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Heat Stress Management

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Production Techniques of Cole Crop:

Knolkhol (*Brassica oleracea var. gongylodes*) in the flood ravaged Lakhimpur District of Assam

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Knolkhol (*Brassica oleracea* var. gongylodes) is known by many names in India. In Assam Knolkhol is particularly known as "Hulkobi". It is mostly grown in the states like Kashmir, West Bengal, Maharashtra, Assam, Uttar Pradesh, Punjab and some parts of south India, but it is not cultivated commercially. It is characterized by the formation of knob which arises from a thickening of the stem tissue above the cotyledons. The fleshy edible portion is an enlargement of the stem, which develops entirely above ground and is used as a vegetable. It is excellent and tasty vegetable if used at its early stage before it becomes hard and tough. The edible portion is globular to a slightly flattened stem. This knob is harvested for human consumption as cooked vegetable, though in some parts, young leaves are also used. In Lakhimpur district of Assam, the annual rainfall is around 2830 mm (Average) which create flood in the whole district. After the flood i.e. in the months of September to October, Knolkhol receives a good response as a cash crop in the whole district.

Climate and Soil

Knolkhol thrives best in a relatively cool, moist climate. It grows well with a monthly average temperature of 15°–20°C, maximum and minimum average temperature being 24°C and 4.5°C. It can withstand extreme cold and frost better than other cool season crops. Knolkhol can be successfully grown on all types of soil. But the sandy loam, silt loam and clay loam soils are best suited for knolkhol crops. The optimum pH is 5.5–6.8.

Land Preparation:-

The land should be well prepared and of

good tilth for sowing of Knolkhol, 5- 6 ploughing should be given to get a good

tilth. After ploughing, planking is essential to make soil pulverized and leveled. Harmful weeds and stubbles of previous crop should be removed before planting of this crop. Malathion 5 % dust @ 3 – 4 kg per bigha should be applied with the last preparatory tillage to check the infestation of termites and cutworm. Generally, 60 cm wide and 2.5m long nursery beds are prepared. For 1 m^2 nursery 100 g of fertilizer mixture containing 15g each N, P and K and 2.5–4kg farmyard manure should be mixed well with soil and raised nursery bed must be prepared with 30cm channel along with the nursery. On light and drought sensitive soils, sunken nursery beds are preferred.

SOWING OF SEED:

a) Time of Sowing:

Generally, Knolkhol is propagated by seeds. The seeds are sown in lots from the end of August to the end of November for a continuous crop. Time of sowing seeds varies according to the variety viz.

Variety	Tine of sowing seed in Nursery	Time of planting in the main field
Early	August	September
Main	September	October
Late	October	November

b) Seed rate:

1 to 1.5 kg seed is sown in nursery to cover one hectare of land.

P**lanting:**

3 to 4 week old seedlings become ready for planting. Ridges and furrow type of layout is used. The seedling is planted at a distance of about 30 cm from row to row and 25 cm from plant to plant. Closer spacing of this vegetable increases the yield. After planting, the seedlings should be watered and protection may be given from sun rays for 4 – 5 days. The yield is more in close spacing but the size of knobs is reduced. The early varieties may be planted at closer spacing while the late ones require wider spacing.

Varieties:

Early: Earliest, white Vienna etc.

Late: Purple, Vienna, etc.

MANURING AND FERTILIZATION

Knolkhol responds well to manuring, as it is a heavy feeder. Mixing of 15 - 20 tones of FYM to soil at the time of land preparation. At the time of planting apply100 kg N, 85 kg P and 175 kg K/ ha, second dose of 50 kg N should be given 1 ½ months after

planting. Excess of N may cause abundant leafy growth and a delayed crop. Split application of N is more beneficial. Half of N along with full quantity of P and K are applied at the time of planting. The remaining half N is applied in 2 equal split doses, 3 weeks after planting and the other at the knob development stage. The farmyard manure is added to soil 4–6 weeks before planting. Optimum N and K

doses are necessary to get good flavoured knobs.

Intercultural Operation:

Intercultural operations are performed mainly to check weed growth, to make the soil loose and to maintain proper moisture condition. Since its root system is shallow, hoeing is done to keep the crop weed-free. Presence of weeds in the early stages reduces yield due to poor growth of the plants. Timely hoeing will help to check weed population. As soon as weeds start appearing, shallow hoeing should be done. Hoeing and weeding should be done after each irrigation when the soil comes in working condition. The plant should be earthen up after 25 – 30 days of planting. Practice of weedicide application is supplemented with 1 or 2 hand hoeing which is useful and economical.

Irrigation:-

Knolkhol requires a continuous supply of moisture for uniform growth and development of knobs. First irrigation is done immediately after planting and thereafter irrigation is done depending upon soil and weather conditions. Irrigations at 15 days interval are adequate. Heavy irrigation should be avoided. Irrigation should be applied when the moisture content of the soil has dropped below 50% of field capacity.

Harvesting:-

The knobs are harvested by pulling out from the land and removing leaves and roots by a sharp knife or sickle. The demand is fairly high for knobs of smaller size of about 5–7 cm diameter. In preparing the produce for market, the root portion is removed and the plants are tied in bunches along with the tender leaves. It is also marketed after removing both leaves and roots. The knobs of early varieties may have an average weight of 200–250g while those of late ones weigh up to 1kg. Generally, the yield may vary from 250-300 quintal per hectare.

Role of Avian Immunoglobulin against Microbial Infections In Chicken

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Immunoglobulins (Ig) are glycoproteins that have antibody (Ab) activity and are found in the blood, lymph and vascularized tissues of all the jawed vertebrates. Their basic unit structure consists of four polypeptide chain includes two heavy (H) and two light (L), that form the monomeric unit (H2L2). Each class of immunoglobulin can form a membrane-bound antigen receptor or a soluble secreted immunoglobulin. Immunoglobulin G (IgG) consists of the basic unit but more complex molecules, such as IgM and IgA are made up from multiples of the unit, e.g. $(H2L2)_n$. The H and L chains are formed from domains, each of about 115 amino acids, which have highly conserved cysteine and tryptophan residues and an intra-domain disulphide bridge that confers the functionally important tertiary structure. The H and L chains are joined by inter-chain disulphide bonds. Domains at the amino-terminal are highly variable (V) and the VHand VLdomain pairings create the antigen (Ag)-binding site which confers the Ab specificity. The Ag-binding cleft accommodates an epitope (antigenic site) of 6–9 amino acids or carbohydrates. Since pairs of H and L chains joined by disulphide bonds form the basic "monomeric" Ig unit (H2L2), it therefore possesses two Ag-binding sites. Very little genetic variability is found in the other domains and these are referred to as the constant region domains (CHor CL).

Types of Avian immunoglobulin

So far, there are three classes of avian immunoglobulin (Ig) have been identified as the homologues of mammalian IgM, IgA and IgG. Although the chicken and duck Ig have been described well in detail, the turkey, pigeon, quail and ostrich Ig described only partially. Avian IgM Chicken IgM is structurally and functionally homologous to its mammalian counterparts. It is the predominant membrane receptor on B cell and it is to be first expressed Ig among all. In chicken mostly the IgM present as tetrameric

$(\mu_2L_2)_4$ rather than a pentameric $(\mu_2L_2)_5$. However, small amount of monomeric Ig also observed in chicken serum. This IgM is predominant isotype Ig produced after initial exposure to any antigen. Avian IgY (IgG). IgY has low molecular weight, high serum concentration and homologous with both mammalian IgG and IgE. The IgY Ig is expressed next to IgM and predominant in chronic as well as secondary infections. Although IgG and IgY are interchangeably used, this low MW Ig has different biochemical properties as compare to other isotypes, so it is mainly used as IgY. Avian IgY has five constant domains (V, C1-C4), thereby it has long H chain.

Avian IgA

The predominant form of antibody in bodily secretions is IgA. In mammals, IgA is a dimer but the avian system has trimer of tetramer form of IgA in secretions. This secretory IgA found in the bile of ducks and geese was shown to resemble IgM both in physically and antigenically. The secretory Ig binds with poly Ig receptor on tissue of epithelial cells and it further protects the IgA from enzymatic degradation within the cells. After secretion, the part of poly receptor bound with IgA called secretary compound. It is protecting birds from invading pathogen at the mucosal surface.

Lack of avian IgD and IgE. Avian immune system lacks the homologue of IgD and IgE with mammalian system. However, avian IgY sensitize the tissues as mammalian IgE.

Half life of Immunoglobulin

Table 1: Duration of Half life of different type of birds

Sl. No.	Type of Birds	Duration of half life
1.	14 week old male	2 days
2.	Adult female	1.55 days
3.	Newly hatched chicken	3 days
4.	Laying hens	1.45 days

Natural antibody (NA)

NA is a type of antigen binding antibody, which is elicited in non-immunized animals. It is mainly produced by CD5+ B cell population predominantly located in the peritoneal cavity and along the intestinal tract of mammalian system. Generally NA have broad specificity and function along with complement system for elimination of pathogen, however it has low binding affinity. NA considered being a part of innate immune system. In chicken, NA could play an important role in both the initiation and the regulation of specific humoral immune response. Moreover, the chicken NA increases in the levels with age, perhaps due to environmental sensitization.

Maternal antibodies

The immune system of newly hatched chick is partially matured; it does not able mount an immune response against pathogen immediately after hatching. Although, innate immune system is fully functional, to achieve optimal adaptive immune response it takes first few weeks after hatching. The emigration of B

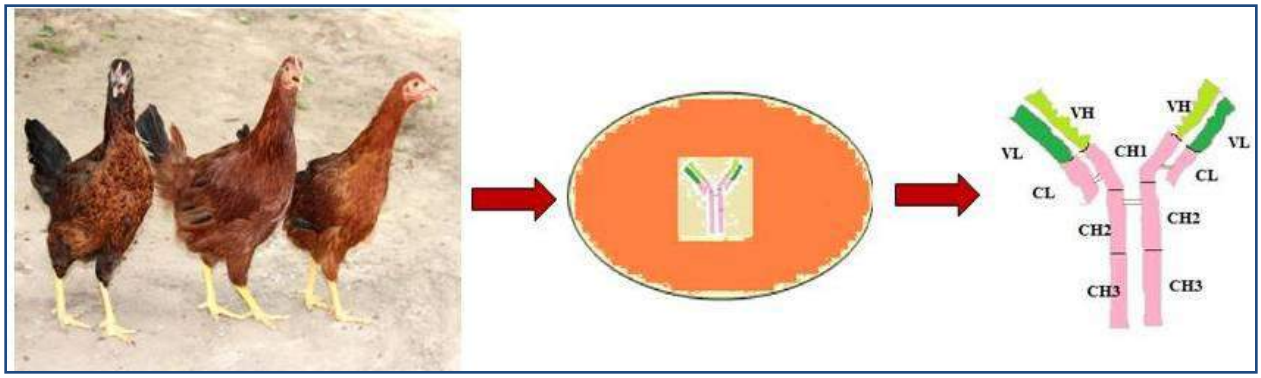

Fig.1. *Represents the transfer of maternally derived antibody through egg yolk*

lymphocytes from bursa starts only about 3 days before hatching, whereas, the emigration of T lymphocytes from thymus first starts around embryonic incubation day (EID) 6, second and third phase of emigration takes place around EID 12 and around the time of hatching.

In the First phase, IgY is selectively secreted from the circulation of the hen into the egg yolk, the amount of IgY transferred across the follicular epithelium into the yolk is proportional to the IgY concentration in serum. **In the Second phase,** the maternal antibody transfer requires absorption of IgY across the yolk sac membrane into the embryonic circulation. This transport process begins at a slow rate around EID 7 and increases steeply during the 3 days before hatching to reach a capacity of 600 µg/day. However, the total of IgY absorbed by the embryo represent only 10% of that deposited into the egg yolk. Though IgY is only found in the yolk not in the albumin, the IgM and IgA is found in albumin. It is directly transferred to gut of chick during imbibing of amniotic fluid by embryo. However, the birds like pigeons, penguins and greater flamingos use the additional pathway for transfer of IgA antibody via transfer of crop milk to chick; thereby it protects the chick from gut associated microorganism.

CONCLUSION

In order to achieve successful vaccination in poultry farm, knowledge about the avian immunoglobulin, Natural antibody and maternal antibody should be thoroughly studied. Most importantly, the half life of immunoglobulin and duration of maternal antibody interference against vaccination at earlier age group should also be known well. This basic knowledge will helpful for improvement of novel strategy like in-ovo vaccination during incubation for hatching.

Ebola Haemorrhagic Fever:

A Re-Emerged Life-Threatening Disease

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Ebola virus was identified in 1976 as a new strain of Marburg virus. The name Ebola virus, was derived from the Ebola River (a river that was at first thought to be in close proximity to the area in Democratic Republic of Congo, previously called Zaire), where the 1976 Zaire Ebola virus outbreak occurred. The disease (EVD) was first appeared in 1976 in 2 simultaneous outbreaks (one in Sudan and the other in Yambuku of Democratic Republic of Congo). The international committee on Taxonomy of viruses classifies the Ebola virus as species *Zaire Ebola Virus* which included the genus *Ebola virus*, family *filoviridae*, and order *mononegavirals*. Ebola hemorrhagic fever (EHF) or Ebola virus disease (EVD); or simply Ebola, is a disease of humans and other primates caused by ebolaviruses. Signs and symptoms of the disease typically start between two to three weeks after infecting the virus as a fever, sore throat, muscle pain, and headaches. Vomiting, diarrhoea is usually occurred along with decreased function of the liver and kidneys. Internal and external bleeding is a typical sign of disease. The disease has a high risk of death about 25 % to 90% of mortality with a average risk of 50%. Death is followed by very low blood pressure by bleeding and heavy fluid loss, and usually occurred six to sixteen days after symptoms appear. Because of its high mortality rate, virus is also listed as a select agent, World Health Organization Risk Group 4 Pathogen (requiring Biosafety Level 4-equivalent containment). CDC Centres Category a Bioterrorism Agent, and listed as a Biological Agent for Export Control by the Australia Group. The ebola virus is single stranded RNA virus which contains approximately 19,000 nucleotides. This genome encodes seven structural proteins like nucleoprotein (NP), polymerase cofactor (VP35), (VP40),GP, transcription activator (VP30), VP24, and RNA polymerase (L). Virus is entered in host cell by the help of two important protein named: a cholesterol transporter protein and the host-encoded Niemann–Pick C1 (NPC1).

EPIDEMIOLOGY

The bats (particularly fruit bats) are considered as the natural reservoir of

Ebola virus, virus is primarily transmitted between humans and from animals to humans through body fluids. As of 2013, whether other animals (chimpanzees, gorillas, fruit bats, monkeys, forest antelope and porcupines) are involved in its spread is not known. Plants, arthropods and birds have also been considered possible viral reservoirs. The Sub-Sahara African tropical region is main outbreak site of virus. From 1976 (when first case was reported) through 2013, the World Health Organization reported 1,716 confirmed cases. Till date, the largest outbreak is occurred in the ongoing 2014 West Africa Ebola virus outbreak, which is affecting Guinea, Sierra Leone, Liberia and Nigeria. As the latest of 29 October 2014, 13,567 suspected cases and 4,960 deaths had been reported.

The incubation period of the disease is usually 2 to 21 days. People are generally infected by direct contact with the blood or body fluids of a person who has developed symptoms of the disease. Saliva, mucus, vomit, feces, sweat, tears, breast milk, urine and semen are main Body fluids that may contain ebolaviruses. The WHO states that only people who are very sick are able to spread Ebola disease in saliva, and whole virus has not been reported to be transmitted through sweat. Entry points for the virus include the nose, mouth, eyes, open wounds, cuts and abrasions.

How the Ebola spreads?

- ✓ Ebola may be spread through large droplets from sick person.
- ✓ By Contact with objects like needles and syringes contaminated by the virus infections.
- ✓ It may be spread by Sexual relationship with the infected person.
- ✓ Breast milk of recovered women is also souse of Ebola.
- ✓ It also spread by contact with Dead bodies of infected peoples during certain Guinean burial rituals.
- ✓ Health-care workers (who doesn't follow appropriate protective clothing such as masks, gowns, gloves and eye protection; or handle contaminated clothing incorrectly) those who are infected are at greatest risk of getting infected themselves.
- ✓ Malnutrition and immune-suppression is the main factor which increase the disease spreading in African country
- ✓ Hospital-acquired transmission has also occurred
- ✓ Air has not been reported to occur during EVD outbreaks.
- ✓ Spread of virus by water or food, other than bush meat, has also not been observed.
- ✓ No spread by mosquitos or other insects has been reported.

SYMPTOMS OF EBOLA VIRUS DISEASE

The main symptoms of disease are usually fever, pain in the muscles and joints, headache, and sore throat and feeling influenza-like stage. The fever is usually higher than 38.3 °C (100.9 °F). This is often followed by vomiting, diarrhea and abdominal pain. Next, chest pain and shortness in breath is observed. In about half of the cases, the skin may develop a maculo-papular rash (a flat red area covered with small bumps). In some cases, internal and external bleeding may occur. Bleeding

from mucous membranes or from sites of needle punctures has been reported in 40–50 percent of cases. All infected people have low blood clotting activity. This may result in the vomiting of blood, coughing up of blood or blood in stool. Heavy bleeding is uncommon, and if it occurs, it is usually located within the gastrointestinal tract. Recovery may begin between 7 and 14 days after the start of symptoms. Death, if it occurs, follows typically 6 to 16 days from the

Table: 1 List of previous Ebola virus disease outbreaks in chronological order.

Year	Country	Ebolavirus species	Cases	Deaths	Case fatality
2014	Western Africa	Guinea, Liberia, and Sierra Leone	872	507	58%
2013	Western Africa	Guinea	49	29	59%
2012	Democratic Republic of Congo	Bundibugyo	57	29	51%
2012	Uganda	Sudan	7	4	57%
2012	Uganda	Sudan	24	17	71%
2011	Uganda	Sudan	1	1	100%
2008	Democratic Republic of Congo	Zaire	32	14	44%
2007	Uganda	Bundibugyo	149	37	25%
2007	Democratic Republic of Congo	Zaire	264	187	71%
2005	Congo	Zaire	12	10	83%
2004	Sudan	Sudan	17	7	41%
2003	Congo	Zaire	35	29	83%
2002	Congo	Zaire	59	44	75%
2001	Gabon	Zaire	65	53	82%
2000	Uganda	Sudan	425	224	53%
1996	South Africa (ex-Gabon)	Zaire	1	1	100%
1996	Gabon	Zaire	60	45	75%
1995	Democratic Republic of Congo	Zaire	315	254	81%
1994	Cote d'Ivoire	Taï Forest	1	0	0%
1994	Gabon	Zaire	52	31	60%
1979	Sudan	Sudan	34	22	65%
1977	Democratic Republic of Congo	Zaire	1	1	100%
1976	Sudan	Sudan	284	151	53%
1976	Democratic Republic of Congo	Zaire	318	280	88%

start of symptoms and is often due to low blood pressure from fluid loss. In general, bleeding often indicates a worse outcome, and this blood loss may result in death. People are often in a coma near the end of life. Those who survive often have ongoing muscle and joint pain, liver inflammation, and decreased hearing among other difficulties.

DIAGNOSIS

The diagnosis of EVD is confirmed by isolating the virus, detecting its RNA or proteins, or detecting antibodies against the virus in a person's blood. Isolating the virus by cell culture, detecting the viral RNA by polymerase chain reaction (PCR) and detecting proteins by enzyme-linked immunosorbent assay (ELISA) are methods best used in the early stages of the disease and also for detecting the virus in human remains. Detecting antibodies against the virus is most reliable in the later stages of the disease and in those who recover. The most common and sensitive diagnostic methods are real-time PCR and ELISA. Filovirions, such as EBOV, may be identified by their unique filamentous shapes in cell cultures examined with electron microscopy, but this method cannot distinguish the various filoviruses. Differential diagnosis of Ebola Virus Disease is very difficult from other infectious diseases such as malaria, typhoid fever and meningitis. Confirmation of the disease can be done by different serological and molecular diagnostic test after symptoms are suspected for Ebola virus infection.

- Antibody-capture enzyme-linked immunosorbent assay (ELISA)
- Antigen-capture detection tests
- Serum neutralization test
- Reverse transcriptase polymerase chain reaction (RT-PCR) assay
- Electron microscopy
- Virus isolation by cell culture.

PREVENTION AND CONTROL

Centers for Disease Control and Prevention was classified Ebolavirus is a biosafety level 4 agent, as well as a Category A bioterrorism agent. It has the potential to be weaponized for use in biological warfare. Prevention of disease spreading is the main aspect of control the disease outbreaks. Following points can be helpful in prevention of disease.

- ✓ Geographical area which has high potential of disease outbreak should be surveillance and contact tracing of diseases patient.
- ✓ Prevention of wildlife-to-human transmission from contact with infected one.
- ✓ Reduce the consumption of the row meat of wild animal.
- ✓ Prevention of risk of human-to-human transmission from direct or close contact with people with Ebola symptoms.
- ✓ Safe burials of dead bodies of human as well as infected animals.
- ✓ Health-care workers should always take standard precautions when caring for patients; include basic hand hygiene, respiratory hygiene, use of personal protective equipment, safe injection practices.
- ✓ Health-care workers should always take standard precautions to prevent contact with the patient's blood and body fluids, and also wear face

protection, a clean, non-sterile long-sleeved gown, and gloves.

- ✓ Laboratory workers should be took precaution in sample collection and processing of Ebola suspected samples. Samples should be processed in suitably equipped laboratories.
- ✓ According to CDC medical and laboratory worker should be trained for biosafty and for proper suit-up and removal of personal protective equipment (PPE).
- ✓ All equipment, medical waste, patient waste and surfaces that may have come into contact with body fluids need to be disinfected.
- ✓ Ebola viruses can be eliminated with heat (heating for 30 to 60 minutes at 60 °C or boiling for 5 minutes)
- ✓ Bushmeat, an important source of protein, should be handled and prepared with appropriate protective clothing and thoroughly cooked before consumption.
- ✓ Isolation and quarantine of those who may have been exposed to a disease until they either show signs of the disease or are no longer at risk.

The control of Ebola disease outbreaks requires the voluntarily community engagement and good coordinated medical facility. The medical services include: rapid detection of cases of disease, contact tracing of those who have come into contact with infected individuals, quick access to laboratory services and diagnosis, proper care and management of those who are infected and proper disposal of the dead through cremation or burial.

TREATMENT AND VACCINES

There is as yet no proven treatment available for EVD. However, a range of potential treatments including blood products, immune therapies and drug therapies are currently being evaluated. No licensed vaccines are available yet, but 2 potential vaccines are undergoing human safety testing. Many Ebola vaccine candidates had been developed in the decade prior to 2014, but as of October 2014, none had yet been approved by the United States Food and Drug Administration (FDA) for clinical use in humans.

Inactivated Ebola virus vaccines were shown to not promote an adequate immune response to the real pathogen. Several promising vaccine candidates that integrate viral subunits have been shown to protect nonhuman primates (usually macaques) against lethal infection.

Phase I clinical trials involve the administration of the vaccine to healthy human subjects to evaluate the immune response, identify any side effects and determine the appropriate dosage. As of October, 2014, such trials had begun for the replication-deficient cAd3-EBO Z vaccine, and for the replication-competent VSV-EBOV vaccine.

MEDICATIONS

A number of antiviral medications are being studied.

- Favipiravir, approved in Japan for stockpiling against influenza pandemics, appears to be useful in a mouse model of Ebola.
- BCX4430 is a broad-spectrum small molecule antiviral drug developed

by BioCryst Pharmaceuticals and undergoing animal testing as a potential human treatment for Ebola by USAMRIID.

- Brincidofovir is a broad-spectrum antiviral drug. Its maker has been granted FDA approval to proceed with a trial to test its safety and efficacy in Ebola patients.
- Lamivudine, usually used to treat HIV/AIDS, was reported in September 2014 to have been used successfully to treat 13 out of 15 Ebola-infected patients by a doctor in Liberia, as part of a combination therapy also involving intravenous fluids and antibiotics to combat opportunistic bacterial infection of Ebola-compromised internal organs.
- JK-05 is developed by the Chinese company Sihuan Pharmaceutical along with the Chinese Academy of Military Medical Sciences. It is reportedly being fast tracked through human trials for Ebola treatment after successful tests in mice.
- Lack of available treatment options has spurred research into a number of other possible antivirals targeted against Ebola, including natural products such asscytovirin and griffithsin, as well as synthetic drugs including DZNep, FGI-103, FGI-104, FGI-106, dUY11 and LJ-001, and other newer agents.

ANTISENSE TECHNOLOGY

Other promising treatments rely on antisense technology. Both small interfering RNAs (siRNAs) and phosphorodiamidate morpholino oligomers (PMOs) targeting EBOV RNA polymerase L protein may prevent disease in nonhuman primates. TKM-Ebola is a small interfering RNA compound, currently being tested in a Phase I clinical trial in humans. Sarepta Therapeutics has completed a Phase I clinical trial with its PMO protecting up to 80-100 percent of the nonhuman primates tested.

ANTIBODIES

ZMapp is a monoclonal antibody vaccine. The limited supply of the drug has been used to treat a small number of individuals infected with the Ebola virus. Although some individuals have recovered, the outcome is not considered statistically significant. ZMapp has proved effective in a trial involving rhesus macaque monkeys. The Bill & Melinda Gates Foundation have donated $150,000 to help Amgen increase its production, and the U.S. Department of Health and Human Services has asked a number of centers to also increase production. There was no confirmation or proof that the ZMapp drug was a factor in the recovery of two American Ebola patients, however; a Spanish priest with Ebola had taken ZMapp but died afterward.

OTHER

Two selective estrogens receptor modulators usually used to treat infertility and breast cancer (clomiphene and toremifene) have been found to inhibit the progress of Ebola virus*es vitro* as well as in infected mice. Ninety percent of the mice treated with clomiphene and 50 percent of those treated with toremifene survived the tests. The study authors conclude that

given their oral availability and history of human use, these drugs would be candidates for treating Ebola virus infection in remote geographical locations, either on their own or together with other antiviral drugs. A 2014 study found that three ion channel blockers used in the treatment of heart arrhythmias, amiodarone, dronedarone and verapamil, block the entry of Ebola virus into cells *in vitro*.

BLOOD PRODUCTS

The WHO has stated that transfusion of whole blood or purified serum from Ebola survivors is the therapy with the greatest potential to be implemented immediately

VACCINE

Many Ebola vaccine candidates had been developed in the decade prior to 2014, but as of October 2014, none had yet been approved by the United States Food and Drug Administration (FDA) for clinical use in humans. Several promising vaccine candidates have been shown to protect nonhuman primates (usually macaques) against lethal infection. These include replication-deficient adenovirus vectors, replication-competent vesicular stomatitis (VSV) and human parainfluenza (HPIV-3) vectors, and virus-like particle preparations. Conventional trials to study efficacy by exposure of humans to the pathogen after immunization are obviously not feasible in this case. For such situations, the FDA has established the "animal rule" allowing licensure to be approved on the basis of animal model studies that replicate human disease, combined with evidence of safety and a potentially potent immune response (antibodies in the blood) from humans given the vaccine. Phase I clinical trials involve the administration of the vaccine to healthy human subjects to evaluate the immune response, identify any side effects and determine the appropriate dosage. In September 2014, two Phase I clinical trials began for the vaccine cAd3-EBO Z, which is based on an attenuated version of a chimpanzee adenovirus (cAd3) that has been genetically altered so that it is unable to replicate in humans. It was developed by NIAID in collaboration with Okairos, now a division of GlaxoSmithKline. For the trial designated VRC 20, 20 volunteers were recruited by the NIAID in Bethesda, Maryland, while three dose-specific groups of 20 volunteers each were recruited for trial EBL01 by University of Oxford, U.K.

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Responses of Poultry to Heat Stress

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The net energy stored in the tissues of a bird equals the difference between energy intake and energy loss. Metabolism of food and high environmental temperature are potential sources of energy while low environmental temperatures and the maintenance of normal body temperature are potential expenditures of energy. Excessive flow of energy into the body and excessive depletion of energy from the body both lead to death, although many birds can survive conditions in which the potential for energy flux is extreme by invoking various adaptive mechanisms that increase or decrease the flow of energy to or from the environment. In many parts of the world, particularly in warm tropical and subtropical regions, poultry are maintained in environmental temperatures which require the involvement of intricate molecular, physiological and behavioural changes that enable domestic birds to cope with the flux of energy into their tissues at high ambient temperatures.

Heat Stress and the Maintenance of Body Temperature

Body temperature of domestic chickens is maintained within a relatively narrow range that is usually reflected by the upper and lower limits of a circadian rhythm in deep body temperature. When exposed to a hot environment and/or performing vigorous physical activity, body temperature might rise by 1 or 2°C as heat is stored. Heat storage cannot continue for extended periods before body temperature increases past the limit that is compatible with life. Conversely, when birds are exposed to very cold environments, heat escapes from the bird and, unless it is replenished by energy from the metabolism of food, body temperature will decline until the bird is incapacitated and dies. As ambient temperature rises and falls, a wide variety of physiological, behavioural, neuroendocrine and molecular responses are initiated to maintain body temperature within the normal limits. In some instances, the responses are short-term measures that are invoked to withstand a brief period of extreme temperature. The responses can also be invoked to develop a longer-term response aimed at acclimatizing the bird to ambient temperatures that fall within the upper regions of the thermoregulatory zone. Finally, the responses can be terminal reactions that can be sustained for only brief periods; these responses are

initiated to cope with extreme and life-threatening environmental conditions.

Behavioural Responses to Heat Stress

During thermal stress, birds alter their behaviour to help maintain body temperature within the normal limits. Behavioural adjustments can occur rapidly and at less cost to the bird than most physiological adjustments. As ambient temperature increases above the comfort zone, chickens devote less time to walking and standing. During exposure to high temperature, chickens consume less feed and more water to compensate for water lost through evaporative cooling, although a reduction in drinking time was observed when heat stress was applied concurrently with other stressors (McFarlane et al., 1989). When exposed to high temperatures, domestic fowl may splash water on their combs and wattles in order to increase evaporative cooling from these surfaces. Heat-stressed birds also spend relatively less time engaging in social behaviour and in changing their posture. When maintained in cages, heat-stressed chickens tend to distance themselves from each other, pant, and often stand with their wings drooped and lifted slightly from the body to maximize sensible heat loss (Mench, 1985). From their studies on domestic fowl, Hooper and Richards (1991) concluded that the relative contribution of operant behaviour to overall temperature regulation was different both qualitatively and quantitatively under heat load and cold load temperatures. Under heat load, the preferential method of thermoregulation included an operant response, whereas under cold exposure the autonomic response (as indicated by increases in oxygen consumption and heart rate) appeared to be the major contributor to thermoregulation.

Physiological Responses to Heat Stress

The physiological responses to heat stress in birds involve the functional integration of several organs to meet the metabolic needs of birds that are trying to dissipate heat and maintain homeostasis.

Acclimatization to high ambient temperature:

Exposure of chickens to high environmental temperatures produces an initial increase in the temperature of peripheral tissues and subsequently in core body temperature. Boone (1968) observed that the body temperature of chickens began increasing when the ambient temperature rose above 30°C if the rate of increase in ambient temperature was rapid. On the other hand, if the ambient temperature rose more slowly (Boone and Hughes, 1971b), the birds maintained their normal body temperature until the ambient temperature reached 33°C. Using the length of time required for body temperature to become constant as a measure of acclimatization, it has been reported that adult chickens required 3 to 5 days to acclimatize to both hot and cold environments.

Consumption of feed and water:

As birds accumulate heat in their tissues, several responses to increase the dissipation of heat are invoked to reduce

the heat load. Water consumption increases when chickens are exposed to high ambient and survival in a hot environment is dependent upon the consumption of large volumes of water. Voluntary feed consumption is diminished in response to high environmental temperatures and fasting for 1–3 days has been shown to progressively increase survival time of chicks exposed to heat stress (McCormick et al., 1979). The increase in water consumption occurs immediately, whereas the reduction in food consumption is delayed until several hours after the birds have experienced high temperatures. The immediate increase in water consumption meets the immediate demands of evaporative cooling from respiratory surfaces, and the associated decline in food consumption reduces the contribution of metabolic heat to the total heat load that requires dispersion.

Sensible heat loss through specialized heat exchange mechanisms:

Heat is dispersed through anatomical specializations in birds that provide increased blood flow to surfaces that can effectively transfer heat by radiation and conduction. The vascular system in the legs and feet of many birds, including domestic fowl, contains arteriovenous heat-exchange mechanisms that facilitate the dispersal of heat through these uninsulated surfaces. The volume of blood that flows through the arteriovenous network, which serves as a heat exchanger, is regulated by shunts in the vascular system (Midtgard, 1989). At high ambient temperatures, these shunts bring cool venous blood in close proximity to arterial blood to dissipate the maximum amount of heat to the environment. In many birds, heat exchange can be increased through the reteopthalmicum, an arteriovenous heat exchanger situated between the optic cavity and the brain (Midtgard, 1989). This anatomical specialization can be used to dissipate heat through the cornea, the eye, the buccal cavity, the beak and the nasal passages. The extent to which domestic birds can disperse heat through this mechanism is not yet established.

Sensible heat loss and feather cover in poultry:

In some breeds, the absence of feathers in the neck is controlled by a single autosomal dominant gene designated as naked neck (Na). In addition to eliminating plumage from the neck, the naked-neck gene suppresses 30–40% of the plumage in all of the other feather tracts. Regardless of the cause of poor feather covering, sensible heat loss is substantially increased. In normal environments, food consumption is increased to offset the increase in heat dissipation and, consequently, the feed efficiency of poorly feathered birds is decreases. At ambient temperatures above 25°C, however, where the ability to dissipate heat is an asset, naked-neck (Na Na) chickens possessed superior growth rate, viability, egg weight and female reproductive performance.

Changes in respiration rate and blood pH:

Panting is one of the visible responses of poultry during exposure to heat. This specialized form of respiration dissipates heat by evaporative cooling at

the surfaces of the mouth and respiratory passageways. Panting enables hens to increase the rate of water evaporation from 5 to 18 g/h in response to a change in ambient temperature from 29 to 35°C with relative humidity of 50–60%. However, it is believed that at an ambient temperature of 32°C and relative humidity of 50–60% hens reach the maximal ability to lose heat through evaporation. Panting increases the loss of carbon dioxide from the lungs, which leads to a reduction in the partial pressure of carbon dioxide (Wang et al., 1989), and thus bicarbonate, in blood plasma. In turn, the lowered concentration of hydrogen ions causes a rise in plasma pH, a condition generally referred to as alkalosis. In laying hens, the reduction in the plasma concentrations of bicarbonate compromises eggshell formation by limiting the availability of the anion required during formation of CaCO3crystals in the shell.

Dissipation of heat by evaporative cooling demands an increase in respiration, while respiratory alkalosis demands a decrease in respiration. Gular flutter and utilization during panting of respiratory passages that are not involved in gas exchange (e.g. nasal cavities, nasopharynx, larynx and trachea) are known to reduce respiratory alkalosis in heat-stressed birds. Gular flutter involves the rapid and sometimes resonant vibration of the upper throat passages, driven by the hyoid apparatus. Respiratory alkalosis can also be combated nutritionally by providing a source of anion via feed or water. For example, Teeter and Smith (1986) have shown that supplemental ammonium chloride in drinking water of chronically heat-stressed birds can return blood pH to normal and enhance weight gain.

Changes in plasma concentrations of ions:

The normal functions of tissues are dependent upon the stability of the total osmolarity of intracellular and extracellular fluids. The major ions of the plasma are sodium, chloride, potassium, calcium, phosphate, sulfate and magnesium. The concentrations of the major ions are also important in determining the pH of the body fluids. Elevation of body temperature to 44.5–45.0°C by exposing chickens to 41°Cambient temperature has been associated with increased plasma sodium and chloride and decreased plasma potassium and phosphate (Ait-Boulahsenet al., 1989). In normally hydrated fowls, however, heat stress (35–45°C for10–12 h) produced no significant changes in the serum concentrations of sodium, potassium, chloride and calcium, or in serum osmolality, although serum phosphate declined (Arad et al., 1983). The increase in serum concentration of sodium and in serum osmolality were exacerbated and associated with a decline in serum concentrations of phosphate when dehydrated birds were subjected to 35–45°C for10–12 h.

Heart rate, cardiac output, blood pressure and total peripheral resistance:

Exposure to high ambient temperature is associated with a decline in blood pressure, an increase in cardiac output and a decrease in peripheral resistance (Darre and Harrison, 1987). As

birds become acclimatized to elevated ambient temperature, however, cardiac output decreases, blood pressure increases and peripheral resistance returns to normal. In chickens exposed to high ambient temperature, blood flow through the comb, wattles and shanks is increased due to peripheral vasodilation, and excess heat is dissipated to the surrounding air. Based upon their observation of cardiovascular response of chickens to acute mild hyperthermia, Darre and Harrison (1987) proposed that the thermoregulatory response starts with a decreased heart rate and peripheral vasodilation, which leads to decreased blood pressure, decreased peripheral resistance and compensatory increases in stroke volume and cardiac output. It is suggested that the large increase in cardiac output during heat stress demonstrates the intense demand placed upon the cardiovascular system to dissipate heat from the bird. Darre and Harrison (1987) concluded that the fine-tuning of the body temperature is accomplished primarily by cardiovascular adjustments to prevent major overshoots or under control.

Hormonal Involvement in Thermoregulation

Hormones are produced by endocrine tissues and transported through the circulatory system to their target tissues. They provide an important link in the flow of information among cells and tissues in an animal to initiate and maintain the physiological and behavioural responses to heat stress. As a bird attempts to cope with heat stress, an intricate series of changes that is mediated by many, if not all, hormonal systems is initiated. The relative importance of each of these systems and the extent to which they are called upon depend on the severity of the heat stress.

Pituitary hormones:

Arginine vasotocin (AVT): The principal neurohypophyseal hormone in birds, AVT, is an antidiuretic hormone in non-mammalian vertebrates. However, AVT is believed to play a role in heat dissipation that is independent of its role in osmoregulation in chickens (Wang et al., 1989). In non-heat-acclimatized fowl, heat stress alone could increase AVT levels, while in heat-acclimatized birds, an increase in plasma osmolality could also be necessary (Wang et al., 1989). The release of AVT in response to heat stress has also been implicated in the mobilization of free fatty acids (FFA). In a recent study with pigeons (John, T.M. and George, J.C.,1978), increases in AVT were associated with increases in plasma thyroxine (T4) level and concomitant decreases in triiodothyronine (T3) level. Basal metabolic rate is determined by plasma T3 in birds and therefore a drop in T3 level would reduce metabolic heat production to alleviate heat stress.

Mesotocin (MT): Mesotocin, the avian analogue of oxytocin, has recently been implicated in thermoregulation in domestic fowl (Wang et al., 1989). Heat stress suppressed the circulating level of MT (Wang et al., 1989), but it is not clear what role MT plays in thermoregulation. Infusion of MT has been shown to produce a dramatic increase in respiratory rate which could enhance evaporative heat loss

during heat stress. It is suggested that the thermoregulatory function of MT is carried out via either the central nervous system or peripheral mechanisms.

Growth hormone (GH): In pigeons deprived of drinking water and subjected to high ambient temperature for 3 days (28°C, 31°C and 36.5°C, respectively), the plasma levels of growth hormone (GH) increased significantly (John et al., 1975). This increase in GH levels is believed to play a role in fatty acid mobilization since GH is a major lipolytic hormone in birds. Although the sequence of events that lead to the release of GH is not clearly understood, the importance of GH in diverting metabolism to provide a high-energy substrate for muscle metabolism is believed to contribute to the support of panting related muscular activity during heat exposure.

Adrenal Hormones:

Corticosterone: Corticosterone is the principal steroid hormone of the avian adrenal cortex. Heat stress stimulates the release of corticosterone from the adrenal glands and increases plasma concentrations of corticosterone in chickens. Both neural and endocrine inputs to the central nervous system stimulate the production of corticotrophin-releasing factor (CRF) from neurons of the hypothalamus. CRF is released into the hypothalamic portal vascular system and transported to the pituitary gland, where it stimulates the production of adrenocortico-trophic hormone (ACTH). ACTH is released into the general circulatory system and is transported to its major target tissue, the adrenal cortex, increasing the production and release of all of the adreno-cortical hormones, although the major hormones are corticosterone and aldosterone. Plasma concentrations of corticosterone increase in response to heat stress, but high levels of corticosterone can only be maintained for short periods to cope with acute exposure to the high temperatures. In chronic cases it subsides to normal levels. Changes in the plasma concentrations of corticosteroids and ACTH affect the lymphoid tissues and consequently the ability of chickens to mount an immune response.

Catecholamines: The catecholamines, epinephrine (E) and norepinephrine (NE) are synthesized and released from adrenal chromaffin cells. Their secretion in response to stress is similar to the corticosterone response, since both adrenal cortical hormones and ACTH stimulate the release of both E and NE. The increase in plasma concentrations of E and NE in response to acute heat stress are probably as transitory as that of corticosterone. Catecholamines may also exert an influence on body temperature more directly than through their role as mediators of a general response to heat stress, since infusion of catecholamines into the hypothalamus of chickens lowers body temperature.

Melatonin: Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine that has been recognized as the major pineal hormone. In birds, as in many other vertebrates, melatonin is also produced in several extra pineal tissues such as the retina and the Harderian gland. Melatonin has been implicated in thermoregulation in

birds and may regulate the circadian rhythm in body temperature since, in pigeons, body temperature is relatively low in the night, when both plasma and pineal levels of the melatonin are high (John et al., 1978). Conversely, body temperature is higher in the day, when the melatonin levels are low. In the presence of high concentrations of melatonin, heat dissipation by peripheral tissues is enhanced by vasodilation and blood flow, particularly to the foot, which is an important site for heat dissipation in birds. Furthermore, melatonin may act centrally by lowering the set-point of the main 'thermostat', which is believed to be present in the hypothalamus.

Reproductive hormones:

The effect of heat stress on reproductive performance in chickens has been indicated by reduced egg production. The diminished egg production is suspected to be at least partly influenced by the ovulatory hormones. In the hen, heat stress reduces serum luteinizing hormone (LH) levels, hypothalamic content of luteinizing hormone-releasing hormone (LHRH) and the preovulatory surges of plasma LH and progesterone. Since the preovulatory surges of LH and progesterone are controlled in a positive feedback loop and since both hormone levels are depressed concomitantly, it is difficult to identify the site or sites of action of heat stress. However, the hypothalamus could be a primary target for heat stress because it receives both neural and endocrine inputs that could be translated into general inhibition of the reproductive system.

Thyroid hormones:

The importance of the thyroid gland in adaptation to heat stress is related to the central role that thyroid hormones play in the regulation of metabolic rate of birds. This effect has been demonstrated by surgical or chemical thyroidectomy of chickens, which produces a decrease in metabolic rate and body temperature and by thyroid hormone administration, which stimulates heat production. In chickens, thyroid hormone secretion is depressed as ambient temperature increases and heat tolerance improves as thyroid function is reduced. The two active forms of thyroid hormones are T4 and T3, and the inactive form is reverse triiodothyronine (r-T3). The selective peripheral conversion of T4 to T3 or r-T3 is believed to play an important role in thermoregulation in domestic fowl; when chickens are exposed to warm temperatures, T4 is inactivated by conversion into r-T3, whereas during cold exposure T4 is converted into T3, which stimulates metabolic rate.

Heat-shock Proteins and Heat Stress

A response of all organisms – animal, plant or microbe – to elevated temperature is the increased synthesis of a group of proteins known as the heat-shock proteins. Heat-shock proteins play an essential role by associating with a variety of proteins and affecting their conformation and location. In a heat-shocked cell, the heat-shock proteins may bind to heat-sensitive proteins and protect them from degradation, or may prevent damaged proteins from immediately precipitating and permanently affecting cell viability.

The most commonly found forms of heat-shock proteins have relative molecular masses of approximately 10,000–30,000, 70,000, 90,000 and100,000 –110,000, and so are referred to as HSP70, HSP90 and so on. Much of the work on the molecular biology of heat-shock-protein expression in vertebrates has been done using the chicken as a model system. Several of the chicken heat-shock-protein-encoding genes have been cloned and characterized, including bsp70 (Morimoto et al., 1986), hsp90 (Catelli et al.,1985), hsp108 (Sargan et al., 1986) and UbI and Ubll, which are ubiquitin-encoding genes (Bond and Schlesinger, 1986). It is tempting to speculate that part of the difference in heat tolerance of various breeds could be attributable to different alleles of heat-shock-protein-encoding genes, resulting in differing ability to respond rapidly to a heat stress, differing final concentrations of the relevant heat-shock proteins in stressed birds or differing ability of various heat-shock-protein iso forms to interact with their normal ligands in the cell.

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Heat Stress Management of Dairy Animals

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Stress is defined as a state of physiological or psychological imbalance resulting from the disparity between situational demand and the individual's ability or motivation to meet those demands. It is inability of an animal to cope with its environment, a phenomenon that is revealed by a failure to achieve genetic potential, e.g. for growth rate, milk yield, disease resistance, or fertility. When environmental temperatures move out of the thermoneutral zone then dairy cattle begin to experience heat stress.

Environmental (heat/cold), nutritional (feed/water deprivation), social and psychological (fear/restrain), internal (disease/toxin/pathogen), physiological (altitude/ pregnancy/ lactation), transportation/ training/ management are different types of stress in dairy animals.

HEAT STRESS

Heat stress defined as the sum of forces external to a homeothermic animal that acts to displace body temperature from the resting state. Heat stress for the dairy cow can be understood to indicate all high temperature-related forces that induce adjustments occurring from the sub-cellular to the whole animal level to help the cow avoid physiological dysfunction and for it to better fit its environment. Heat stress is the significant burden to animal in most areas of world and sub-tropical and tropical parts of our country. Heat stress is primarily by high air temperature, but intensified by high humidity, thermal radiation and low air movement.

IMPACTS OF HEAT STRESS

a) It itself increase in magnitude if continued global warming occurs.

b) World's population growing faster in tropical and subtropical regions than temperate regions and a greater proportion of the world food animals will live in hot climates.

c) Changes in genetics and physiology of food animals for increased production are making these animals less able to regulate body temperature i.e less adapted to warm environments especially dairy cattle (Saini and Chandrahas, 2013). Heat stress of the animal can be calculated using the THI. THI crosses 72 indicate the animal is under stress (Hafez, 1968).

EFFECTS OF HEAT STRESS ON DAIRY ANIMALS

a) Body temperature is elevated due to disturbed heat dissipation mechanism.

b) Increased respiration rate or Panting is first sign of heat stress (>70/min). It impairs the acid base balance as well as calcium homeostasis, this leads to increased incidence of hypocalcaemia and milk fever (Pejman and Habib., 2012).

c) Increased maintenance energy requirement – through various mechanisms animal tries to dissipate the excess heat and maintain body temperature. Due to this the maintenance energy requirement may increase by 20-30% in animals under heat stress. This decrease the energy available for production functions.

d) Dry matter intake – decreases, depending on the severity of heat stress may be reduced by 8-12% or more, because decreased blood flow to the rumen and intestines, to avoid increased heat production from feed digestion, increased water intakes that fill the stomach (Saini and Chandrahas, 2013).

e) Decreased milk production.

f) Decreased reproductive performances – elevated temperature impairs the follicle and embryo development. Redistribution of blood flow from the viscera to the periphery during heat stress, leads to reduced perfusion of placental vascular bed, retarded foetal growth (Alejandro et al., 2014).

ECONOMIC IMPACTS OF HEAT STRESS

The economic loss is direct result from heat stress reducing milk production, reproductive performance and milk quality, heifer growth, increasing cow and calf mortalities and health care costs. Urdaz et al., (2006) conducted a study on dairy cows which reared under feed line sprinklers (a) and feed line sprinklers with shade & fans (b). He found that (b) produced increased 60 d milk yield, decreased postparturient disorders and serum non-esterified fatty acid concentrations and b gained annual profits by 8.92$/cow.

WAYS TO DISSIPATE HEAT

In order for an animal to survive a given environment over a long period of time the energy gain must equal the energy lost.

a) Conduction: Heat move from a warmer to a cooler surface. It needs a direct contact with a surface. If the animal is lying on ground which is warmer than his surface temperature, then he will gain heat from the ground by conduction. If the ground is cooler than the animal, then he will lose heat.

b) Convection: The layer of air next to the skin is replaced with cooler air, where thermal currents leave the cows body. If the air temperature is warmer than is external surface temperature, the animal will gain heat by convection, but if the air temperature is cooler than his surface temperature, he will lose heat.

c) Radiation: heat can radiate from a warmer environment to a cooler environment, where the cow radiates heat, such as the cool night sky. If the sun is shining on the animal, he may be gaining more energy from radiation than he is emitting (Hafez, 1968).

d) Evaporation: sweat or moisture is evaporated from the skin (sweating) or respiratory tract (panting). If the animal is sweating, then he is losing heat through evaporative cooling. If dew forms on the animal, he may gain a small amount of energy from the latent heat of condensation. Under high environmental temperature, the sensible heat loss

become the main avenue of heat loss, accounting for approximately 85% of the total heat loss, while the rest is lost by respiratory evaporation (Maia et al., 2005).

MANAGEMENT OF HEAT STRESS

Physical modification of the every environment is based on two concepts (Pejman and Atrian et al., 2012):

i) Protecting the cows from the factors contributing to heat stress.

ii) Enhancing evaporative heat loss by the animal Heat abatement system such as shades, fans, for misters and sprinklers are use to alleviate heat stress of high producing cows during summers. Depression in wet bulb temperature (DWB) may be used as an index for turning adiabatic coolers in different parts of India during summer. Places with high DWB are more suitable for evaporative cooling and may help in reducing thermal stress of livestock by using evaporative coolers.

A) SHELTER MANAGEMENT: Lowering the environmental temperature by modifying the structure of the shed where the animal are kept or by introducing cooling facilities to facilitate heat loss from animals.

Fig 1: Custom designed animal shed

SHADES

The most effective shades are trees, as they provide protection from sunlight combined with beneficial cooling as moisture evaporates from the leaves (Da silva, 2004). The radiant environment in a shade has four constituent parts: the cold ground in the shade; the hot ground outside the shade; the lower (inner) surface of the roof; the sky. Areas with clear, sunny afternoon's shades should be 3 to 4.5 m high in order to permit maximum exposure to the relatively cool

WALLOWING TANKS

Swine, as well as water buffaloes, are naturally wallowing animals and wallows for them have been shown to improve performance (Dasilva, 2004). The primary mode of heat loss in Most notable of these are leptospirosis and mastitis caused by variety of organisms, particularly *Prothecn* species (Nagpal et al., 2005). cooling during 5-10 min after exiting the pond. But access to streams and farm ponds has been associated with a number of infectious disease and some toxicity to dairy cattle.

Fig 2: Wallowing tank

ROOF COOLING

If the roof is sprinkled, the temperature of the roof can be reduced upto 28 C by application of 1.5-1 water per hour and per square meter roof area. If a wall or a roof is wet, energy and therefore heat will be used to evaoporate the water. Therefore radiated sun energy will be reduced.

FANS

High velocity blast fans are to be mounted on sidewalls can help in reducing thermal stress on animals and body temperature. Cows reared under electric fan produced 1.2 kg/d more milk during the early lactation (Suriyasathaporn et al., 2006). The fan system in the holding pen should be capable of moving 1,000 cubic feet of air per minute per cow (cfm/cow). The total ventilation rate is based on the maximum number of cows in the holding pen. Most 30- and 36-inch fans will move between 10,000 and 12,000 cfm per fan, so install one fan per 10 cows or 150 square feet of holding pen area. It may be better to use more 30- or 36- inch fans than 48-inch fans to minimize dead air zones in the holding pen.

Fig: 3 Ceiling fan

Install fans near the eves of the sidewalls tilted downward at 15 to 30 degrees. Adjust the angle so that air movement directly in front of the fan can be felt the width of the holding pen and at the height of a cow's back (Murphy et al., 2002).

Fig4: Sidewall fan

SPRINKLERS

Sprinkler cooling is a process whereby water droplets are applied to wet the cow's hair coat to the skin, and fans are used to force dry the cow. Sprinkling does not attempt to cool air as in the case of fogging and misting but instead uses large water droplet size to wet the hair coat to skin. Cooling is accomplished as water evaporates from hair and skin. In combination with forced air, sprinkling increases the loss of body heat over that possible by sweating alone (Nagpal et al., 2005). Sprinklers cows before entering a shade reduced respiration rate by 65-81% and body temperatures by 46-50% over shade alone. Using sprinklers in combination with supplemental air flow proved to be superior to a fan alone or sprinkling. A combination of fans and misters is as effective as fans and sprinklers at maintaining intake and milk yield. However, the fan/sprinkler system used about 10-fold more water than did the fan/mist system (Urdaz et al., 2006). It is found that cooling using fans and sprinklers improved DM intake by 7 to 9%, milk yield by 8.6 to 15.8%, reduced rectal temperature by 0.8-1.0°C.

MISTERS

This system does not work well in humid environments, because the mist droplets are too large to evaporate before they reach the floor and the bed or feed becomes wet. Another complication with misters is that if the system does not wet the hair coat through to the skin, an insulating layer of air can be trapped between the water layer and the skin.

Fig:-5 Mister

However, high pressure fogger/fan systems are available which spray a fog at high pressure into the fan stream. This cools the air, does not wet the cow. The best climatic conditions during the hottest hours of the day were found in the pen with fans and misters (FM) (Frazzi et al. 2002). In mist system, the fine mist particles stay suspended in the air and evaporate before being deposited on the ground, thus cooling the surrounding air; some small droplets may be deposited on the hair coat of cattle (Nagpal et al., 2005).

FOGGERS

- Foggers disperse very fine droplets of water which quickly evaporate and cool the surrounding air, while raising the relative humidity. A ring of fog nozzles is attached to the exhaust side of the fan and then the cooled air is blown down over the animal's body. This system is expensive and requires a lot of maintenance and the water must be kept very clean or the fogging nozzles will plug. A fogger system is effective in areas with low humidity and is not recommended in humid climates. In an environment which is saturated with water the droplets cannot evaporate and a "steam bath" effect is created.
- **Desert coolers:** Most effective during dry hot conditions.
- **Air conditioners:** Though it is very effective the cost associated with this facilitates makes this system are rare today.

CONCLUSION

- *The heat stress impacts on animal performance and profitability, by*

lowering feed intake, nutrient utilization and production.

- *We can't control the weather in case of heat related stress, but we can do everything reasonably possible to reduce various kinds of the stress effects on animals.*

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Mutation Related Disease In Farm Animals

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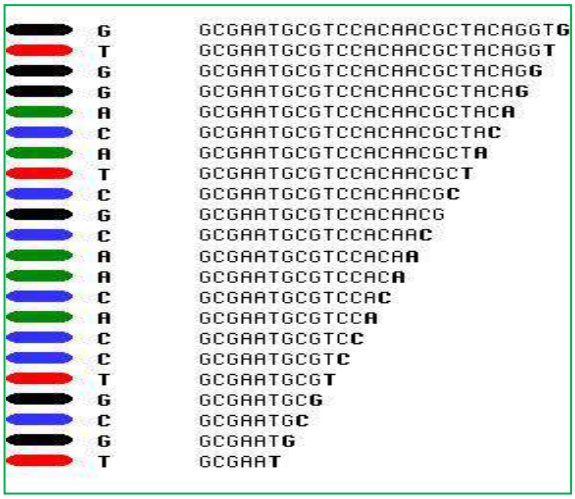
Mutation is the sudden heritable changes in the genetic material which provides new genetic polymorphism that allows organisms to evolve. Mutation can be caused by Spontaneous (natural process) or Induced (mutagens). It can arise in one of the two ways. First from mismatch that is from errors in DNA replication. Second from structural alteration induced by physical and chemical agents (mutagens). There are various types of mutation. First type is mutation at the DNA sequence level. Point mutation/SNP which are occur by replacement of a single nucleotide by another nucleotide. These are of two types. One is transition which is a change of purine to purine or pyrimidine to pyramidine. Another is transversion which is a change of purine to pyramidine or vice-versa. eq. A to C or T, C to A or G. Second type is insertion or deletion which is addition or removal of one or more base pairs. Third type is translocation which is transfer of a part of chromosome or a set of genes to a non-homologous chromosome. Fourth type is inversion which is excision of a piece of DNA and re-insertion in the same position but in the reverse orientation.If we understand mutation at gene level we can categorise them as silent mutation that is a base change that does not cause a change in the specified codon. A missense mutation



that is a base change which causes a change in the specified codon. e.g, sickle cell anaemia. Nonsense mutation which is a base change that causes a change in the specified codon to become a stop codon. Frameshift mutation which is a base loss or gain causes a change in the reading frameand thus changes codons thereafter. e.g, Tay-Sachs disease.

Mutagenesis is a process by which the genetic information of an organism is changed in a stable manner, either in nature or experimentally by the use of chemicals or radiations.There are various types of mutagenesis. One is directed mutagenesis where organisms respond to environmental stresses. Second is insertional mutagenesis which is mutation of DNA by insertion of one or more bases. One is virus insertional mutagenesis other is insertional inactivation in which plasmid is used.

Third is signature tagged mutagenesis a genetic technique to study gene function. In this gene in question inactivated by insertional mutation and by use of transposons containing different tag sequencesinactivated genes are detected. Fourth is transposon mutagenesis, allows gene to be transferred to a host organism's chromosomesand modify the functions of a gene on the chromosome. Last one is site directed mutagenesis, most commonly used methodology. In this technique mutation is created at a defined site in a DNA molecule. There are three ways to conduct site directed mutagenesis, primer extension, cassette mutagenesis, PCR method.

Applicationsof Mutagenesis in farm animals

In animal breeding, genetic disorders are one of the most important issues for breeders.Due to the negative influence of such disorders on animals eq. through abnormal anatomy, or reduced production, breeders and breeding associations need to control the impact on the population.Known inherited disorders in cattle are mostly caused by autosomal recessively inherited genes.The characteristics feature of autosomal recessive genes is that they are only expressed as a diseased phenotype if both alleles are present.Knowing the molecular bases of a defect, the direct detection of carriers is possible at the genetic level, thus preventing unintended breeding of the animal. At present, several inherited bovine disorders are identified as eq. bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine monophosphate synthase (DUMPS), complex vertebral malformation (CVM), bovine citrullinaemia (BC) and factor XI deficiency (FXID)

Bovine leukocyte adhesion deficiency

BLAD is a lethal autosomal recessive disorder and known to affect Holstein cattle breed. Affected cattle have severe and recurrent mucosal infections such as pneumonia, gingivitis, periodontitis, loss of teeth, papillomatosis, pus formation, delayed wound healing, stunted growth.The molecular basis of BLAD is a single point mutation (A to G) of nucleotide 383 in the CD 18 gene located bovine chromosome 1

Deficiency of uridine monophosphate synthase

DUMPS is a hereditary lethal autosomal recessive disorder in Holstein cattle causing early embryonic mortality during implantation in the uterus.UMP synthase is necessary for the de novo synthesis of pyrimidine nucleotide which are constituents of DNA and RNA.Growth and development is arrested leading to embryonic mortality around 40 days post- conception. DUMPS is caused by single point mutation (C to T) at codon 405 located bovine chromosome 1

Complex vertebral malformation

CVM is a recessively inherited disorder leading to frequent abortion of affected fetuses or perinatal death associated with vertebral abnormalities. Typical sign are shortened neck and bilateral symmetrical moderate contraction of carpal joints, slight lateral rotation of fetlock joints.CVM is caused by single base transversion of guanine to thymine located at position 559 of bovine chromosome 3

Bovine citrullinaemia

BC is an autosomal recessively inherited disease that prevents the synthesis of argininosuccinatesynthetase, the enzyme that catalyses the conversion of citrulline and aspartate to argininosuccinate at the consumption of ATP.Cattle affected by BC appear normal immediately after birth, however by the 2nd day of life become depressed and feed poorly. By 3rd day aimless wandering or standing with their head pressed against a wall.Between the 3rd and 5th day, the disease progress rapidly and calves appear to be blind and finally collapse.BC is caused by a transition of cytosine (CGA/Arginine) into thymine (TGA/Stop codon) at codon 86 located at bovine chromosome 11

Factor XI deficiency

Factor XI is one of more than dozen proteins involved in blood clotting.Its deficiency results in prolonged bleeding from the umblical cord and anemia. Prolonged oozing of blood following dehorning or castration.Affected cows frequently have pink-colouredcolostrums. Causative mutation for FXID consists of a 76 bp segment insertion into exon 12 in bovine chromosome 27

Oculocutaneous albinism

Oculocutaneous albinism (OCA) is an autosomal recessive hereditary pigmentation disorder affecting humans and several other animal species.The main clinical findings were photophobia and a lack of pigmentation of the hair, skin, horns, hooves, mucosa, and iris.In the OCA buffalo, a single-base substitution was detected at nucleotide 1,431 (G to A), which leads to the conversion of tryptophan into a stop codon at residue 477.

Spider lamb syndrome

Spider lamb syndrome is characterized by serious skeletal deformities making it difficult for a lamb to stand/walk.It is the result of a mutation in a gene controlling bone development; lambs exhibiting spider lamb syndrome have two copies of this mutation. Lambs carrying only one copy of the mutation appear normal. The gene codes for a protein called fibroblast growth factor receptor 3 (FGFR3).

Booroola Merinos

Booroola Merinoscarry a mutation that causes them to have larger litters of lambs. If they carry two copies of the mutation they have even more lambs than if they only carry one copy. In this one copy of the mutation results in more lambs, but two copies result in no lambs.

Influence on cattle milk production

Some dairy cattle carry a mutation in a gene which causes them to produce milk with a higher percentage of fat.There are many genes that influence milk production. Mutation in one of these has a very strong effect on milk fat. eq. A lysine to alanine substitution (K232A) in the diacylglycerol acyl transferase 1 (DGAT1) gene.

Double muscling

Mutations in a certain gene are associated with double muscling in cattle. These mutations damage the signaling protein and muscle growth continues causing a condition known as double muscling. These cattle certainly produce more meat. Double muscling is usually considered a disease because these cattle often have serious problems, such as difficulty giving birth.

Role Of Nanovitamins In Animal Production

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Nanotechnology is widely anticipated as one of the key technologies of the 21st century. Nanotechnology has the potential to provide the tools and the research to change the future of food and feed technology. Applying the principles of nanotechnology, researchers can produce more nutritious food and beverages; improve food and feed packaging and develop special biosensors. These biosensors can monitor food or feed safety and the health of crops, forest areas, fish ponds, and livestock (Morris, 2005). This new technology deals with controlling the properties of matter with lengths between 1 and 100 nm. One nanometer is equal to one billionth of a meter, and is about the size of small molecule. Therefore nanotechnology focuses on the characterization, fabrication and manipulation of biological and non-biological structures smaller than 100 nm. Structures on this scale have unique and novel functional properties. It has the potential to significantly influence our economy and poised to have a major impact on science, food systems, agriculture, medicine, and the environment (Discher, 2006). Major food producers are using nanotechnology to improve the quality of their foods.

Nanoparticles can be categorised into different types based on their ability to carry different ingredients and react to different environmental conditions. They can be divided into two broad categories, inorganic and organic, based on the chemical characteristics of the nanoparticles. *Organic nanoparticles*, (sometimes referred to as nanocapsules when used as vehicles for delivery of, e.g. essential nutrients or pharmaceuticals) are likely to be used to enhance the nutrient value of feed systems through improvement or alteration of feed functionality. *Inorganic nanoparticles* has ingredients manufactured at the nanoscale and are of additives already approved for use in feed, e.g. titanium dioxide, a feed colorant, can be used as a UV protection barrier in feed packaging when used as a nanoparticle.

Applications of nanotechnology in animal feed (Food Safety Authority of Ireland, 2008)

Vitamin and enzymatic nano bioengineering has application in the agri-food/feed sector. Many vitamins and there precursors, such as

carotenoids, are insoluble in water. However, when formulated as nanoparticles, these substances can easily be mixed with cold water and their bioavailability increases. Currently, food additives with nanoingredients are used. These additives may imply that nanoparticles are present in the food and the additives are mainly aimed at the diet, sports and health food markets and contain minerals with a nano-formulation, such as silicon dioxide, magnesium, calcium, etc. The particle size of these minerals is claimed to be smaller than 100 nanometre so they can pass through the stomach wall and into body cells more quickly than ordinary minerals with larger particle size. Nano-additives can also be incorporated in micelles or capsules of protein or another natural food/feed ingredient

Vitamin E is a generic term used to describe all *tocopherol and tocotrienol* derivatives that exhibit the biological activity of alpha tocopherol. The structure of vitamin E is sensitive to light, heat and oxygen. Its stability can be affected when stored in food for relatively short periods of time. Natural sources of vitamin E include vegetable and soybean oils. Synthetic versions are less expensive, but have lower biological activity. Incorporation of high viscosity liquid form (vitamin E 97% Aldrich) was assessed gravimetrically by increase in weight (Kuzma, 2005).

Milk production/industry sector (nanomilk)

Nanotechnology is a new technological tool in modern raw milk production and pasteurization. Recent and ongoing advances in biomedical technology will assist in advancing our understanding of disease prevention and health promotion, as well as medical diagnostics and therapeutics (Brody, 2003). Likewise, these advances will also open the barn door and accelerate our understanding of identical aspects in relation to the milking cow considering the ongoing progress within livestock genomics.

Carbon nanotubes have been widely used as a nonfood application of nanotechnology. These structures have been used as low-resistance conductors or catalytic reaction vessels among other uses. It has been shown that certain globular proteins from milk (such as hydrolyzed α-lactalbumin) can be made to self-assemble into similarly structured nanotubes under appropriate environmental conditions (Graveland- Bikker and de Kruif, 2006).

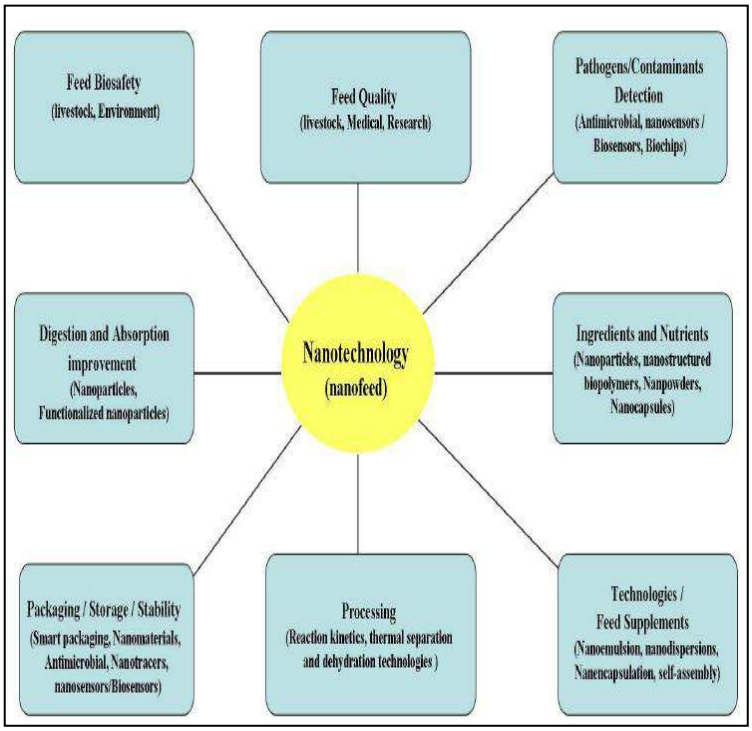


Fig 1 shows the application of Nanotechnology in various fields

According to Hans Westerbeek, R&D/QA manager of Campina, Netherlands statement calcium enriched fermented dairy drink contains a milk mineral complex at submicron level that is enabled by nanotechnology. Enriching milk with calcium salts would give the drink a 'sandy' mouth feel, and the insoluble calcium salts would precipitate as well, leading to unequal amounts of calcium per serving and it is mainly due to particle size. Another application of nanotechnology is making healthier ice cream. Unilever is developing low fat ice creams by decreasing the size of emulsion particles that give ice-cream its texture. Unilever believes that by halving the size of particles that make up the emulsion of ice cream could use 90% less of the emulsion. This would result in ice cream which traditionally consists of 8 to 16% fat – with less than 1% fat. This works by creating a tighter matrix that reduces the size of ice crystals, which then allows for a creamier, smoother product with better freeze/thaw stability.

Egg production/industry sector (Nanoegg)

There is shortage of knowledge in use of nanotechnology innovation in egg poultry production sector. Poultry meat and eggs are often the source of foodborne pathogens, like *salmonella*. Early detection of foodborne pathogenic bacteria is critical to prevent disease outbreaks and preserve public health. Several methods have been developed in order to detect this pathogen; however, the biggest challenges remain detection speed and sensitivity. Now, a novel nanotechnology-based biosensor is showing great potential for foodborne pathogenic bacteria detection with high accuracy.

Role of nanotechnology in designer eggs

Designer eggs are already in the market. The share of the designer eggs in the total share of the egg market will rise to more than 30% in about 2020. Supply of the cholesterol free eggs, yolkless or reduced yolk eggs which can be of high value protein source, immune eggs with the predetermined antibodies and therapeutic eggs with predetermined physiological factors for treatment purposes are possible outputs.

The tools and techniques currently used will not give the solution for these challenges. They can only be met out by the emerging nanotechnology, which deals not only at the molecular level but also at the atomic level (Kannaki and Verma, 2006).

Animal products packaging quality and technology (Nanopackaging)

Nanotechnology has the potential to improve food quality and safety significantly. There are many new applications for improving food safety. One of these is packaging. Packaging is a critical key for insuring the safety of food products. Nanotechnology can involve food packaging in order to improving packaging material and their functionality and consequently food safety and consumer protection. Some of the potential uses of nanotechnology in food packaging include modifying the permeation behavior of foils, increasing barrier properties, improving mechanical and heat resistance properties, developing active antimicrobic and antifungal surfaces. Another food application is the

detection of food-borne pathogens and their toxins (German, 2006).

Nano•E™ provides highly bioavailable natural vitamin E to horses through a unique delivery system

The availability and necessity of vitamin E in equine diets is well established. When healthy horses are given frequent access to fresh green forage such as good-quality pasture, there is little need for supplementation of vitamin E. However, now-a-days many horses are managed without fresh forage in their diets. These horses can survive in such management systems, but they will not be consuming sufficient vitamin E for optimal health. Vitamin E is first and foremost an antioxidant, an agent that keeps free radicals from forming and potentially weakening cells and tissues. In addition to its antioxidant responsibilities, vitamin E is vital to immune, cardiovascular, circulatory, neuromuscular, and reproductive functions. Therefore, supplementation with an effective vitamin E supplement is recommended for all horses that do not consume a significant quantity of green pasture (Chen et al., 2007).

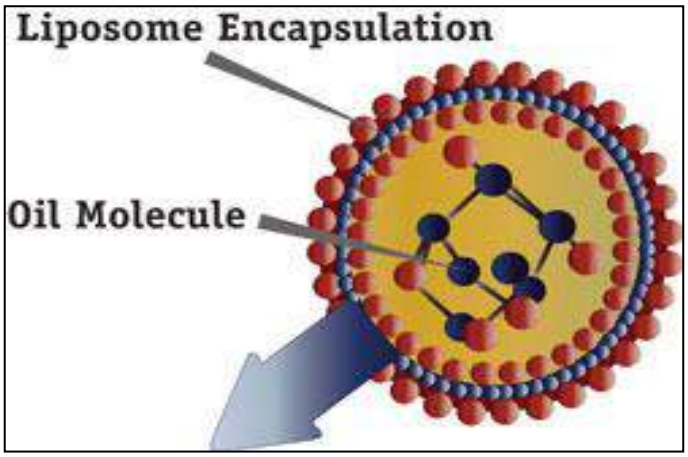
Delivering Vitamin E Eficiently

Fat-soluble vitamins like vitamin E must be offered to horses in a way that maximizes absorption. Because it is not mixed with other ingredients, the vitamin E in Nano•E™ does not need to be protected by esterification, but it must become water-soluble or dispersible in liquid.

Each water-soluble vitamin E nanoparticle in Nano•E™ is encapsulated or surrounded by liposomes, which allows the nanoparticle to mix evenly throughout a water-based environment such as the gastrointestinal tract

Researchers have developed a method by which fat-soluble vitamins can be absorbed with great efficiency. Because of its oily nature, vitamin E is hydrophobic (water-shunning). To overcome this, use of proprietary technology to encapsulate the vitamin E in nanoparticles and then surrounding each nanoparticle with a hydrophilic (water-loving) outer layer is preferred. The hydrophilic outer layer allows the nanoparticles to be rapidly and evenly released in water-based environments such as the gastrointestinal tract. The greatest benefit of this unique delivery system is an increase in the bioavailability of vitamin E. The more vitamin E that is absorbed into the

Figure 2 shows the 1 Nano•E™ for supplemting horses.



bloodstream, the more will be available for use as a body-wide antioxidant essential for tissue repair and for its imperative roles in various body systems.

Controlled studies at Kentucky Equine Research indicate that Nano•E™ is superior to synthetic and other natural vitamin E sources.

Appropriate for broodmares and stallions, Nano•E™ provides optimal antioxidant protection. The beneficial effects of Nano•E™ in broodmares are increased fertility and increased transfer of passive immunity to their foals. Vitamin E supplementation has been linked to increased libido and semen quality in stallions.

Veterinarians and researchers have found that vitamin E supplementation often helps in recovery from certain neurological problems. Nano•E™ provides natural-source vitamin E in a form that is quickly absorbed. Prior to relocation, long-distance travel, weaning, or other stress-inducing events, Nano•E™ provides a boost to antioxidant and immune defenses. Following injuries such as lacerations puncture wounds, or burns, the addition of Nano•E™ to the diet will increase antioxidant activity and enhance recovery.

Feeding Directions

Daily Use: give 4-8 mL (1000-2000 IU) per 1100 lb (500 kg) horse by mouth or top-dress on feed.

Pre-Competition: give 12-20 mL (3000-5000 IU) per 1100 lb (500 kg) horse 6-12 hours prior to competition or stressful event.

Guaranteed Analysis: 250 IU d-alpha-tocopherol per mL

CONCLUSION

Nanotechnology is in constant development and its applications are specific, with a high potential for improving livestock production. However, a great amount of research is still required to support its effectiveness in various fields of animal production

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Effect and Mode of Action of Saponin on Microbial Population and Fermentation in the Rumen

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Saponins are the diverse group of glycosides or plant secondary compounds that have presented the possibility to modify rumen fermentation and enhance animal production (Patra and Saxena, 2009). Chemically, saponins are a group of high molecular - weight glycosides in which saccharide chain units (1–8 residues) are linked to a triterpene or steroidal aglycone moiety. These saccharide chains are commonly attached at the C3 position (monodesmosidic), but some sapogenins contain two saccharide chains (bidesmosidic) attached at the C3 and C17 position. Most of the saponins are monodesmosidic or bidesmosidic (Sarnthein *et al.,* 2004).

Saponins are widely distributed in the plant kingdom and reported to occur in nearly 100 plant families in 754 species of central Asian plants. These are mainly found in the seeds, roots and bulbs of the plants, whereas some are present in the assimilatory parts of the plants (Vincken *et al.,* 2007). Saponin content in different plant species varies widely and is influenced by age, physiology, environment as well as agronomic factors. Saponins aresynthesized by a common metabolic pathway similar to cholesterol and other steroids where squalene acts as common intermediary precursor.

TYPES OF SAPONINS

1. Triterpenoid Saponins: These saponins are most widely distributed having 30 carbon skeleton comprising mostly four or rarely five units. These are found in higher amount in beans, peas, soybean, lucerne, tea, spinach, sugar beet, horse chestnut and ginseng.

2. Steroid Saponins: These saponins are less widely distributed, having 27 carbon skeleton which consists of either 6-rings (spirostane) or 5-rings (furostane). These saponins are found in yucca, oat, capsicum, pepper, aubergine, tomato, alliums, asparagus, yam, fenugreek and ginseng (Sparg*et al*., 2004)

BIOLOGICAL ACTIVITIES OF SAPONINS

Saponins reveal anextensive spectrum of biological activities like haemolytic, anti-inflammatory, insecticidal, antioxidant, vasorelaxant, anti-oedematous, antitumor, cytotoxic, antiviral, antibacterial, antifungal, anticancer, anti-allergic, anthelmintic, molluscicidal, piscidal and immune-modulatory action. Saponin administration in ruminants drop the plasma cholesterol and blood glucose levels, form insoluble complexes

with cholesterol and bile acids by sequestration in the intestine and also reduce blood glucose (Waheed *et al.,*2012).

SAPONIN METABOLISM IN RUMINANTS

Old perception was that the saponins are not suitable for animal use has been changed in recent years following demonstration of their beneficial effects in different ruminant species under various dietary conditions.Poorly absorbed in intestine due to their large molecular mass (> 500 Da), high hydrogen-bonding capacity and high molecular flexibility. Due to this reason, most of their effects are exhibited in the gut or rumen only (Meagher *et al.,*2001).Most of the saponins and their degradation products are transported further, along the intestinal tract and finally excreted in the faeces. However, there are reports that these may be absorbed in the duodenum and transported to the liver via the portal vein (Flaoyen *et al.,*2002).No adverse effects on feed intake on inclusion of saponins in ruminant diets. Interestingly, increase in feed intake is observed following inclusion of saponins in diets of dairy cows and sheep (Holtshausen*et al.,*2009; Abreu *et al.,* 2004).Saponin extracts or saponin-containing plants did not alter digestibility, but decreased CH_4production (Santoso*et al*., 2004). At low dose saponins, exhibit anti-methanogenic effects without affecting digestibility, while at higher doses reported the reduction of both digestibility and methanogenesis(Wang *et al*., 2009).

Effects of Saponins on Rumen Microbial Population

CILIATE PROTOZOA

Saponin-containing plants or extracts have inhibitory effects on protozoal population depending upon the concentrations in the diet with a small increase in bacterial numbers (Klita*et al.,*1996). Theysuppress the growth of rumen protozoa *in vitro* without affecting dry matter (DM)disappearance and other rumen microbial populations(Hristov*et al.,*1999). Oral administration of saponins from the plant *Biophytumpetersianum* decreased protozoal numbers in goats by 35 and 40% at the dose of 13 and 19.5 mg/kg body weight, respectively(Santoso*et al.,*2007).

BACTERIA AND FUNGI

*Y. schidigera*extract inhibited the growth of pure cultures of *Butyrivibriofibrisolvens*and *Streptococcus bovis*, while the growth of *Prevotellaruminicola*was stimulated and the growth of *Selenomonasruminantium*was unaffected by Yucca extract(Wallace *et al.,*1994*).* Low doses of saponins might increase the permeability of the cell membrane in a controlled manner, thus allowing the increased absorption of nutrients into the bacterial cells(Sen *et al.,*1998). However, fungal numbers increased when sheep were fed with diets containing *Sapindussaponaria/ Enterolobiumcyclocarpum*(Diaz *et al*., 1993).

RUMEN ARCHAEA

The reduction of methane production by *Sapindussaponaria*was observed in a defaunated state without any change in methanogen counts, indicating that saponins may directly influence the activity of methanogens(Hess *et al*.,

2003). Tea saponins had no effect on the growth and expression of the methyl coenzyme M reductase subunit A gene (mcrA) of *Methanobrevibacterruminantium* (Guo*et al.,*2008).

Mechanism of Action of Saponins in Rumen Ecosystem

Saponins reduce methane production via inhibition of either protozoa or methanogens or both.Their anti-protozoal action is manifested through interaction with cholesterol in the cell membrane leading to its disruption, breakdown, lysis and finally cell death (Wina*et al.,*2005).Anti-methanogenic activity of saponins is believed to occur by limiting hydrogen availability to methanogens and re-channelling of metabolic hydrogen from methane to propionate production in the rumen (Goel *et al.,*2008).Saponins cause lesser fermentation of feed matter in the rumen which consequently resulted in more extensive fermentation in hindgut thus making conditions more favourable for acetogenesis through diversion of hydrogen from methanogenesis pathway(Patra & Saxena 2009).The major mechanism suggested for the antifungal activity of saponins is their interaction with sterols followed by pore formation and loss of membrane integrity. Some saponins may show a specific inhibitory activity to fungi but not to bacteria(Francis *et al.,*2002).The metabolism of saponins in the rumen involves deglycosylation and structural changes of the aglycone nucleus (Wang *et al.,*2000).

Effects of Saponins on Rumen Fermentation

Saponins or saponin-containing plants decreased rumen ammonia N concentrations and increase the efficiency of microbial protein synthesis in an *in vitro* fermentation system containing hay as a substrate. Oral administration linearly decreased ruminal ammonia N concentrations, and increased retained N as a proportion of N digested, efficiency of microbial N synthesis and microbial N supply in goats fed a diet consisting of elephant grass silage and concentrate(Santoso *et al.,*2007).Variable effects were seen on VFA production, increase in the proportion of propionate and a reduction in acetate, butyrate and branched chain VFA(Castro *et al.,*2011).Further, the effects of saponins on VFA are pH and diet dependent where more pronounced effects were seen at low pH.Some reduced VFA levels (Derivatives of *Quillajasaponaria*) whereas some enhanced VFA levels (*sarasaponin*). *Yucca* extract increased VFA levels when hay and straw were used as substrates(Lila*et al.,*2003).Theasaponin caused an increase in propionate production, while methane and isobutyrate production were decrease(Ye *et al.,*2001).Methane production also decreased linearly with increasing concentrations of Yucca saponins in an *in vitro* medium containing different substrates such as potato starch, maize starch and hay-concentrate (Lila *et al.,*2003).

CONCLUSION

By decreasing protozoal populations, saponins enhance propionate production, microbial biomass synthesis, microbial nitrogen flow and gluconeogenesis. Thesesaponins found to be an effective

and promising agent for CH_4 mitigation from livestock. Plants or their extracts with high concentrations of saponins appear to have the potential to act as natural rumen manipulators.Despite their multifunctional roles, there is a need for further evaluation to elucidate their exact mechanism of action, absorption, toxicity, effects at gene levels and dose-activity relationships.

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Health Benefits of Pomegranate

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Pomegranate has been used for medicinal purposes in the middle and Far East regions for over thousands of years. It was used as a tonic to heal ailments like ulcers and diarrhea. The juice of Pomegranate contains Antioxidants like Anthocyanin and ellagic acid, compounds like Gallic acids, and flavonoids like Quercetin which offer protection from diabetes, heart diseases, osteoarthritis and several kinds of cancer.

A) Benefits of Pomegranate in curing diseases

1. Heart benefits: Pomegranate juice can have a great impact on health, particularly on theHealth of the heart, by keeping the arteries flexible and decreasing the inflammation in the lining of the blood vessels. It is known to reduce atherosclerosis, which is one of the leading causes of heart disease. It lowers the risk of blockage in the arteries which can cause a restriction in the flow of blood to the heart and brain.

2. Maintains blood sugar levels: Although Pomegranate juice contains fructose, it does not elevate the blood sugar level as other fruit juices do.

3. Maintains blood pressure: The juice of Pomegranate reduces lesions and the inflammation of blood vessels in heart patients. It is a natural aspirin, which keeps the blood from coagulating and forming blood clots. It even acts as a blood thinner allowing for an unrestricted flow of blood through the body.

4. Protection from cancer: Pomegranate juice eliminates free radicals from the body and inhibits the growth and development of cancer and other diseases. Its high contents of anti- oxidants stimulate the white blood cells to neutralize toxins in the body thereby promoting a strong and healthy immune system.

5. Soothes the stomach: Pomegranate juice is used in the treatment of diarrhea and dysentery as it plays a vital role in the secretion of enzymes which aids proper digestion.

6. Boosts immunity: Pomegranate juice has strong anti-bacterial and anti-microbial properties which help fight viruses and bacteria and boost our immunity system. It significantly reduces microbes that are found in the mouth commonly responsible for cavities and staph infections.

7. Prevents anemia: Anemia is a condition caused by the deficiency of red blood cells in the body. Since Pomegranate juice

contains ample amount of iron it helps in surmounting a deficit of red blood cells in the body if any.

8.Improves digestion: Pomegranate juice aids in the smooth functioning of the stomach, heart and liver. This juice induces hunger and can even control thirst. Therefore it is a great choice of drink during summers. It also soothes urinary tract infection and eases the flow of urine. The high amount of dietary fiber, both soluble and insoluble in Pomegranate juice helps to improve digestion and regulate bowel movement.

9. Helps in cartilage regeneration: Pomegranate juice acts as an inhibiter on enzymes that are responsible for damaging the cartilage. It is therefore highly recommended for patients suffering from osteoarthritis, a chronic condition characterized by the breakdown of the joint's cartilage. **10. Good for prenatal care:** Pomegranate juice is extremely beneficial for pregnant women. It is a great source of a number of vitamins and minerals including folic acid which is a crucial element of a pre natal diet. The anti-inflammatory property of Pomegranate juice ensures a healthy blood flow, which is essential for the overall development of the fetus. The potassium content in this juice can also help prevent leg cramps that are generally associated with pregnancy.

14. Boost Digestive Condition: Pomegranate juice helps to secrete enzymes with anti-bacterial properties that aid digestion and help to fight off hemorrhoids, nausea, dysentery, intestinal parasites, piles and diarrhea.

15. Reduce Inflammation: The anti-inflammatory properties of Pomegranates stems from its high vitamin C content that will help to manage asthma, sore throat, cough and wheezing.

B) Benefits of pomegranate in curing Skin problems

Pomegranatejuice can be a secret potion to get a healthy, vibrant and youthful skin.

1. Anti-aging benefits: A diet high in Pomegranate juice can help to slow down the process of ageing by reducing wrinkles and fine lines on the face caused by constant exposure to sun. It also helps to sustain the regeneration of the skin cells and prevent hyper pigmentation and occurrence of dark spots.

2. Suited for all skin types: Pomegranate juice works wonders for dry skin. Its smallMolecular structure penetrates deeply into the skin to provide ample moisturisation and hydration to the skin. Thus it soothes dry and irritated skin. Its content of punicic acid, an omega 3 fatty acid keeps the skin constantly hydrated by sealing in the moisture. Pomegranate juice is beneficial for oily and pimple prone skin too. Application of Pomegranate juice on oily skin prevents the outbreak of pimples and controls the production of sebum.

3.Helps to heal scars: Pomegranate helps in the regeneration of cells in the epidermal andDermal layers of the skin. It has an ability to hasten the process of healing of wounds. Even the oil of Pomegranate seeds is loaded with skin friendly nutrients which are beneficial for the strengthening and regeneration of the epidermis. It also protects the skin from sun burns and heals the damage caused to

the skin due to constant exposure to the sun.

4. Improves the texture of skin: Pomegranates are also known to extend the life of Fibroblasts which are responsible for the production of collagen and elastin that tighten the skin and prevent the formation of fine lines and wrinkles. Collagen and elastin provide strength and support to the skin. When the fibers of collagen and elastin break down, the skin develops laxity which causes wrinkles and jowl. Pomegranate is also very useful for lightening the skin tone. Drinking a glass of Pomegranate juice daily gives a fair and glowing skin tone.

5.Pomegranate face mask for glowing skin: Mix1 teaspoon each of powdered greenPapaya, grape seed oil, and grape seed extract with2 teaspoons of Pomegranate juice and apply it on the face. Leave it on for about an hour and wash it off with lukewarm water.

6.Prevents skin cancer: Pomegranate contains Anthocyanins and hydrolysable tannins which possess strong anti-oxidant and anti-tumor promoting properties. When applied directly on the skin, ellagic acid, a Polyphenol Antioxidant found in Pomegranates inhibits the growth of cancer of the skin.

7. Regenerate Cells: Pomegranate protects the epidermis and dermis by encouraging skin Cell regeneration, aiding in the repair of tissues, healing wounds and encouraging circulation to skin that is healing.

8.Protect from the Sun: Consuming Pomegranate provides the skin with compounds thatHelp to protect against free radical damage which can cause sun damage, cancer and sunburn. The oil of a Pomegranate contains the Antioxidant ellagic acid that can help to inhibit skin tumors to protect the body from skin cancer.

9.Slow Aging: Pomegranates can help to prevent hyper pigmentation, age spots, fine lines and wrinkles that are often caused by sun damage.

10.Produce Youthful Skin: Because Pomegranates help to soften the skin and produce additional elastin and collagen it can make your skin look more firm, smooth and youthful.

Allelomimetic Behaviour and its Implications in Practical Farm Animal Management

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Each animal species has a characteristics way of performing certain functions and rarely departs from it. The behaviour associated with certain specific function is termed as behavioural pattern. Each pattern of behaviour has a definite special adaptive function, which can generally be related to one of the nine general functions (Scott, 1958). A group of behaviour patterns with a common general function compromises of system of behaviour. Allelomimetic behaviour is one of the nine systems of behaviour described by Scott, 1958. This involves two animals doing the same thing at the same time with some degree of mutual stimulation. This behaviour is very common in all domestic animals especially in sheep and chickens with an exception in rabbit and cats. It's most general function is to maintain a social group and to provide safety, for when one animal sees danger all become alerted by responding to his behaviour. It is more important in dogs in hunting and group attacks on prey, since several animals working together are more likely to be successful than one alone. Allelomimetic behaviour can also be referred as ***'Allelomimetism', 'Copy Cat Behaviour', 'Mimicking', 'Activity synchronization'.***

1. BASIS OF ALLELOMIMETIC BEHAVIOUR

The Allelomimetic behaviour is based on '***Social facilitation***' in animals. By stimulating each other, animals produce the phenomenon of '***Social facilitation',*** which is defined as any increment of activity resulting from the presence of other individuals (Crowford, 1939; Simmel *et al.,* 1968). More specifically "social facilitation" is defined as behaviour by an indivisual that is initiated or increased in rate or frequency by the presence of another indivisual carrying out that behaviour (Zentall, 1996) .Within group, the activity of certain individuals usually rapidly followed by the majority seems to direct behavioural policy for all. This group effect serves as a basis for the holistic strategies of group behaviour. Social facilitation in flocks and herd is involved in daily movements and in stampedes, marches and migrations that persists as outstanding behavioural phenomena in animals. Social facilitation is more likely where there is adequate association, ability to communicate and react, a potential for mimicking activities, similarity of motivational state and suppression of intra-species aggregation.

One of the classical examples of social facilitation seen in dairy cows, when one cow eats, another might be stimulated to do likewise, whether she is hungry or not (Curtis and Houpt, 1983). When animals eat in groups they eat more than if they are fed separately.

Synchrony can be produced by social facilitation (Clayton 1978), when individuals match their behaviour to that of other animals in the group. According to Clayton (1978) where environmental stimuli only provide gross synchrony, socially facilitated behaviour will provide finer-scale synchrony, and, what is functionally important, greater cohesion of the social group'. synchrony is more likely to result from imitation (Wechsler & Brodman1996; Rajaratnam and Redman 1999) through a process called allelomimetism (Scott 1956; Deneubourg and Goss 1989), which embodies the case of equally shared, mutual social facilitation (Clayton 1978).

2. IMPORTANCE OF ALLELOMIMETIC BEHAVIOUR

2.1 Feeding

Allelomimetic behaviour means that animals of a species tend to do the same thing at the same time. Cattle and sheep tend to graze at the same time and rest and ruminate at the same time. Range cattle gather at the watering place at about the same time each day because one follows another. It has been well documented that animals eat more, when they are in groups than if they are fed separately. If cattle, sheep or pigs are taken from their groups and housed individually they eat less (Cole *et al*., 1976).

Cattle:

Cows fed in groups, are likely to be less fearful, and hence, more contented, healthier, and more productive. The common practice of feeding and milking cows in groups thus has a sound psychological basis. (Scott, 1962).This could be the response to lack of companions in general or to lack of companions at feeding time. Even when food is continually available, social animals usually synchronize their feeding. The duration of grazing is much more constant when animals graze in a herd than when they graze individually. Hughes (1971) found that chickens in cages synchronized their feeding much more often than would be expected by chance. Social facilitation strongly influences eating bouts and feed consumption in cows reared in group housing compared with isolated cows (Albright and Arave 1997).

The rate of feeding is affected by presence of one or more companions. A calf fed on a milk replacer was fed alone early in the morning, but its companion calf was not fed. If the companion was then reintroduced to the adjacent pen and given milk, which it drank, the first calf-which could see the second drinking-consumed more milk replacer. In a subsequent experiment, the first calf was again fed alone then the second calf was introduced to the same pen but was muzzled. When milk replacer was made available, the muzzled calf tried to drink and stimulated the first calf to take even more milk. These reports suggest that food intake by young calves can be increased when others can be seen and heard feeding. Hence, since competition is relatively unimportant in these calves,

the chances that any calf will receive too little milk replacer is reduced if several teats (about one per two calves) are provided close together (Barton and Broom, 1985).

Studies showed that pen-mates transferred foraging information to other animals more efficiently than non-pen-mates. In another study, reports suggest that movements of a cattle herd during grazing were directed by active movement of cattle with high-social ranking or the independent movement of low-ranking cattle.

Chicks:

Chicks pecked more frequently and ingested more when a companion was present (Tolman and Wilson, 1965) and an apparently satiated hen took more food if a hungry hen was introduced to its cage (Katz and Revesz, 1921).

Pigs:

Allelomimetic behaviour is also seen in pigs. After one piglet in the litter has started eating, other piglets may also learn from this experienced piglet what and where to eat. Seeing another individual eat increases the motivation to also start eating, a phenomenon called social facilitation (Keeling and Hurnik, 1996).Several studies in pigs suggest increase in feed consumption when they are housed in groups than in individual housing

Furthermore, Nielsen *et al.*, (1996) described that pigs housed in isolation spend more time rooting than pigs housed in groups and their pattern of eating is also affected. The results suggest that exploration and social contact may to some extent substitute for each other. Thus, the absence of a social partner may increase the demand for straw, whereas it may have no effect or even have an opposite effect on other resources.

Dogs:

In dogs the presence of other members of a group can lead to individuals consuming 50% more food than would occur if they were alone and when previously satiated dogs were joined by hungry indivisuals, they often ate more food (Manteca, 2002).

2.2 Forage selection

In maternal gregarious herbivores, evolutionary process has favoured a development where social learning plays an important role in the evolving foraging skills of the infants (Launchbaugh and Howery, 2005). Calves also learn from their mother. Hessle, 2009 reported that calves in the company of experienced conspecifics were more active during grazing compared with calves kept in groups by themselves (score 124 vs. 99, P = 0.005).

Animals learn preferences for foods (Provenza *et al*. 1990). We can refine their learning process and teach them to avoid eating specific troublesome poisonous plants through the process of social facilitation. In a study conducted by Ralphs and Olsen, 1990, eight Hereford cattle trained to avoid larkspur (a poisonous plant) were put with four non averted (control heifers and offered larkspur. Four of the averted heifers were reinforced by infusion with lithium chloride whenever they ate larkspur, the other four heifers were not infused. Reinforced heifers continued to be averted, whereas the aversion was soon extinguished in the non- reinforced animals. All heifers were then taken to larkspur infested rangeland. When four

remaining averted heifers observed control animals feeding on larkspur three of them began eating it also. When heifers were returned to the facility and the situation that led to the initial aversion, that aversion was reinstated. This gives evidence of the strong influence of ***"Social facilitation" or "Copy cat behaviour"*** in a new environment in overcoming a prior aversion to eating a poisonous plant species.

Higher grazing activity was observed in naive calves the company of grazing-experienced co specifics (Hessle, 2009). The initial higher foraging activity in calves kept together with older steers may be due to an increased feeling of security in these animals, besides social learning, which may be indicated by the shorter time span to first ruminating at turn-out of these calves. Young gregarious ruminants are aware of the anti-predator effect of proximity of older conspecifics (Green, 1992).

2.3 Postural synchrony

The postural synchrony (lying /standing) is a collective behaviour which occurs in the herd by the process of ***"social facilitation***" or" ***allelomimicry****"*, where individual animals mimics and adapts the posture (lying or standing) of other animals (Clayton, 1978; Deneubourg and Goss,1989). The postural synchrony (lying/standing) of cattle is significantly more with that of the near neighbour as compared to randomly chosen members of herd suggesting that cattle in a herd actively synchronizes their postures with that of their neighbours (Stoye *et al,* 2012). Stoye *et al*, 2012 also reported that the degree of synchrony varied significantly with time of day and the herds were most synchronous in the evening (96.76%), least synchronous in the middle of the day (89.47%), and had an intermediate degree of synchrony in the morning (92.65%). The understanding of synchrony of lying in cattle can be a useful tool for assessing cattle welfare. Previous studies suggest that when ample amount of recourses (space) is available, synchrony of lying takes place and insufficiency of the above (space) may restrict the animal from lying down, which may lead to increase incidence of lameness. So the knowledge of synchrony of lying in cattle can help in improving the performance of cattle.

Girard *et al*., 1993 reported that adult cattle experience REM (rapid eye movement) sleep, almost exclusively at night, during 2-4 % of the nyctohemera of 8-10 episodes, each 24 hours. Younger cattle (6 months) logged 9 % REM sleep during 9-15 episodes, some of which occurred during daylight. There is a significant social facilitation to lie/rest as evidenced by 92% of calves to be doing so when calves in neighbouring stalls were lying (Coe *et al*., 1991).There is evidence that, in some dairy cows, when one animal defecates or urinates, others may commence to do likewise.

2.4 Protection from predators

Any animal which normally lives in a flock or herd will become either lonely and depressed or frightened and agitated if kept by it. This forms a special problem with indivisuals riding horses or milking cows and with such solitary pet animals as dogs which are left by themselves for any length of time. Human companion will substitute in part, but in general two animals get along better than one. When animals are in group they are in less fear

from predators because if one animal finds any danger from predators it also alarms other animals. Dairy cattle are social animals that operate within a herd structure and follow a leader (leader-followership) to and from pasture, the feed through, and milking parlour. Such behaviour can be beneficial (following a leader onto a scale) or detrimental (stampede).

PRACTICAL IMPLICATIONS OF ALLELOMIMETIC BEHAVIOUR

Allelomimetic behaviour is of practical importance because the producer can then observe the herd or the flock with little difficulty, notice anything that is wrong with a particular animal, and have that animal brought in for treatment. Some practical implications of Allelomimetic behaviour in practical farm management are as follows:

- Cows fed in groups, are likely to be less fearful, and hence, more contented, healthier, and more productive.
- The common practice of feeding and milking cows in groups has a sound psychological basis. Because when cows are milked in groups, let down of milk is better, this aids in better production in the animals.
- Rest is 'vital to the animals in its integration and mediation with its environment' (Fraser, 1983). Resting time in cattle is mostly spent lying, and a larger part of the day is diverted towards this behaviour (Arnold, 1984).Resting time can be increased by postural synchrony in dairy cows.
- Postural synchrony also can reduce the incidence of lameness in the cows
- The incidence of abnormal behaviour can also be reduced. As the animals stay in social group, there is more copy cat behaviour or mimicking of activities from the neighbouring animals.
- Social facilitation strongly influences eating bouts and feed consumption in cows reared in group housing compared with isolated cows. This clearly suggests that the cows should be housed in groups.
- The process of social facilitation can be used to make the calf learn, to drink milk from pail by mimicking of older experienced calves.
- The best time to locate the range cows in heat is when they gather at the watering place. Allelomimetic behaviour is useful in driving groups of animals from one place to another.
- Cattle can be trained to avoid eating harmful poisonous plants during grazing; this is achieved by social stimulation of nearby cows.

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Implications of Coal Fly Ash In Agriculture

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Fly ash is regularly generated as a by-product by coal burning or thermal power stations. The potential of fly ash as a resource material in agriculture and related areas is now a well-established fact. The appreciable application of fly ash in agriculture, because of its favorable physicochemical properties, including content of essential plant nutrients is beneficial. While compare to soil, fly-ash consists all the elements except organic carbon and nitrogen. The purpose of this paper is to provide an overview of characterization and utilization of fly ash in agriculture. The effect of fly ash on soil properties, improvement in the crop yields, heavy metal uptake by the plants and ground water contaminations were mentioned.

The coal fly ash is a residue of burning of coal, the organic source of energy. Fly ash one of the major waste material of Thermal Power Station. Fly ash constitutes very fine and light particles. Size ranges from 0.5-100 micron. Fly ash is a mixture of aluminium and silicate. Fly ash is dominantly made up of Al, Si, Ca, Fe, Mg, Na, S, As, Cd, Cu, Zn, Cr, Ni, etc. 108 million tons fly ash produce per annum. Coal is the most abundant, extensively used and important source of energy for power generation in the world. Among the total power generated annually in India, about 70% is produced by thermal power plants. The generation of fly ash depends on the type and ash content of the coal being used. Generally lower quality coal is used in Indian power plants, which contains a high percentage of ash. In India, studies have been carried out toward management of fly ash disposal and utilization. Fly ash is utilized in the cement and construction industries with the remainder trucked to landfills or piped to settling ponds. However the rate of production is greater than consumption. The disposal of such a huge amount of fly ash is one of the major problems of developing countries. Dumping of fly ash affects the quality of the surface and ground water, soil and vegetation of the area. Besides these, the use of fly ash in agricultural fields is a good alternative. Fly ash contains essential nutrients such as K, Ca, and Mg which has an alkaline effect.

Amending such alkaline fly ash can reduce soil acidity at a certain level and it is suitable for agriculture, and it can increase the availability of important nutrients. The use of fly ash can promote plant growth by increasing soil conductivity, organic carbon contents, and microbial activity, soil porosity and water holding capacity. Fly ash contains essential nutrients for plant growth and it can be used as a fertilizer to complete the deficiencies of several elements. Use of fly ash in agriculture provides a feasible alternative for its safe disposal to improve the soil environment and enhance the crop productivity

COMPOSITION OF FLY ASH

Fly ash occurs as very fine particles, having an average diameter of less than 10 μm, low to medium bulk density, high surface area and very light texture. The chemical composition of fly ash varies depending on the quality of coal used and the operating conditions of the thermal power stations. Approximately 95% to 99% of fly ash consists of oxides of silicon, aluminum, iron and calcium. About 0.5% to 3.5% consists of sodium, phosphorous, potassium, sulphur and the remainder of the ash is composed of trace elements. Thus, fly ash practically consists of all the elements present in soil except organic carbon and nitrogen.

BENEFITS IN AGRICULTURE

Fly ash improves the physical health of the soil. It serves as a soil modifier and also enhances the water retaining capacity and fertility of the soil. It improves the plant's water and nutrient uptake, helps in development of roots and soil binding, stores carbohydrates and oils for use when needed, protects the soil from soil-borne diseases and detoxifies contaminated soil. Use of fly ash in agriculture has been shown to increase the yield of cereals, oil seeds, pulses, cotton and sugarcane by 10-15% and vegetables by about 20-40% as observed in experiments carried out under varied climatic conditions and soil types across the country with different doses of fly ash and pond ash with and without organic manure in various crops.

It was found that the fly ash application caused changes in soil properties due to modifications in macro- and micro-pore size distribution, which contributed to the increased crop yields. It has recently been reported that paddy grain yield differed significantly due to treatments. The most effective treatment noted for paddy grain yield was the combination of FA+FYM (92% increase over control) followed by FYM and FA. Besides enhancing agricultural yields, reclamation of wastelands, degraded lands, acid and saline alkaline soils, eroded soils etc. has also been demonstrated. Since fly ash has physical and chemical properties similar to those of soil, it can be used directly on the soil, or in land reclamation, with organic matter, lime or gypsum, in composts, or made into granulated materials or potassium silicate fertilizers. Fly ash increases moisture retention and aeration in poor soils. It provides the micronutrients for plant growth, but lacks

potassium and only supplies a limited amount of nitrogen.

Agricultural lime application contributes to global warming as Intergovernmental Panel on Climate Change (IPCC) assumes that all the carbon in agricultural lime is finally released as carbon dioxide to the atmosphere. Use of fly ash instead of lime in agriculture practices can reduce net carbon dioxide emission, thus reducing the problem of global warming.

Also, less fertilizer, gypsum and irrigation are required after fly ash treatment, which reduces costs (as long as the ash does not need to be transported for long distances). For example, a fly ash treatment of 100 tonnes per hectare on sandy soils in Australia reduced the water consumption of the soil by around 75%. Improved water retention also reduced the rate of leaching of any fertilizers used. Cooperative effects have been shown between coal ash and organic substances that improve the soil and promote plant growth. Various biosolids, including treated sewage sludge, have been shown to complement fly ash in composts. Fly ash composted with earthworms improves yield so that expensive chemical fertilizer applications could be reduced. Fly ash dispersed in powdery form in various crop fields (paddy crop, mustard etc.) kills pathogenic microbes and other harmful insects and pests.

KEY FACTS OF ADVANTAGES

- ✓ Improves permeability status of soil.
- ✓ Improves fertility status of soil/agriculture yield.
- ✓ Improves soil textural properties and soil aeration.
- ✓ Reduces soil bulk density and crust and compact formation.
- ✓ Improves water-holding capacity /porosity.
- ✓ Makes favorable and optimum soil pH for crops.
- ✓ Provides several micronutrients such as Mo, B, Mn, Fe, Zn, Cu, etc.
- ✓ Source of many macronutrients like Mg, S, K, P, Ca, etc.
- ✓ Alternative for gypsum for reclamation of sodic soils and lime for reclamation of acidic soils.
- ✓ Improves soil microbial activities in combination with other organic amendments.

RISKS OF FLY ASH IN AGRICULTURE

There are certain risks too. Fly ash has particle per million (ppm) level concentration of heavy metals. When applied to soil these elements may get absorbed by plants grown on it and may ultimately enter into the food chain. Despite fairly intensive research over the last few years, the data on trace element accumulation in plants is rather sketchy and inconsistent. Boron in fly ash is readily available to plants. Investigators consider boron to be the limiting factor in unweathered fly ash utilization for agriculture. Trace and heavy metals in fly ash may also percolate into the soil and pollute ground water. The solubility of these elements is less than ten percent. It

has been observed in laboratory experiments on leaching potential that 5-30% of toxic elements, especially Cadmium, Copper and Lead, are leachable. At least 10% of total Cadmium would be solubilized in the acidic pH range of 3 to 5. It is unlikely that these will have any major effect on the quality of ground water. However, monitoring of these parameters is advisable. There have been several reports of the presence of radionuclides in fly ash but studies on their impact have been limited. The radiochemical pollution of Uranium and Thorium series is always present in fly ash. Radium is another common constituent of fly ash. The half-life of radium is more than a thousand years. Think about basements put on land where fly ash had been previously disposed! Thus, soil amendments on the periphery of expanding cities are potentially hazardous. Several crops grown in quantities of fly ash (5 to 20 % of soil weight) have been found to absorb toxic metals according to a study by Indiana State University researchers. When the amount of fly ash increased, the crops absorbed higher concentrations of arsenic and titanium.

KEY FACTS OF DISADVANTAGES

- ✓ Uptake and accumulation of toxic heavy metals by crop plants.
- ✓ Fatal effects on humans and cattle due to consumption of heavy metal contaminated crops.
- ✓ Ground water pollution due to heavy metal percolation down to earth.
- ✓ Higher doses of FA in agriculture field may cause soil infertility.
- ✓ The radiochemical pollution present in FA.

FUTURE STRATEGIES

The use of fly ash in agriculture is a craftlike practice, because it depends on several factors. The source and quality of fly ash needs to be matched with the soil or spoil being treated, the crop being grown as well as the local climate. Regulation needs to account for these findings. Rather than exempting fly ash from hazardous waste regulations (as is the current practice), it should instead be managed commensurate with the actual metal levels measured in each batch of fly ash. Fly ash has great potential in agriculture due to its efficacy in modification of soil health and crop performance. However, since there is a potential for harming the environment and human health, long term confirmatory research is necessary before planning agriculture as a venue for fly ash utilization.

Disease Management In Bovines

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Disease is the inability to perform physiological functions at normal levels even though nutritional and other environmental requirements are provided at adequate levels. Management practices aimed at identifying and resolving the disease problems early are the single most direct and cost effective way to improve health of the animals. The focal point of any diseases in animals is the making of a diagnosis and the critical part in making that decision is clinical examination of the individual animal or group of animals. There has been considerable emphasis on clinical and laboratory examination of individual animals affected with clinical disease or that have not performed normally. The predisposing factors and other etiological agents produce a disease with a specific pathognomonic lesion. Several agents such as bacteria, virus, protozoa, fungus, nutritional and managemental factors are the primary causes of disease in animals especially in bovines. Some of the commonly encountered disease conditions in bovines are:

i. Rumen Acidosis

Rumen acidosis is a metabolic disease of cattle that occurs due to fall in the rumenal pH below 5.5. The fall in pH causes rumenal atony that may lead to depressed appetite and production. Secondly, the change in acidity changes the rumen flora, with acid-producing bacteria producing more acid, making the acidosis worse. The increased acid is then absorbed through the rumen wall, causing metabolic acidosis, which in severe cases can lead to shock and death.

Cause

- Feeding a high level of rapidly digestible carbohydrate, such as barley and other cereals
- Feeding increased concentrates compared to forage.

Symptoms

- Acute acidosis often results in death.
- Cattle may become depressed, go off feed, and have an elevated heart rate or diarrhoea.

Sub-acute:

- Reduced feed intake
- Poor body condition and weight loss
- Unexplained diarrhoea
- Temperature

- Pulse rate and respiratory rate may rise
- Lethargy

Treatment

There is no specific treatment for subacute ruminal acidosis. Secondary conditions may be treated as needed.

Prevention

- The key to prevention is reducing the amount of readily fermentable carbohydrate consumed at each meal that requires both good diet formulation (proper balance of fiber and nonfiber carbohydrates) and excellent feed bunk management.
- Including long-fiber particles in the diet reduces the risk of subacute ruminal acidosis by encouraging saliva production during chewing and by increasing rumination after feeding.
- Ruminant diets should also be formulated to provide adequate buffering. This can be accomplished by feedstuff selection and/or by the addition of dietary buffers such as sodium bicarbonate or potassium carbonate.
- Supplementing the diet with direct-fed microbials that enhance lactate utilizers in the rumen may reduce the risk of subacute ruminal acidosis. Ionophore (eg, monensin sodium) supplementation may also reduce the risk by selectively inhibiting ruminal lactate producers.

ii. Mastitis

The mammary gland and udder tissue is an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm (commonly through bedding or contaminated teat dips), and can also occur as a result of chemical, mechanical, or thermal injury to the cow's udder. Mastitis is a multifactoral disease, closely related to the production system and environment that cows are kept in.

Symptoms

- Somatic cell counts measure milk quality and can be used as an indicator of mastitis prevalence.
- The most obvious symptoms of clinical mastitis in the udder are swelling, heat, hardness, redness or pain.
- Milk takes on a watery appearance, flakes, clots or pus is often present. A reduction in milk yields, increases in body temperature, lack of appetite, and a reduction in mobility due to the pain of a swollen udder are also common signs.

Treatment

NSAID are widely used for the treatment of acute mastitis. Aspirin, flunixinmeglumine, flurbiprofen, carprofen, ibuprofen, and ketoprofen have been studied as treatments for experimental coliform mastitis or endotoxin-induced mastitis. Orally administered aspirin should be used with caution in acute coliform mastitis because it may lead to severe rumen atony.

Prevention

- Hygienic teat management
- Prompt identification and treatment of clinical mastitis
- Dry cow management and therapy
- Culling chronically affected cows
- Regular testing and maintenance of the milking machine
- Good record keeping

iii. Calf Scour

It is the most important disease problem in dairy calves that causes more financial loss to the calf producer than any other syndrome. Rotavirus is most common cause of diarrhoea, with over 30% lab diagnoses being rotavirus. Controlling rotavirus can thus significantly reduce the losses due to calf scour.

Prevention of rotavirus and other scour problems can be done by:

- Maximisation of hygiene
- Feeding of colostrums to the calves
- Vaccination

iv. Peri-Weaning Diarrhoea

Peri-weaning (or post-weaning) diarrhoea is a syndrome in calves around the time of weaning that cause of the disease is unknown, infectious agents, managemental, environmental and nutritional factors have all been implicated. The diarrhoea is usually first seen fairly soon after group housing, particularly if the animals were previously kept in outdoor hutches.

Clinical Signs

- Profuse watery grey diarrhoea.
- Most calves affected, but death usually rare
- The calves tend to be bright and continue eating
- Dehydration tends to be moderate
- Pot-bellied appearance common in 'recovered' calves
- Reduced growth rate, and uneven batches of calves has significant economic effect.
- Significant fecal staining of the coat and appearance of 'ill thrift'.

Diagnosis

- Clinical signs
- Diagnosis of 'exclusion'

Treatment

- Treatment, except for electrolytes to treat dehydration, is often ineffective.
- Many antibiotics have been tried but none are routinely effective.

Prevention

- Avoid abrupt weaning and ensure calves get colostrum for at least the first week after birth.
- Avoid the use of calf pellets that are rapidly fermented in the rumen.

v. Bovine Viral Diarrhoea (BVD)

BVD is a common cause of respiratory and reproductive issues in the herd. It causes a number of transient infections which are often the cause of animal health and economic problems.

Cause

Bovine viral diarrhea is a viral disease of cattle and other ruminants that is caused by the bovine viral diarrhea virus (BVDV). It is transmitted either through a congenital infection of the fetus or after birth.

Symptoms

Signs of acute infection include fever, lethargy, loss of appetite, ocular discharge, nasal discharge oral lesions, diarrhea and decreasing milk production. Chronic infection may lead to signs of mucosal disease. In calves, the commonly recognized signs are:

- Ataxia
- Tremors
- Wide stance
- Stumbling

Treatment

Treatment of BVD is limited primarily to supportive therapy. Once identified, infected animals should be culled.

Prevention

- Minimisation of BVD transmission is to make infected cattle less infectious that can be achieved by increasing the antibody titer.
- Persistent culling of infected animals from the herd.
- Strategic vaccination and high-quality colostrum could also decrease the proportion of susceptible cattle.

vi. **Calf Diphtheria (Necrobacillosis)**

There are two forms of calf diphtheria. The most common is an acute oral (mouth) infection, usually seen in calves less than 3 months old. The second form is usually seen in older calves and affects the larynx (or voice-box), Both forms are caused by the bacteria *Fusobacterium necrophorum*.

Clinical Signs

Oral form

- Initial presenting sign may just be a swollen cheek
- Calf may be otherwise bright and active with no temperature
- Examination of the inside of the mouth shows a foul-smelling ulceration and swelling of the cheek
- Temperature may be normal at the start

Laryngeal form:

- Coughing : Moist and painful
- High temperature
- Loss of appetite and depression
- Difficult breathing, chewing and swallowing
- Pneumonia

Diagnosis

- Clinical signs
- Bacteriological examination
- A post-mortem can confirm the ulcerative nature of the disease, particularly in calves with the laryngeal form

Treatment

- Early prompt treatment is important as early treatment is much more effective
- Separate the infected animals and isolate them
- Antibiotics and pain killers are effective in most cases

Prevention

- Thorough cleaning and disinfecting of all calf feeders.
- Young calves must be examined daily to identify early stages of the disease.

vii. Joint ill (Navel ill)

Navel or joint ill is a disease of young calves, usually less than one week of age that occurs as a result of infection entering via the umbilical cord at, or soon after, birth.

Symptoms

- Swollen and painful navel that does not dry up.
- An abscess may develop from which pus may burst.
- High temperature, reduced appetite and depression
- Swollen stiff painful joints
- In some calves infection spreads from the navel to the liver causing a liver abscess.

Treatment

- Infected animals should be separated and treated with antibiotics and pain killers.
- For large navel abscesses, veterinary intervention to drain and remove the infected tissue is often necessary.

Prevention

- Ensuring that the cow calves are in a clean environment will significantly reduce the risk of joint ill
- Proper planning and preparation can prevent the build-up of disease that occurs in too many calving areas.
- Applying a disinfectant such as iodine to the navel can reduce the risk of bacteria entering via the navel.
- The cattle those are born in a nice clean environment should not be moved to other pens or contaminated pastures until their navels have dried completely.
- Ensuring of receive of appropriate amounts of colostrum within the first 24 hours of birth by the young animals.

viii. Rotaviral Diarrhoea

Rotaviruses are the most common cause of neonatal diarrhoea in calves. The virus is present in most cattle herds and typically causes diarrhoea in calves between 5 to 14 days old.

Symptoms

- Pale yellow diarrhoea is common, sometimes with mucous and blood flecks.
- Calves are dull and reluctant to drink.
- Calves become dehydrated and pick up secondary infections.

Treatment

- Oral fluids are the most important line of treatment.
- The use of antibiotics to treat calf diarrhoea is controversial, particularly if rotavirus is the main cause. Antibiotics will not kill the virus, but they can reduce secondary bacterial infection. Antibiotics are probably best used when the calf does not respond well to oral fluids.

Prevention

- Hygiene
- Ensuring of receive of appropriate amounts of colostrum within the first 24 hours of birth by the young animals.
- Vaccination

ix. Infectious Bovine Rhinotracheitis (IBR)

Infectious Bovine Rhinotracheitis (IBR) is a highly contagious, infectious respiratory disease that is caused by Bovine Herpesvirus-1 (BHV-1). It can affect young and older cattle.

Symptoms

- Fever
- Coughing
- Depression
- Loss of appetite
- Hyperaemia of the mucosae
- Mucosal lesions
- Nasal discharge
- Conjunctivitis
- Drop in milk production
- Infertility
- Abortion

Treatment

There is no direct treatment for viral diseases. Infected animals should be isolated from the rest of the herd and treated with anti-inflammatory drugs and antibiotics for secondary infections if

necessary. Carrier cattle should be identified and removed from the herd.

Prevention

- Use of vaccines.
- Appropriate biosecurity will also reduce risk on farm.

x. Calf pneumonia

Calf pneumonia is a multifactorial disease caused by the infectious agents that include Mannheimiahaemolytica, Haemop hilussomnus, Infectious Bovine Rhinotracheitis (IBR), bovine Respiratory Syncytial Virus (RSV) and Parainfluenza III Virus (PI3), along with many other bacteria and mycoplasma species and viruses. Environmental factors include low environmental temperatures and high humidity and poor ventilation and also direct draughts are the predisposing factors of this disease.

Symptoms

- Dull and depressed
- High temperature
- Raised breathing due to lung damage
- Nasal discharge
- Coughing
- Reduced food intake

Treatment

Antibiotics, anti-inflammatories or anthelmintics can be prescribed for treatment.

Prevention

- Improved husbandry, ventilation and good nursing care can all reduce risks of pneumonia.
- Ensuring of receive of appropriate amounts of colostrum within the first 24 hours of birth by the young animals.
- Vaccination

CONCLUSION

Properly conducted heard health programs and planned animal health and production programs maintain accurate records on all matters of production and health. Early diagnosis and treatment of a disease condition along with prevention that includes maintenance of proper hygiene, vaccination schedule and strict enforcement of quarantine laws can reduce the risk of occurrence of dangerous bovine diseases.

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Metabolomics:
Clinical Biomarkers For Metabolic Disease Monitoring In Dairy Cattle

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Metabolomics has been defined as a global holistic overview of the metabolic status and could provide new insight into pathophysiologic mechanisms in diseases. It can allow for measuring the comprehensively small molecule endogenous metabolites in easily accessible biofluids and biomarker discovery (Nicholson and Lindon 2008). The metabolome of a cell, tissue, organ, or organism is represented by its small molecular metabolites (molecular weight less than 1000 Da), which are the end products of cellular processes. Variation of the metabolome reflects the interaction of changes in upstream molecules, such as genes and proteins with environmental factors. Metabolic profiling, which is the high-throughput characterization of the metabolome, can be used to assess health status and is a potential diagnostic tool for diseases. It allows for a global assessment of a cellular State within the context of the immediate environment, taking into account gene expression, genetic regulation, altered kinetic activity and regulation of enzymes, and changes in metabolic reactions (Griffin *et al.,*2004). Thus, metabolomic studies compared with genomics or proteomics reflect changes in phenotype and function of a particular tissue, or organism. The change in the expression of genes and proteins will surely produce changes in the metabolic profile of a cell, tissue or organism (Spratlin *et al.,* 2010). Metabolite changes that are observed in diseased individuals as a primary indicator have been an important part of clinical practice. Thus metabolomics offers potential advantages that classically diagnose approaches do not, based on the discovery of a suite of clinically relevant biomarker that is simultaneously affected by the disease.

Techniques used to measure metabolomics

Common metabolomic technologies include gas chromatography- mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), liquid chromatography - mass spectrometry (LC-MS), capillary electrophoresis–mass spectrometry (CE-MS) and metabolites chip are widely used as analytical tools (Nicholson *et al*., 2002). Among the analytical techniques that can be employed for metabolomics applications, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the most common.

Metabolomics in metabolic diseases

The productive life span of a dairy cow largely depends on her metabolic health status. High disease incidences including fatty liver, ketosis, milk fever, mastitis, metritis, displaced abomasum, and downer cow syndrome cause a substantial decline in the profitability of most dairy operations. Most metabolic diseases have been related to perturbation of one specific metabolite. For example milk fever has been linked to perturbation of calcium homeostasis; fatty liver has been explained with development of a negative energy balance (NEB) during peripartum and to increased release of non-esterified fatty acids (NEFA) from adipose tissue and subsequent storage in the liver in the form of triglycerides (TG); ketosis has been related to increased release of ketone bodies from liver hepatocytes as a result of oxidation of NEFA in mitochondria. Metabolomics has been introduced as part of 'omics' technology to better Understand interaction between nutrition and disease (wishart, 2008). A major advantage in the application of metabolomics stems from an improved ability to detect up to many hundreds of metabolites in parallel, which provides an efficient method for monitoring altered biochemistry. Changes in biological status is then based on the detection of perturbations in the concentrations and fluxes of specific endogenous metabolites involved in a number of key disease-related or other specific cellular pathways. Thus, metabolomics can reveal crucial information that is closely related to the current disease or theraputic status.

Metabolomic evaluation of Milk fever in plasma of dairy cows

Milk fever (MF) is a complex metabolic disorder, usually of dairy cows, that occurs around parturition. The biochemical characteristic of this condition is severe hypocalcemia (usually <1.5 mmol/L), which most likely explains the clinical signs associated with milk fever (Goff, 2006). Investigation of this disease has chiefly focused on blood calcium, ionized calcium, or total calcium contents in the transition period because of the close connection between blood calcium and milk fever. Blood phosphorus status has also been researched because of the prominent interaction between calcium and phosphorus at the onset of lactation (Larsen *et al*., 2001). Several other parameters, such as magnesium, alkaline phosphatase, hydroxyproline, osteocalcin, parathormone, calcitionin, and 1,25-dihydroxy-vitamin D, which are associated with the regulation of calcium metabolism can be analysed. Cows with milk fever had greater plasma Serum amyloid A (SAA) and lower concentrations of calcitonin-gene related peptide (CGRP) in the plasma compared with clinically normal cows (Ametaj *et al*., 2003). SAA is a protein released by the liver that binds and neutralizes endotoxin whereas CGRP lowers plasma calcium and increases glucose in the blood.

There is upregulation of endopin 2B and serpin peptidase inhibitor(SPI) proteins in the plasma from cows with milk fever. Endopin 2B (E-2B) is a component of secretory vesicles where neuropeptides are produced. Endopin 2 inhibits the cysteine protease, papain, and the endogenous secretory vesicle cathepsin L which participates in the

production of the biologically active enkephalin peptide neurotransmitter (Hook and Hwang, 2002). Serpin peptidase inhibitors (SPIS) are a family of protease inhibitors participate in a series of physiological and pathological processes including digestion, degradation, fertilization, embryogenesis, fibrinolysis, hormone activation, complement activation, cellular and humoral immunity, and maintenance of homeostasis (Krem and Dicera, 2002). There is downregulation of proteins in the plasma from cows with milk fever; namely, fibrinogen beta chain (FG-β), IGg heavy chain C-region (IGg -CH), and albumin. Downregulation of plasma FG-β indicated that milk fever has a negative effect on blood coagulation and signal transduction, which may be closely related to hypocalcemia whereas downregulation of IGg –CH demonstrated that milk fever contributes to an increase in infectious diseases which may be related to decreased synthesis of IGg caused by hypocalcemia at calving.

Metabolomic evaluation of Fatty liver in plasma of dairy cows

The transition time around parturition and early lactation involves critical physiologic changes in dairy cows. An excessive demand for nutrients due to the increased performance required for milk production results in a negative energy balance (Hammon *et al*. 2009; Mullins *et al*., 2012). One major adjustment consists in the rapid mobilization of energy sources from tissue depots in the form of non-esterified fatty acids (Ingvartsen 2006; Geelen & Wensing 2006). Although in cattle the major site of fatty acid synthesis is the adipose tissue, the liver plays a central role in coping with sudden increases in the energy requirement (Kreipe & Deniz 2011). Fatty liver disease develops when, during this critical transition period, the hepatic uptake of non-esterified fatty acids liberated from the adipose tissue exceeds their elimination from the liver, thus causing their hepatic storage as triacylglycerols (Bobe *et al*., 2004). In cattle, the ability to release hepatic triacylglycerols as very low-density lipoproteins (VLDL) circulating in the blood is extremely low compared to other species (Katoh 2002). Excess storage of triacylglycerols in the liver causes progressive hepatocyte damage and,consequently, membrane leakage that results in the increased release of liver enzymes and bile constituents into the blood (Bobe *et al*., 2004). Thus high producing cows with increased milk yields are, therefore, particularly susceptible to the development of the fatty liver syndrome (Reid & Collins 1980).

Hepatic parameter changes in cows suffering from FLD

Parameter	Changes
TAG	↑
Total lipids	↑
Glycogen	↓
Phospholipids	↓
Cholesterol	↓
Enzymes of the gluconeogenesis	↓
Enzymes of the β-oxidation	↓
Enzymes of the glycolysis	↓
Enzymes of the TAG-synthesis	↑

↑ Respective concentration or activity is elevated in cows with FLD
↓ Respective concentration or activity is

reduced in cows with FLD Phosphatidylcholines may be reduced in fatty liver disease which is an important precursor for the synthesis of triacylglycerols (Jacobs *et al*., 2013) indicating that their level may decrease in response to an enhanced triacylglycerol production. Reduced phosphatidylcholine level may directly cause an excessive accumulation of triacylglycerols in the liver. The abnormal decline of certain specific phosphatidylcholines and sphingomyelins could be regarded as a promising biomarker indicative of fatty liver disease. There is reduction of the fibrinogen serum conten in periparturient cows suffering from hepatic lipidosis. As this fibrin precursor is produced in the liver, its lower level may represent a direct consequence of hepatocyte dysfunction due to excessive lipid deposition, but may also result from coagulopathy linked to liver disease (Pluta *et al*.,2010). Amino acids glycine and glutamine that have already been linked to metabolic disorders and chronic inflammatory conditions (Oberbach *et al*., 2011), are key hallmarks of hepatic lipidosis. Although changes of phosphatidylcholine and sphingomyelin blood levels have previously been associated with metabolic disorders (Floegel *et al*., 2012) or chronic liver diseases (Cantoni *et al*., 1975), Changes of lipid levels involve an increase of those phosphatidylcholines that carry relatively short fatty acid moieties (PC aa C30:2 and PC aa C32:2), whereas the quantity of phosphatidylcholines containing larger fatty acid components (≥34 carbons) was reduced. Cows experiencing fatty liver have presence of an inflammatory state as indicated by greater plasma concentrations of TNF-α and SAA.

Metabolomic profiling of dairy cows affected with ketosis

Ketosis is one of the most prevalent metabolic diseases of dairy cows during transition period (Grummer, 1993). B-hydroxybutyrate (BHBA) is widely considered the golden standard for diagnosing ketosis in dairy cows. In addition, blood glucose levels (Glc), total triglycerides (TG), nonesterified fatty acids (NEFA), and aspartate aminotransferase (AST) can also be analyzed to monitor ketosis related complications (Oetzel., 2004). Metabolic disturbances of ketosis involve in multi-biochemical pathways such as glycolysis, gluconeogenesis, amino acids metabolism, fatty acids metabolism, pentose phosphate pathway. Lactic acid (LA), glucuronic acid (GLCA), l-alanine (L-ala), glycolic acid (GA), ribitol, pyroglutamic acid (PGLU), galactose (Gal), 2,3,4-trihydroxy butyric acid (THBA), and glucose (Glc), decreased from low to high in both Clinical and Sub Clinical Ketosis. Low PGLU values may indicate glutathione deficiency due to oxidative stress. Thus, gluconeogenesis decreased in both Clinical and Sub Clinical Ketosis because of a decrease in these glucogenic amino acids. In addition, glucuronic acid, ribitol, Gal, and Glc can play a role in energy metabolism by entering glycolysis via the pentose phosphate pathway (Lankinen *et al*., 2011). Decreased ribitol levels may be related to riboflavin deficiency (Lankinen *et al*., 2011). Vitamin C may also be involved in energy metabolism due to glucuronic acid, which is derived from vitamin C (King *et al*., 2000). Therefore, a

low level of plasma carbohydrates plays an important role in the development of ketosis, and low vitamin C and B_2 levels, as well as oxidative stress, may also be contributing factors. GLCA and LA plays important roles in maintaining energy balance were maximally down-regulated in both Clinical and Sub Clinical Ketosis. Metabolites mainly 3-hydroxybutyric acid (BHBA) and non esterified fatty acids (NEFA), including palmitic acid (PA), heptadecanoic acid (HA), stearic acid (SA), trans-9-octadecenoic acid (T-9-OA), myristic acid (MA), cis-9-hexadecenoic acid (C-9-HA), which belong to the families of ketone bodies, long chain unsaturated fatty acids, and saturated fatty acids are increased confirming that a great amount of fat mobilization resulting from hypoglycemia may cause ketosis. Some up-regulated amino acids and their catabolic products, such as L-Ile, Glycine, 2- piperidine carboxylic acid (2PC), increased, suggest that proteolysis increases to meet body energy demand in both Clinical and Sub Clinical Ketosis. Those cows can not effectively utilize them, rendering them susceptible to infectious diseases and oxidative stress. However, it might be that the host responds to sickness by increasing those metabolites. Although Clinical Ketosis and Sub Clinical Ketosis had many of the same metabolites some different metabolites were also found between the two groups, including 2-methyl-3-hydroxybutyric acid (2Me3HB), β-oxidation of fatty acids, xylitol, and an intermediate of the pentose phosphate and glycolytic pathways. The decreased metabolites included 3-hydroxyisovaleric acid (3HIV), γ-amino butyric acid (GABA), melibiose, erythritol and L-serine. The results suggest that these carbohydrates and amino acids may be important factors in the development of Clinical Ketosis. In addition, some metabolites were up-regulated in Sub Clinical Ketosis and included 4-hydroxyproline (4-HYP), and l-leucine (L-leu), (Nelson *et al*., 2005). Some metabolites that decreased in Clinical Ketosis compared to Sub Clinical Ketosis namely GABA, L-serine, 4-hydroxy proline, citrate, 3,4-dihydroxybutyric acid (DHBA), L-proline. Therefore, a decrease of the above mentioned metabolites, especially citrate, can further aggravate ketosis due to interruption of TCA cycle and lack of amino acids. Thus, those metabolites may become potential biomarkers for diagnosing ketosis including Clinical and Sub Clinical Ketosis in dairy cows.

Metabolomic evaluation of displaced abomasums in plasma of dairy cows

An NMR-based metabolomics approach was extended to test its ability to identify and quantify physiologically relevant metabolites of displaced abomasums Metabolites identified and quantified by NMR analysis were valine, 3 β-hydroxy butyrate, alanine, glutamine, glutamate, and succinate. Among these parameters succinate decreased significantly in cows with right displaced abomasums (RDA). Pronounced findings between LDA and RDA groups included significant changes in glutamine, glutamate, and 3 β-hydroxy butyrate. Furthermore, 3 BHB concentrations were positively correlated with valine, glutamin, and glutamate concentrations in the LDA group. plasma from LDA cattle exhibited significantly higher free fatty acid and BHB, lower glucogenic amino acids such as methionine, alanine, and serine, and a

higher ratio of ketogenic amino acids among blood-free amino acids, such as leucine and lysine (Martínez *et al*., 2011). The positive correlation between glucogenic amino acids (valine, glutamine, and glutamate) and BHB levels in the LDA group may be attributed to a contribution to glucose production, against ketosis. Decreased levels of glutamine may reflect increased hepatic dysfunction and/or destruction of hepatocytes (Martínez *et al*., 2011).

CONCLUSIONS

The emerging field of "metabolomics," in which a large number of small molecule metabolites from body fluids or tissues are detected quantitatively in a single step, promises immense potential for early diagnosis, monitoring for various metabolic diseases in dairy animals. Metabolomics of cows with metabolic diseases can be used to understand the metabolic changes that occur in the pathogenesis of the disease. New biomarkers of metabolic diseases are urgently needed to facilitate diagnostic procedures, identify distinct stages of the disease, monitor the response to treatment regimens and allow for the design of prevention strategies. Hence, metabolomic discoveries can evolve into discrete diagnostic tests that deliver clinical utility.

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Precision Dairy Farming

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Presently, India is leading the world as top milk producer132.4 million tonnes (Dept. of A.H., MoAGoI2012-2013). Actually this is an elusive pleasure, since it account for about 111.09 million milch cows and 118.59 million milch buffaloes (19th Livestock Census). This indicates that in spite of large dairy animal population with superior genetic potential, there is lack in our managementstrategies.This altered population-production ratio is probably due tohigh feed and labour cost, few large dairy operations, narrow profit margin and animal are managed by few skilled workers*etc*.Simultaneously present consumers demand quality assurance, organic and pathogen free product. Besides increasing production potential, it is also important to emphasize on wellbeing of the livestock. Keeping two sides of the same coin, a new concept of Precision dairy farming (PDF) is being adopted.

INTRODUCTION

Application of technologies has shifted the dairy farming into dairy industry. Precision dairyfarming is the set of these technologies. Actually the concept "Precision Dairy Farming" is the use of technologies to measure physiological, behavioural and production indicators of individual animals to improve management strategies of the farm (Bewley,2010). The main objectives of Precision Dairy Farming are maximizing individual animal potential, early detection of disease and minimizing the use of medication through preventive health measures.Behaviour oriented management and thorough surveillance of farm animals are the prospects which can aid in keeping the animals healthy and productive. These prospects may be lying behaviour, ruminal pH, heart rate, global positioning systems, feeding behaviour, blood analyses, respiration rates, rumination time and locomotion scoring using image analysis *etc*.Before availing these we have to do lot of research in this area. Inspite of these body condition scoring, daily milk yield recording, milk component monitoring (e.g. fat, protein,SNF, Lactose,SCC), pedometers, automatic temperature recording devices, milk conductivity indicators, automatic estrus detection monitors and daily body weight measurements *etc,* are such examples which can be successfully applied in dairy farm and aid in widening the profit margin as well as assure the quality of products.

Ways of utilising the outcomes of these technologies:

Observation of farm endows lot of ideas about how to unravel the drawbacks in our management strategies. Here is the list of some approaches for precision dairy farming.

	Difference between quarters with highest and lowest conductivity(mS/cm)	Conductivity in individual quarter (mS/cm)
Healthy udder	<0.6	<5.5
Doubtful quarter	0.6-0.9	5.5-6.4
Subclinical disease	>1	>6.5
Milk not delivered	>1	>8

Body condition score:

BCS is an indicator of **Range of ideal Body Condition Score** amount of stored energy reserves. It is prime requisite to evaluate BCS of farm animals for fine tuning of feeding management. Adoption of BCS in dairy farms can aid in reduction of incidence of productive and reproductive diseases like ketosis, milk fever, metritis*etc*.For instance, by minimizing BCS, conception rate at first service can be improved which will reduce investments on dairy animals(Kellogs*et al.,* 2010)

Somatic Cell Score:

Subclinical mastitis is **SCC level as indicator of mastitis** 15-40 times more prevalent than clinical mastitis. Usually it goes unnoticed by the dairy farmers. In long run it becomes the cause of highest economic loss. SCC is a useful predictor of intramammary infection (IMI).

Leucocytes in milk increase inresponse to bacterial infection, tissue injury and stress. Monitoring of farm (www.qconz.co.nz) animals by SCC is the sensitive as well as specific way to combat the economically important disease like mastitis.

Electrical conductivity:

Electrical conductivity in healthy and ill quarter

This test measures increase in conductance in milk caused by the

Average cell count	Probability of subclinical mastitis
0-149000	Very low
150000-249000	Low
250000-499000	High
500000+	Very high

elevation in level of Na^{+},K^{+}, Ca^{2+},Mg^{2+} and Cl^{-} during inflammation.This increaseis caused(Janzekovic*et al.*, 2009)

Stage of lactation	Score
Drying-off	3.5-4
Calving(Older cow)	3.5-4
One month postpartum	2.5-3
Milk lactation	3
Late lactation	3.25-3.75
Calving(first lactation)	3.5

by destruction of tight junctionsand the activation-pumping system. This destruction of junction is basically due to destruction of mammary cells. This destruction mainly caused disease like mastitis.

pH test:

The rise in milk**Milk pH value of healthy and mastitic animals** pH, due to mastitis, is detected using pH meter. One of the advantage of this method is that it is user friendly, cost effective and rapid.But it is not sensitive as other tests.

Milk Composition

In India composition of milk **Composition of cow milk** hasimmense importance. Since here price of milk is decided on the basis of fat% but it is not the only advantage of estimating milk composition rather it can provide idea about the diseases like mastitis.

Pedometer

This device determineshow far an animal has walked. Animals in heat areusually restless and may walk long distances which is accounted by pedometer.Cow inseminated after 10-18 h after estrus according to pedometer reading have higher conception rate (90%) than those inseminated according to a.m/p.m rule (58.4%).

Lying behaviour

Lying behaviour of dairy cow

Healthy cows typically spend approximately 12-13 hours per day resting. Although lying time is usually increased during illness and it is thought to help the animal to save energy, (Siivonen*et al.*, 2011) but cows with mastitis show a reduction in lying time due to udder pain and this has important negative effects on their welfare and production.

	Healthy	Subclinical	Clinical mastitis	References
H.F.	6.59± 0.02	6.69± 0.08	--	Batavani*et al.*,2007
Sahiwal	6.55± 0.19	6.63± 0.27	6.93± 0.37	Bahera*et al.*,2011
Cross bred	6.68± 0.23	6.79± 0.34	7.17± 0.43	Bahera*et al.*,2011

	Normal	Mastitis
Fat%	4.54	2.46
Protein %	2.96	3.53
Lactose %	4.69	3.62
NEFA (mmol/lt)	0.15	0.08
Chloride(mg%)	108.25	165.48

Walking time

Through **Mean activity of healthy and sick cows** deviation from the cow's normal state, mastitis, illness can be detected by walking activity.Siivonen*et al.*, 2011 reported that in mastitis, stepping activity increases as compared to normal cows (1413±86 v/s 1160±86).(Edwards and Tozar, 2004) Conversely Hogewerf*et al.*, 1992 reported that activity has no influence with mastitis but decrease in activity with metabolic diseases.

Heat detection efficiency	Heat detection accuracy
70-100%	49-90%

	Mastitic cow	Normal cow
Total standing duration	759.59	638.61
Lying down(min)	670.30	800.43
Total standing duration(%)	52.70	44.38
Total standing bout duration	36.63	27.73

Lactation	Healthy cow Mean activity (steps/h)	Sick cow Mean activity (steps/h)
1	174	165
2	151	137
3	145	137
4 ≤	139	128

CONCLUSION

In most part of our country,this notion is just an idea which has tremendous opportunities for connecting consumer protection, animal welfare, quality control and economic sustainability.Since it focuses on the transition from a group-oriented perspective to a perspective where intensive consideration is given to individual cows, and where particular goals, such as tapping individual potential, diagnosing diseases early and using minimum medication are pursued. Combination of all the devices will provide comfortable environment in dairy farm which can be explorated for better health, lower cost of animal rearing, increase animal longevity and boosting milk yield. Still researches should continue on the development of new technologies that monitor jaw movement, feeding behaviour, hormone level, heart rate, sleeping time etc. One thing we should keep in mind that, these researches are conducted in research environment, care must be taken in trying to transfer these results directly to commercial settings. Field experiments may need to be conducted to alleviate this issue. Because of the gap betweenimpactsof Precision Dairy Farming technologies in research versus commercial settings, additional effort needs to be directed toward implementation of management practices needed to fully utilize information provided by these technologies. To gain a better understanding of technology adoption shortcomings, additional research needs to be undertaken to examine the adoption process for not only successful adoption of technology but also technology adoption failures. Keeping all aspects of PDF, it is the area where lot of researches should be done. So that we can enhance dairy management to limitless potential.

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Health Benefits Of Milk And Milk Products

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Milk has been known as nature's most complete food. Indian ancient Vedic texts describe the virtues of milk and dairy products, as is validated by modern scientific principles and proofs. Therefore, milk has been considered as one of the most natural and highly nutritive part of a daily balanced diet. Currently, the integration of advanced scientific knowledge with traditional information is gaining incredible drive toward developing the concept of potential therapeutic foods. Furthermore, new advances toward understanding the therapeutic roles of milk and milk products have also given a new impetus for unraveling the age old secrets of milk. At present, the best-known examples of therapeutic foods are fermented milk products containing health promoting probiotic bacteria. In the present article, we have tried to review the various aspects of the therapeutic nature of milk and fermented dairy products..Hence, we have made an attempt to highlight the various health beneficial aspects of milk and milk-based foods..

INTRODUCTION

Milk is surrounded with emotional, cultural, and religious importance, which is our first food. We are habituated since child-hood about of milk as "nature's most perfect food." Milk and dairy products have long been acknowledged as an important constituent of a balanced diet. In addition, evidence of health benefits of milk products allied with the presence of specific components or bacteria are progressively gaining established scientific credibility. It is, therefore, logical that among the best-known examples of functional foods are fermented milks and yogurts containing probiotic bacteria (Kurien et al., 2005). Milk and other dairy foods provide nine essential nutrients. The USDA defines an "essential nutrient" as a dietary substance required for healthy body functioning. Essential nutrients must come from the diet because the human body can't manufacture them in sufficient quantities to meet daily needs. From helping repair muscle tissue to maintaining healthy red blood cells, the

nine nutrients in milk and other dairy foods work together to help keep the body in optimal health.

1. **Calcium**: Helps build and maintain healthy bones and teeth.
2. **Potassium**: Helps to regulate the body's fluid balance and maintain normal blood pressure. It's also needed for muscle activity.
3. **Phosphorus**: Helps strengthen bones and generate energy in the body's cells.
4. **Protein**: The protein in milk, yogurt and cheese builds and repairs muscle tissue, and serves as a source of energy and satiety.
5. **Vitamin D**: Promotes the absorption of calcium and enhances bone strength. It is one of the nutrients Americans lack most.
6. **Vitamin A**: Helps maintain normal vision and skin. It is also important for bone growth.
7. **Vitamin B-12**: Helps maintain healthy red blood cells and nerve cells.
8. **Riboflavin**: Helps convert food into energy the body can use. Also known as vitamin B2, it's important for normal eyesight and healthy skin.
9. **Niacin**: Helps bodies digest carbohydrates and fatty acid

MILK AND HEALTH

Milk is not only nature's food for a newborn infant, but also a source for a whole range of dairy products consumed by mankind. Milk contains about 87% water and 13% solids. The fat portion of the milk contains fat-soluble vitamins. The solids (other than fat) include proteins, carbohydrate, water-soluble vitamins, and minerals. Milk products contain high quality proteins. The whey proteins constitute about 18% of the protein content of the milk. Casein, a protein found only in milk, contains all of the essential amino acids and accounts for 82% of the total proteins in milk. Milk also contains calcium, phosphorus, magnesium, and potassium. The calcium found in milk is readily absorbed by the body; Vitamin D plays a role in promoting the calcium absorption and its utilization. Milk is also a significant source of riboflavin (vitamin B2), which helps promote healthy skin and eyes (Dairy Facts, 2003). Dairy products such as yogurt, cheese, and ice cream contain nutrients such as proteins, vitamins, and minerals. Therefore, consumption of dairy products has been associated with decreased risk of osteoporosis, hyper-tension, colon cancer, obesity, and insulin resistance syndrome. The main dietary source of calcium and vitamin D are dairy products (Weaver, 2003). Table 1 shows the milk constituents with their putative physiological effects.

Among the various food products, milk has primarily been identified as having a high potential for the health improvement of human beings. Aside from nutritional values of milk, milk-borne biologically active compounds such as casein and whey proteins have been found to be increasingly important for physiological and bio-chemical functions that have crucial impacts on human metabolism and health (Gobbetti *et al*., 2007).

Table 1: Milk Constituents with Putative Physiological Effects

Milk Component	Health Effect
Butyric acid	Reduce colon cancer risk
CLA (Conjugated linoleic acid)	Modulate immune function, reduce risk of cancer (stomach, colon,breast and prostate)
Sphingolipids	May reduce risk of colon cancer
Stearic acid	May modulate blood lipids to reduce risk of cardiovascular and heart disease
Triglycerides	May enhance long-chain fatty acid and calcium absorption
Whey proteins	May modulate immune system, reduce risk of heart disease and cancer, lower blood pressure
Glycomacropeptide	Prevent dental caries, gingivitis, antiviral, antibacterial, bifidogenic
Immunoglobulins	Antibodies against diarrhea and GI tract disturbances
Lactoferrin	Toxin binding, antibacterial, immune modulating, anticarcinogenic, antioxidant, iron absorption
Lactoperoxidase	Antimicrobia
Lysozyme	Antimicrobial, synergistic with immunoglobulins and lactoferrin
Lactose	Calcium absorption
Calcium	Prevent osteoporosis and cancer, control hypertension

Source: (Hoolihan, 2004)

It is an optimally rich source of essential nutrients such as proteins, fat, lactose, vitamins, minerals, enzymes, hormones, immunoglobulins, and cells. Dairy products are consumed not only for meeting the nutritional requirements of the consumers, but also for their role in preventing various disorders such as obesity, osteoporosis, dental caries, poor gastrointestinal health, cardiovascular diseases, hypertension, colorectal cancer, bone ailments and ageing (Nagpal *et al*., 2012). Milk can be converted into a number of dairy products, and most of these products have already achieved a reputed health-oriented image in the mind of the consumer, and have proved to be an excellent solution for formulating superior nutrition. Overall, dairy foods can be divided into three groups:

- Basic products, that is, milk, fermented milk, cheese, ice-cream, etc.
- Value-added products, in which the milk composition has been changed, for example, low- lactose or lactose-free products, hypoallergenic formulae with hydrolyzed protein for milk-hypersensitive infants, milk products enriched with Ca, vitamins, etc.
- Functional dairy products, primarily those containing pro-biotic bacteria, and quite frequently enriched with prebiotic carbohydrates

MILK: SOURCE OF BIOACTIVE COMPONENTS

Milk contains a wide range of proteins that provide protection against enteropathogens or are essential for the manufacture and characteristic nature of certain dairy products.

Milk has been shown to contain an array of bioactivities, which extend the range of

influence of mother over young beyond nutrition (Gobbetti *et al*., 2007). Peptides are in a latent or inactive state within protein molecules but can be released during enzymatic digestion. Biologically active peptides released from caseins and whey pro-teins contain 3 to 20 amino acids per molecule. Researchers for the last decade have demonstrated that these bioactive peptides possess very important biological functionalities, including antimicrobial, antihypertensive, antioxidative, anticytotoxic, immunomodulatory, opioid, and mineral carrying activities. Beyond essential nutrients milk seems thus capable of delivering many health benefits to humans of all ages by provision of specific bioactive components. Figure 2.1 give an overview of these. A schematic representation of bioactive milk components and their potential applications for promotion of human health is presented in Figure 1.

FERMENTED MILK PRODUCTS

Originally fermented milks were developed as a means of preserving nutrients of milk. Fermented milks including dahi, yogurt are considered as an ideal vehicle for the delivery of many beneficial microorganisms viz probiotics and prebiotics in addition to the microflora of human gastrointestinal tract, therefore fermented milk is the most popular group of functional food. Fermented milk products contain various nutritional components such as bioactive peptides, antioxidants, vitamins, specific proteins, oligosaccharides, organic acids, highly absorbable calcium, conjugated

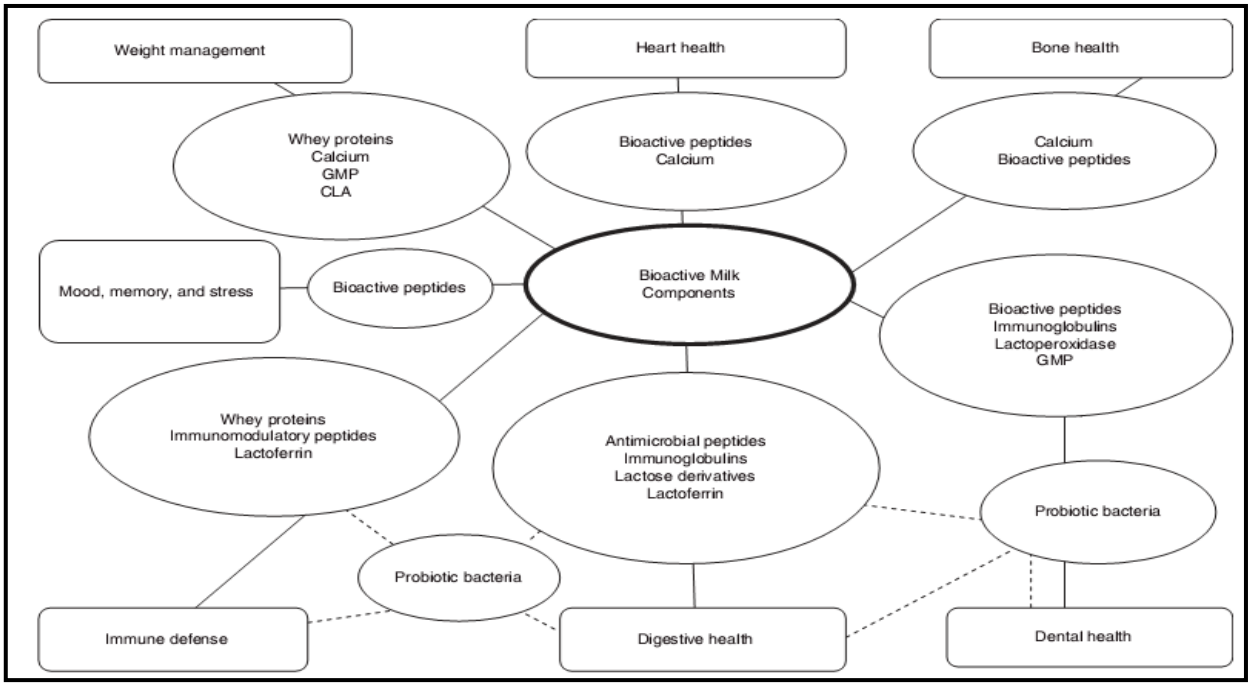

Figure 1: Bioactive milk components and their potential applications for health promotion

linoleic acid and other biologically active components with an array of bioactive functions: modulating digestive and gastrointestinal functions, haemodynamics, controlling probiotic microbial growth and immunoregulation. Many changes occur to the components of milk during fermentation. It has been proved that fermented milks have a higher nutritional composition compared to regular milks, as a result of bacterial fermentation (mainly lactic acid bacteria) under controlled conditions. Furthermore fermented milks have a large variety of flavour and resulting from lactic acid production and the presence of specific aromatic components. Acetaldehyde, diacetyl and acetone give particular flavour to the fermented milks. The nutritional and health benefits of fermented milk products are the result of biologically active components that are present in native milk and also, due to their suitably modulated activities produced by the action of lactic acid bacteria. Health effects are divided into two groups: nutritional function and physiological function. The nutritional attribute is expressed as the function of supplying nutrition sufficiently. The physiological function refers to prophylactic and therapeutic functions beyond nutritional function. Potential nutritional and health benefits of fermented foods are listed in Table 2.

Table 2: Potential Nutritional and Health Benefits of Fermented Foods

Beneficial Effect	Possible Causes and Mechanisms
Improved digestibility	Partial breakdown of proteins fats and carbohydrates
Improved nutritional value	Higher levels of B-vitamins and certain free amino acids, viz. methionine, lysine and tryptophan
Improved lactose utilization	Reduced lactose in product and further availability of lactase
Antagonistic action toward enteric pathogens	Disorders such as diarrhoea, mucous colitis, ulcerated colitis; prevention of adhesion of pathogens
Anticarcinogenic effect	Reduction of carcinogen-promoting enzymes; inhibitory action toward cancers of the gastrointestinal tract by degradation of precarcinogens; stimulation of the immune system
Hypocholesterolemic action	Production of inhibitors of cholesterol synthesis; use of cholesterol by assimilation and precipitation with deconjugated bile salts
Immune modulation	Enhancement of macrophage formation; stimulation of production of suppressor cells and -interferon

Source: Gomes and Malcata, 1999

FERMENTED PROBIOTIC MILK

Lactic acid bacteria are widely used as starter cultures in fermentation of milk, beverages, and bakery products.

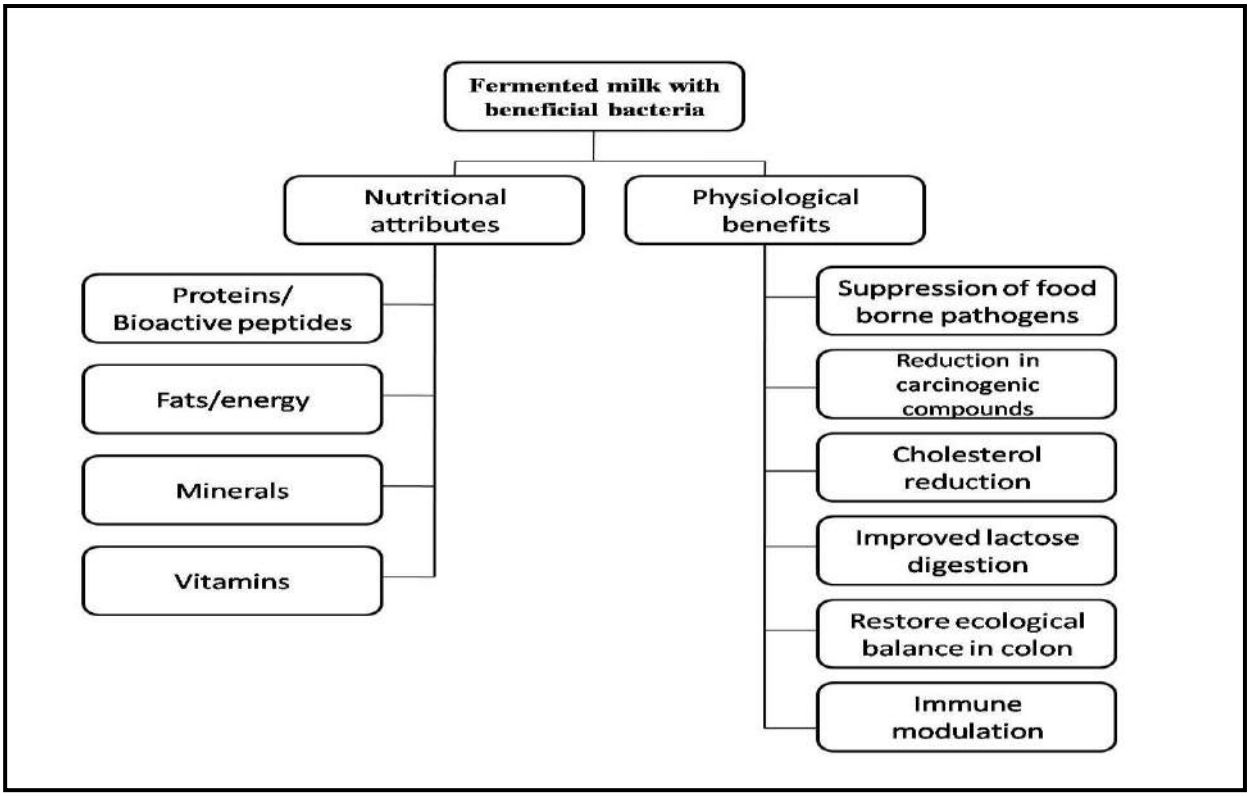

Figure2: Health attributes of probiotic fermented milks. (Chandan, 1999)

Fermentation with lactic acid bacteria results in altered composition, improved flavor, and prolonged shelf life. Lactic acid bacteria are widespread in nature, and are found primarily in the environment with high concentration of carbohydrates, peptides and amino acids, and vitamins. The use of probiotic organisms such as *Lactobacillus acidophilus* and *Bifidobacterium* spp. in fermented milks became popular by the end of 1970s as a result of increased knowledge about these organisms. New fermented products containing *Lb. acidophilus*, *Bifidobacterium* spp., *Lactobacillus casei* Shirota, *Lactobacillus rhamnosus* GG, and *Lactobacillus reuteri* have been developed. However, *Lb. acidophilus* and *Bifidobacterium* spp. are most commonly used as probiotics. It is estimated that over 70 products containing *Lb. acidophilus* and *Bifidobacterium* spp. including yogurt, buttermilk, frozen desserts, and milk powder are produced world-wide. Probiotic organisms are also available as powders, capsules, and tablets (Mittal andGarg, 1992). A number of health benefits are claimed in favor of pro-biotic organisms including antimicrobial properties, control of gastrointestinal disorders, improvement in lactose metabolism, anticarcinogenic properties, and reduction in serum cholesterol. Figure

2 shows the health and nutritional attributes of probiotic fermented milks.

CONCLUSIONS

Milk and dairy products have long been recognized as an important component of a balanced diet. Milk is a rich source of nutritive compounds that can be enriched and/or further modified to give the best benefit to the consumers. The current global interest in developing health promoting functional foods provides a timely opportunity to tap the myriad of innate bioactive milk components for inclusion in such formulations. It is important to remember that therapeutic dairy products are mainly for supplying nutritive foods for everyday consumption. Fermented dairy products render tremendous potential as carrier of functional ingredients required for health and wellness of the human beings.

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