jelly like formation on seeds |
| 19. | Potato | Days from emergence & drying of top/leaves |
| 20. | Radish & Carrot | Root diameter, length and days to harvest |

**Table2. Time taken from flowering to horticultural maturity in vegetable crops**

| S.no. | Vegetables | Time to harvest maturity (days) |
|---|---|---|
| 1. | Ridge gourd | 5-6 |
| 2. | Pumpkin (mature) | 65-70 |
| 3. | Squash | 7-8 |
| 4. | Brinjal | 25-40 |
| 5. | Okra | 4-6 |
| 6. | Pepper (green) | 45-55 |
| 7. | Pea | 30-35 |
| 8. | Tomato (mature green) | 35-45 |
| 9. | Potato | 2-3 month from emergence |
| 10. | Cassava | 8-12 month |

Non-climacteric fruits are those in which respiration shows no dramatic change after harvesting and ethylene production remains at a very low level. Non-climacteric fruits once

harvested do not ripen further; produce very small amount of ethylene and do not respond to ethylene treatment, also, there is no characteristic increase in rate of respiration or production of carbon dioxide eg. cucumbers and strawberries. Such fruits are harvested when they attain horticultural maturity.

**ETHYLENE AND RIPENING**

Fruit ripening is a complex, genetically programmed process by which fruits attain their desirable flavor, quality, color, palatable nature and other textural properties. In tomato and other climacteric fruits such as melons the ethylene burst is required for normal fruit ripening (Ayub *et al*., 1996). Ethylene is the dominant trigger for ripening in climacteric fruit, it has been suggested that both ethylene-dependent and ethylene-independent gene regulation pathways coexist to co-ordinate the process in climacteric and non-climacteric fruit (Lelievre *et al*., 1997; Pech *et al*., 2008). Thus there are some physiological processes during ripening which have been defined to be dependent on ethylene whereas others are either ethylene independent or extremely sensitive to low levels of ethylene. System 1 is functional during normal vegetative growth, here ethylene is auto-inhibitory and is responsible for producing basal ethylene levels that are detected in all tissues including those of non-climacteric fruit. System 2 operates during the ripening of climacteric fruit and senescence of some petals when ethylene production is autocatalytic (Lin *et al*., 2009). Ethylene is also synthesized as a result of stress in the environment, flooding, drought etc. which leads to senescence i.e. fruit and leaf fall. As a result, plant respire less and there is less loss of water and energy. During drought condition ethylene synthesis is auto cut and in flood condition more ethylene synthesis occur which results in fruit ripening (Taiz & Zeiger, 2006).

**ETHYLENE PROS AND CONS**

Some beneficial and detrimental effects of artificially applied ethylene in fruits and vegetables have been listed below:

a) It enhances taste and flavor by stimulating fruit ripening.
b) For enhancing the appearance of many fruits by stimulating their ripening and color development (e.g. apricots, avocados, melons, pears and tomatoes).
c) For degreening in citrus fruits; it accelerates chlorophyll degradation and the appearance of yellow or orange colors in citrus.
d) For stimulating chlorophyll loss and appearance of yellow color promoting ripening of the pulp in bananas.
e) For blanching or whitening the celery by enhancing chlorophyll loss.
f) Removal of $C_2H_4$ or inhibition of its action can delay color changes in storage and prolong the storage life of selected commodities.
g) $C_2H_4$ exposure detrimentally affects the crisp texture by promoting unwanted softening in cucumbers and peppers, or toughening in asparagus, and sweet potatoes.

h) Exposure to ethylene causes russet spotting in lettuce; secretion of isocaumarin (bitter principle) in carrot and sprouting in onion in stores (Figure 3).
i) In asparagus, $C_2H_4$ exposure stimulates phenyl-propanoid metabolism resulting into accumulation of phenolic compounds and lignification of the tissue.
j) Sweet potatoes exposed to $C_2H_4$ during curing or storage develops hardcore, a condition in which the flesh becomes hard and inedible when cooked.

Figure3. Undesirable effects of ethylene a) Sprouting of onions;b) Lignification in asparagus; c) bitterness in carrots.

**Commercial use of ethylene:** For commercial use ethylene is available as:
a) Cylinders of ethylene or banana gas ($C_2H_4$ in $CO_2$)
b) Ethylene-releasing chemicals : Ethephon (2-chloro ethano phosphonic acid) or ethrel.
c) 2, 4–Chlorophenythio triethyl amine hydrochloride (CEPTA) is also ethylene releasing.
d) 'Ethephon' (2-chloroethyl-phosphonic acid) (also called 'Ethrel').
e) "Smart Fresh" (1- methylcyclopropene): It is acceptable to mix commodities that had been treated with the ethylene action-inhibitor 1-MCP, with untreated commodities since it does not migrate from treated to untreated produce (Blankenship & Dole, 2003).

Recommended conditions are: a) 20-21$^0$ C temperature; b) 90- 95% RH; c) 100-150ppm $C_2H_4$; d) Ventilation: one air change per 6 hours; e) Air circulation.

**CONTROL MEASURES FOR POST HARVEST LOSS BY ETHYLENE**

The various control measures to reduce post harvest losses are as:
1) **Cold Storage:** These structures are extensively used to store fruits and vegetables for a long period and employ the principle of maintaining a low temperature, which reduces the rate of respiration and thus delays ripening.
2) **Waxing:** It is used as protective coating for fruits and vegetables and help in reduction in loss in moisture and rate of respiration and ultimately results in prolonged storage life. Paraffin wax, Carnauba wax, Bee wax, Shellac, Wood resins and Polyethylene waxes are used commercially.

3) **Modified atmosphere packaging (MAP):** These packaging modify the atmospheric composition inside the package by respiration. This technology is successful to extend the shelf life of banana, carrots, capsicum, green chilies and tomatoes by 15, 14, 13, 8 and 15 days.
4) **Controlled Atmosphere (CA) storage:** It is based, on the principle of maintaining an artificial atmosphere in storage room, which has higher concentration of $CO_2$ and lower concentration of $0_2$ than normal atmosphere. This reduces the rate of respiration and thus delays aging.
5) **Irradiation:** It is the newer technologies to reduce postharvest losses and extend storage life of fruits and vegetable. When fruits and vegetables expose to ionizing radiation (such as gamma rays) at optimum dosage delays ripening minimizes insect infestation, retards microbial spoilages, control sprouting, and rotting of onion, garlic and potato during storage. It is also used as a disinfection treatment and controls fruit fly on citrus, mango seed weevil and papaya fruit fly (Thompson, 1996).
6) **Waxing:** It is used as protective coating for fruits and vegetables and help in reduction in loss in moisture and rate of respiration and ultimately results in prolonged storage life.
7) **Fungicide application:** Fumigation of stored materials with sulphur dioxide ($So_2$) can prevent the spread of fungi like botrytis. Other fumigants like ethylene oxide, propylene oxide, bi-phenyl, acetaldehyde, nitrogen trichloride can also be used.
8) **Transgenic technology:** Transgenic tomato varieties "Flavr Savr" expressing an antisense polygalacturonase gene showed a reduction in PG transcripts as well in enzymatic activity during ripening and it was seen that with antisense PG the degradation of cellular wall pectins was inhibited but other aspects of maturation, such as ethylene production and lycopene accumulation were not affected. Some of the pleiotropic tomato mutants that have aided the elucidation of ripening control mechanisms include: *ripening-inhibitor* (*rin*), *Never-ripe (Nr), non-ripening* (*nor*), *Green-ripe* and *Colorless non-ripening* (*Cnr*). It has been shown that ethylene affects the transcription and translation of many ripening-related genes (Giovannoni, 2007).

## CONCLUSION

Elucidating the mechanisms involved in the ripening of climacteric fruit and the role that ethylene plays in this process have been central to fruit production and the improvement of fruit quality. Ethylene, in association with other hormones and developmental factors plays a major role in fruit ripening, a complex developmental process. Fruit ripening processes is important in developing new strategies on fruit and vegetable conservation and in obtaining biotechnological products of high aggregated value by genetically engineering commercial plant varieties.

## REFERENCES

Ayub, R., Guis, M., Ben Amor, M. and Pech, J.C. (1996). Expression of ACC oxidase antisense gene inhibits ripening of Cantaloupe melon fruits. *Nat. Biotechnol*., 14: 862-866.

Blankenship, S.M. and Dole, J. M. (2003). 1-Methylcyclopropene: a review. *Postharvest Biology and Technol.,* 28: 1-25.

Bradford, K.J. and Yang, S.F. (1980). Xylem transport of ACC, an ethylene precursor, in water-logged tomato plant. *Plant Physiol*., 65: 322-326.

Chadha, K.L. (2009). Handbook of Horticulture. IARI Publications, New Delhi.

Giovannoni, J.J.(2007).Genetic regulation of fruit development and ripening. *Plant cell*, 16: 170.

Kende, H. (1993). Ethylene biosynthesis. *Plant Physiol. Plant Mol. Biol*., 44: 283-307.

Lelievre, J.M., Latche, A., Jones, B., Bouzayen, M. and Pech, J.C. (1998). Ethylene and fruit ripening. *Physiol. Plant.*, 101: 727–739.

Lin, Z., Zhong, L. and Grierson, D. (2009). Recent advances in ethylene. *J. Exp. Botany,* 60(12): 3311–3336.

Pech, J.C., Bouzayen, M., Latche, A. (2008). Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Sci.,* 175, 114–120.

Pech, J.C., Latché, A. and Balagué, C. (1992). Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, Netherlands, 385 pp.

Taiz, L. and Zeiger, E. (2006). Plant Physiology. Fourth edition. Sinauer Associates, Inc.

Thompson, A.K. (1996). Post harvest technology of fruits and vegetables. Blackwell Science Ltd., London.

# Importance of Selenium in Bovine-

## An overview

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Selenium (Se) is a chemical element and trace mineral, discovered in 1817 by Swedish chemist Jons Jacob Berzelius. It behaves antagonistically with sulphur in humans and animals inhibiting the uptake and function of it. Se is a bioaccumulator which means that plant and animals retain the element in greater concentration than are present in environment. The indicator plants include certain species of Astragalus, Prince's plume and some woody asters. The indicator plants may accumulate Se up to 3000 parts per million (ppm). The liver and glandular tissues have the greatest Se concentrations among all the body tissues (NRC, 2001). Arid environment with alkaline soils in times of drought or where less irrigation water is predispose to high Se levels and a greater uptake of Se by plants. Trace amounts of Se is essential for cellular functions in all animals. It is required for growth to aid resistance to diseases and involved in the production of antibodies for the killing of microorganisms engulfed by macrophages. The nutrient requirement for Se for dairy cattle has been set at 0.3 mg/kg, while requirement for beef cattle if 0.2 mg/kg. Animal health is affected either by deficiency or excess in the diet.

**Importance of Selenium**

1. Se forms a vital component of the biologically important enzyme *glutathione peroxidase* which prevents cellular damage from free radicals produced during oxygen metabolism in body.
2. It is an important component of amino acid like selenocysteine, methylselenocystine and selenomethionine.
3. Some selenoproteins regulate thyroid function and play important role in immune system.
4. Se is necessary for growth and fertility in animals.

**Causes of Selenium deficiency**

1. Pastures short in natural Se levels.
2. Low level of Se in the soil.
3. Seasonal variation in Se nutrition of grazing livestock.
4. High level of sulphur and lead, as well as higher or lower levels of calcium in the diet negatively impact Se absorption in ruminants (NRC, 2005).

**Signs/ Symptoms of Se deficiency**

1. Nutritive muscle disease (NMD) or White muscle disease (WMD) is a sure signs of Se deficiency in calves with lesions occurs in skeletal and/or heart muscle. Commonly affected skeletal muscles are the upper fore and hind limbs.
2. Animal walks with a stiff- legged gait or unable to stand.
3. In older cattle Se deficiency is often linked to other diseases like cystic ovaries, decreased calf weaning weights, immune suppression, retained fetal membranes, anestrous, early and late embryonic death, mastitis etc.
4. Deficiency of either Vitamin E or Se has been associated with increased incidence and severity of intramammary infections.

**Treatment**

Administration of organic Se such as Selenomethionine and Selenite in the form of injections, dietary supplements, salt licks and drenches proved useful. With normal Vitamin E status concentration of Se in feedstuffs needed is 0.03-0.05 mg/kg for all ruminants. However, International standards for Se requirement for cattle are in a range of 0.1 to 0.18 mg/kg dry matter.

**Prevention**

Organic sources of Se, which include Se-enriched yeasts is a more effective way to increase Se status and the activity of the *glutathione peroxidase* enzyme in livestock, compared to feeding inorganic selenium sources (NRC, 2001). By increasing the supply of Se- Supplementation can be done via injection, dietary supplementation and pasture top dressing.

- ❖ Injection- can be given 20 days before calving for the prevention of retained fetal membranes
- ❖ Dietary supplementation – Dietary requirement in cattle is 0.1 mg/kg of Se, with the inclusion of selenium in feeds or salts or mineral mixes.
- ❖ Pasture topdressing- Application of sodium selenate to pasture @ 10 gm/ha, can be used as an economic alternative to individual dosing

**Selenium toxicity**

Toxic effect of Se was first discovered in 1930. Selenium had a very narrow margin of safety between the toxic and deficient doses. Animals having blood Se level >1.5µg/ml is impending Se toxicosis (Deore *et al.,* 2002).

1. Potentially toxic dietary concentrations in ruminant feeds are considered to be 2 to 5ppm.
2. Acute Se toxicity results when a grazing animal consumes a sufficient amount of selenium accumulating weeds (*Astragalus* sp.). Death from acute Se toxicity usually occurs due to respiratory failure. Signs of acute Se toxicity include: Uncertain gait, lowered head, elevated body temperature, diarrhoea, weak rapid pulse, laboured respiration, prostration.
3. Alkali disease results from a chronic form of selenium intoxication in ruminants, usually from the consumption of forages containing 5 to 50 ppm Se over a period of weeks or months. Signs of chronic Se toxicity include: loss of hair (loss of tail switch), malformation and sloughing of hooves,

anemia, reduced fertility, stiffness of joints, kneeling while grazing (painful feet).

**Treatment and prevention of Se toxicity**

1. Administration of reduced glutathione peroxidase (GSH) I/V @ 5mg/kg of the body weight reportedly arrested the toxic signs.
2. A daily dose of 30 gram of pentasulfates (1 kg $MgSO_4$ + 166 gram $FeSO_4$ + 24 gram $CuSO_4$ + 75 gram $ZnSO_4$ and 15 gram $CoSO_4$) per adult animal could be given until recovery noted (Arora *et al.,* 1975).
3. Feeding high protein diets and a balanced mineral mixture that contains sulphur and copper can reportedly reduce Se toxicity (Fessler *et al.,* 2003).
4. Farmers can be trained to identify Se accumulator plants so that they move/graze their animals to safer areas.

**CONCLUSION**

In conclusion, Se is an essential mineral for cattle, however, care must be taken to ensure the supplementation. The range between dietary toxicity and deficiency is narrow, therefore understanding Se concentrations in your feeds and forages is important when considering supplementation.

# Malignant Catarrhal Fever

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Malignant catarrhal fever (MCF) is also known as malignant head catarrh, bovine malignant catarrh and snotsiekte..The disease is characterized by catarrhal and mucopurulent inflammation of eyes and nostril, erosion of nasal mucosa, rapid emaciation, corneal opacity, enlargement of lymph node and nervous symptoms. MCF is seen in bovids, cervids and other ruminant species and caused by gamma-herpesvirus. Alcelaphine herpesvirus 1 (AlHV-1) and ovine herpesvirus 2 (OvHV-2) are the two most widely prevalent herpesviruses causing MCF. The AlHV-1 naturally infects wildebeest causing wildebeest associated MCF (WA-MCF) in cattle in regions of African sub-continent. The OvHV-2 is prevalent in all varieties of domestic sheep as a sub-clinical infection causing sheep associated MCF (SA-MCF) in susceptible ruminants in most regions of the world. The disease has worldwide distribution and also has been reported from india.

**Etiology**

- MCF is caused by gamma-herpesvirus of genus Macavirus having double-stranded, linear DNA genome
- OvHV-2 and AlHV-1 are the two most widely prevalent causative organisms
- OvHV-2 is enzootic worldwide in domestic sheep and causes sheep-associated MCF
- AlHV-2 mainly infect hartebeest and topi

**Susceptibility**

- Bali cattle and Père David's deer are extremely susceptible
- Most species of deer, bison and water buffalo are much more susceptible
- Bos taurus and Bos indicus cattle are relatively resistant
- AlHV-1- associated disease has been reported mainly in sub-Saharan Africa
- OvHV-2- associated disease occurs throughout the world

**Sources of virus**

- Nasal and ocular secretions mainly
- Also reported in faeces and semen (OvHV-2 DNA has been detected in semen of domestic rams)

**Transmission**

- Transmitted mainly by the respiratory route, probably in aerosols
- Shed intermittently in nasal secretions particularly by 6 to 9 month old lambs
- Close contact of susceptible host with sheep is usually required
- Some cases have been reported when sheep and cattle were separated by 70 metres, and in bison herds up to 5 km from a lamb feedlot

**Public Health importance**

There is no evidence that any of the MCF viruses can infect humans

**Incubation period**

Variable, ranging from 11–34 days or up to 9 months

**Clinical signs**

- In per acute cases either no clinical signs or depression followed by diarrhoea and dysentery may develop 12–24 hours prior to death
- High fever(106-107$^0$F)
- Inappetance
- Decreased milk yields
- Increased lachrymation and nasal exudate progressing to profuse mucopurulent discharge
- Progressive bilateral corneal opacity, starting at the periphery, is characteristic
- Skin ulceration and necrosis may be extensive or restricted to the udder and teats
- Salivation and oral hyperaemia
- Erosions of the tongue, hard palate, gums and, characteristically, the tips of the buccal papillae
- Superficial lymph nodes may be enlarged and limb joints may be swollen

Nervous signs

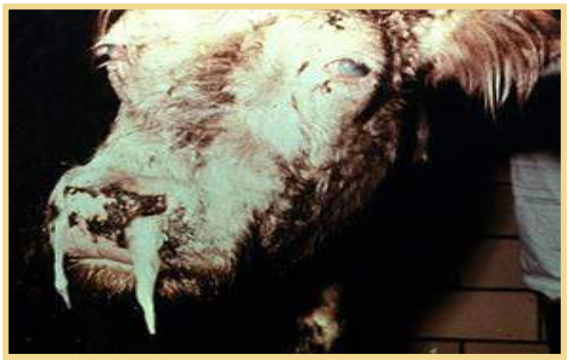
- Hyperaesthesia
- Incoordination
- Nystagmus
- Head pressing may occur alone or with signs described above
- A few infected animals may recover

**VACCINATION**

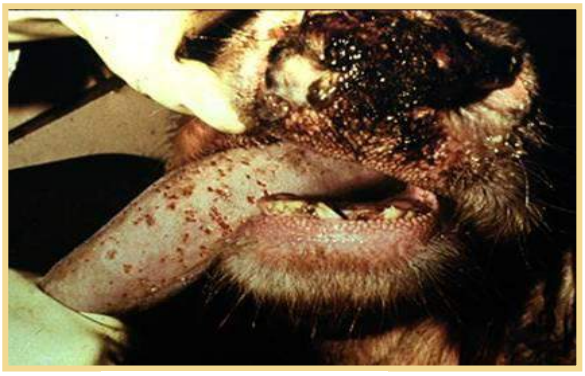
- No effective vaccine has been developed
- Developing a vaccine has been difficult because the virus will not grow in cell culture and until recently it was not known why

**Diagnosis**

- Diagnosis of MCF is based on clinical signs, gross, histologic lesions and laboratory confirmation such as viral neutralization, immunoperoxidase, immunofluorescence, ELISA and PCR



**Nasal discharge**



**Erosions on toung**

- ELISA is currently the most specific and detects antibody against all of the known MCF group viruses. Only PCR can discriminate between the different viruses.
- The test of choice for clinical diagnosis is PCR to detect viral DNA

**TREATMENT**

- No specific antiviral therapy is available
- Antibiotics to control secondary infections and supportive therapy may occasionally helpful

**PREVENTION AND CONTROL**

- Separate susceptible animals from sheep, goats, wildebeest or other suspected reservoir hosts
- Cattle should not graze pastures where wildebeest or sheep have grazed and given birth
- Wildebeest should also be segregated in zoos
- Access to contaminated fomites must be avoided, especially when the species is highly susceptible
- During outbreaks, susceptible animals should be separated immediately from the suspected source
- Because the incubation period can be very long, cases can continue to occur for months

**CONCLUSIONS**

- More research is needed for understanding pathogenesis of MCF disease
- Vaccine is yet to be developed
- Natural host and susceptible host should not be reared together
- Cattle and other susceptible hosts are thought to be dead end hosts, and do not need to be culled
- PCR is recommended method for diagnosis