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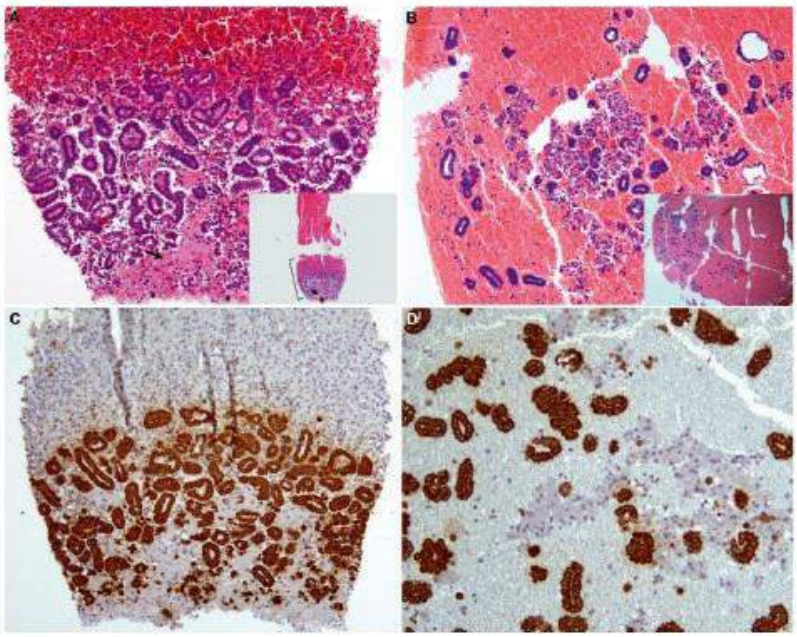

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Reading the Sequence of DNA

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Abstract

Reading DNA sequence means determination of the order of arrangement of nucleotides in the DNA. The four nucleotides that makes up DNA are: Adenine, Guanine, Cytosine, and Thymine. The different generations of DNA sequencing are: first generation that consists of Sanger sequencing method and Maxam-Gilbert sequencing method; second generation consisting Roche 454, Illumina Solexa, and ABI-SOLiD techniques; third generation consists of Helicos, PacBio and Ion Torrent and fourth generation consists of Nanopore sequencers. The first-generation sequencing methods have limitations which are that it could sequence very less number of DNA sequences in one go and that the cost per base is very high. Therefore, development of the high throughput new generations of sequencing are required to sequence the genome of huge number of individuals or organisms for the diagnosis and treatment in short period of time with low cost.

Key words: Animals, Health problem, Slaughterhouse.

Introduction

Sequence of DNA can be read by different methods. Reading DNA sequence means determination of the order of arrangement of nucleotides in the DNA. The four nucleotides that makes up DNA are: Adenine, Guanine, Cytosine, and Thymine. The first or early DNA sequencing methods includes Sanger Sequencing method and Maxam-Gilbert Sequencing method. DNA sequencing can help in determination of the sequence of individual genes, clusters of genes, full chromosomes, or entire genomes of an organism. This can be applied in study genomes and the proteins they encode to identify changes in genes, associations with diseases and phenotypes, and identify potential drug targets; genetic testing to determine if an individual has any risk of genetic diseases; in identification of an organism from any source and in forensic study.

Generations of Sequencing

The methods of DNA sequencing can be of different generations. These different generations are: first generation that consists of Sanger sequencing method and Maxam-Gilbert sequencing method; second generation consisting Roche 454, Illumina Solexa, and ABI-SOLiD techniques; third generation consists of Helicos, PacBio and Ion Torrent and fourth generation consists of Nanopore sequencers. The first-generation methods are the mostly used sequencing methods but have limitations. These limitations are that it could sequence very less

number of DNA sequences in one go and that the cost per base is very high. Therefore, development of the high throughput new generations of sequencing are required to sequence the genome of huge number of individuals or organisms for the diagnosis and treatment in short period of time with low cost. While the advantages of the new generations include sequencing of larger number of sequences instantly with lower cost per base and provides high throughput data within lesser time and more accuracy.

First Generation of Sequencing

Fredrick Sanger gave the first method for DNA sequencing, called Sanger sequencing or chain termination method back in 1977. Allan Maxam and Walter Gilbert developed the chemical method of DNA sequencing the same year. Sanger sequencing depends on incorporation of specific chain-terminating dideoxynucleotides by DNA polymerase. In Sanger sequencing method, DNA primer is used for the DNA synthesis, this primer is complementary to the template DNA (that is the DNA to be sequenced) and the four deoxynucleotide triphosphates (dATP, dGTP, dCTP, and dTTP) are used to extend the primer by adding the complementary dNTP to the template DNA strand using polymerase, then, the four terminating nucleotides – dideoxyucleotide triphosphates (ddNTPs: ddATP, ddGTP, ddCTP, and ddTTP) labelled with a distinct fluorescent dye are used to terminate the DNA synthesis thereby sequencing the DNA. Whereas Maxam-Gilbert is based on chemical modification of DNA and subsequent cleavage at specific bases. Maxam-Gilbert sequencing method requires radioactive labelling at one end of the DNA to be sequenced, chemical treatment with specific chemicals for specific nucleotides break the DNA to produce small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G, C, C+T); different chemicals may be Dimethyl sulfate for Adenine and Guanine, Piperidine for Guanine, Hydrazine NaCl for Cytosine and Hydrazine for Cytosine and Thymine, therefore, a series of labelled fragments is generated from the radiolabelled end to the first cleaved site in each molecule. The sequencing is then done by gel electrophoresis.

New Generation Sequencing Methods

Development of advanced sequencing is important with the increasing population and the increase in different disease conditions both in human and animals. The new generation methods are the advanced methods of DNA sequencing which run faster and more accurate with high throughput data.

Second-generation sequencing technology: They are also known as next generation sequencing technology and includes Roche 454, Illumina Solexa, and ABI-SOLiD. Roche 454 depends on the principle of pyrosequencing, Illumina Solexa depends sequencing by synthesis on and ABI-SOLiD depends on sequencing by ligation. They all follow four general steps for sequencing – Library preparation, cluster generation, DNA sequencing and Data analysis. They permit running of millions of sequencing reactions in parallel on the same solid surface which may be beads or glass slide and do not require the physical separation of reaction in different well or tube but spatially separated. Hence, thousands of different reactions can occur simultaneously. So, they are able to produce enormous amount of data at very economic cost and expediture. Moreover, these technologies are more rapid than traditional method that whole genome of small organisms can be sequenced in a single day.

Third-generation sequencing: They are also known as next next-generation sequencing or Long Read sequencing and includes Helicos and PacBio and Ion Torrent. This generation refers to those technologies which do not require amplification step and are capable of sequencing single DNA molecule in real time. These platforms have the capability to provide single run at very low cost as well as made the preparation of sample easier. Further, third-generation platforms produce generally longer read of about some kilobase length, which resolve the problem of assembling the reads.

Fourth-generation sequencing: This generation includes Nanopore sequencers offered by Oxford Nanopore Technologies (ONT), namely, GridION and MinION. They are based on principle of ligation chemistry. These methods have the ability to in situ (perform sequencing directly in the cell) sequence the fixed tissue and cells and are able to offer simultaneous visualization and quantitative analysis of the transcriptome in the fixed tissues.

Conclusion

DNA sequencing can give the whole genetic information of the organism. This has a large range of application in human, animal and plant biotechnology – in studying the evolutionary biology to understand the mechanism of adaptation to the changing of the environments; in studying the genetic diseases of the different organisms; in genetic testing of the organism whether it has the genes for a disease or not and in development of personalized medicine as every individual has different genetic make-up, so, each will have different treatment and management of the condition. Sequencing of the genes has a few challenges. The lack of computing skill, requirement of different hardware's and expensive equipment and reagents are the main challenges of the present sequencing methods. DNA sequencing is one of the foremost technologies required in the development of human, animal and plant biotechnology.

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Alopecia and Hypotrichosis in Goat: Causes, Diagnosis and Therapeutic Management

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Abstract

Alopecia and hypotrichosis in goat is very common in farm condition. The condition may start even in the early ages to adult animal. The pre4valence may vary in different seasons. There are several causative agent for the conditions. Infectious causes may be bacteria, viruses, parasites, nutritional deficiencies, photosensitivity, traumatic injury and even autoimmune disorders**.** The disease may be transmitted if it is infectious. Clinically the Animals show baldness on the skin surface where previously there were hairs. Alopecia may be confined to a particular area or all over the body surface depending on the severity and nature of agents. Diagnosis of the disease could be done by clinical signs and manifestations. Isolation and identification of bacteria, virus, and parasites could be done by cultural, staining of culture, serological, biochemical characterization and molecular means. Early diagnosis and therapeutic intervention may restrict the production loss. Alopecia causes economical loss through production loss, morbidity and medical expenses.

Key words: Alopecia, Goat, Skin lesion, therapy

Introduction

Alopecia is a condition when a complete loss of wool or hair is lost from a part of body particularly where the body surface is covered with hairs and wool. Hypotrichosis implies a less densely population of hairs in comparison to normal conditioning a species of animals. Hypotrichosis may a condition occurs due to hereditary traits. Folliculitis is the Inflammation targeting the hair follicle that leads to destruction or damage of the hair shaft. Alopecia the pathological condition in goats is frequently seen. This may be due to loss of hair follicle as well as less production of hair follicles due to inherited conditions like hypotichosis, follicular dysplasia

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and dyserythropoiesis and dyskeratoniosis. Due to wide ranges of etiology it is difficult to diagnose of the condition and effective treatment lagged behind. Therefore, alopecia may be due to various reasons may be described as follow

Causes of alopecia

This could the due to broad two categories. Traumatic and non-traumatic origins. The traumatic causes are many which may be due to injury of skin. It may be due to scratch, rubbing of skin against hard objects, over groom, and accidental exposure with sharp tools.

Non traumatic causes of alopecia may be due to wide range of etiology. It could be virus, bacteria, fungi, parasite, neurogenic damage of peripheral nerve damage, metabolic alopecia due to deficiency, poisoning (thallium, selenium, arsenic, mercury), sterile eosinophilic folliculitis in cattle, wool slip and various other diseases like parakeratosis, hyperkeratosis, cutaneous neoplasia, hypothyroidism, hyper-adrenocorticism, several other causes like nutritional, metabolic disorders such as iodism, zinc deficiency, Selenium toxicosis, chronic hepatic disorders.

Infectious causes of skin disorders are much prevalent in goats. Several infectious agents are responsible for alopecia in skin particularly on face, head, thigh, back, abdomen, and tail and so on. In goat infections with virus, bacteria, fungus, parasites are main organisms those cause primary as well as secondary alopecia.

Virus- Goat pox virus, FMD, vesicular stomatitis, malignant catarrhal fever virus.

Bacteria- *Dermatophilus congolensis, Staphylococcus aureus, Spherophorus necrophorus, Actinomyces pyogenes.*

Fungus- *Microsporum nanum, Trichophyton verrucosum,Candidia sp*

Parasite- *Sarcoptes scabiei var caprae, Demodex caprae are common* while surface-feeding mites *Psoroptes cuniculi* and *Chorioptes bovis*, chorioptic scab mite (*C. bovis*) may cause dermatitis and alopecia. Goat biting lice (*Bovicola crassipes*) and *Bovicola limbata,suckling lice Linognathus stenosis* and *Linognathus africanus*, biting flies such as *Stomoxys calcitrans*,blackflies, midge

Photosensitivity- Photodynamic agents like soap, detergent, olaquindox (fattening), Phylloerythrin derivative of chlorophil can cause alopecia.

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Nutritional- Deficiency of Zinc, iodism, copper, selenium both hypo and hyperselenosis, vitamin A, vitamin B2,B6,Niacin, malnutrion with protein and aminoacids.

Autoimmune disorders- Autoimmune blistering diseases (AIBD) with pemphigus vulgaris (Ackerman 1985) and bullous pemphigoid (BP) (Anhalt et al,1983). These skin conditions are typically characterised by autoantibodies against structural proteins in the epidermisand/or the basement membrane on cutaneous and mucosal surfaces. Currently, four variants of pemphigus are recognized naely vulgaris, vegetans, foliaceus, erythematosus.There two types of pemphigoid such as bullous and cicatricial are prevailing

Transmission and pathogenesis

The causes of the disease are not always infectious ones. Therefore it not always transmitted from infected animal to other animals directly. Infectious agent such as virus, bacteria, parasites, fungus can be transmitted from one host to other via direct contact, indirectly through feed, fodder, water and even through aerosol transmission in case of viral agents. Prolong wetting on skin causes sebaceous gland torsion and secondary bacterial invasion may occur in case *Dermatophilus congolensis* and other bacterial infection. Continuous wetting helps in activation of the zoospores present in the skin may also transport the spores to other non-infected sites on the goat and other animals.

Normal shedding of hairs from the animal is regular process during environmental changes such as long winter, high temperature summer, high exposure the sunlight etc. In inherited alopecia there may reduction in numbers of hair folicles in the skin. Infectious etiologies such as dermatophytosis, demodicosis, inflammatory diseases, traumatic episodes cause death of hair follicle and autolysis of hair follicle and surrounding tissues. Several diseases that cause slow growth of hair follicles are nutritional deficiency (Protein, vitamin A helps to prevent breakdown of collagen, vitamin C helps collagen formation), hypothyroidism, hyper-adrenocorticism and excess estrogen production. Other vitamins such as biotin, riboflavin, pyridoxine, folate and pantothenic acid have role for maintenance of skin health

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Clinical signs

The general clinical signs in alopecia are the partial or complete absence of hair from an areas of the body where it normally grows; or earlier there were hairs those have been denuded .Alopcia may be distributed all over the body parts or to a limited area. The condition may vary depending on the aetiologies and its severity. Crushing lesion against the hard object can be seen on the skin,in some cases greasy or crusty flecks may be prominent on the affected areas. Inflammatory changes such as: lichenification, hyperpigmentation, erythema may be seen in infectious alopecia. The clinical signs vary with different atiological factors. When fibres fail to grow on the skin surface it appears shynny and clean. In congenital alopecia the hair covering is absent in patches of skin. Viral alopecia may severe in cases goat pox, FMD and vesicular stomatitis. Bacterial alopecia along with other clinical involvements may be there. As general alopecia is discrete with pus formation on the skin surface. Fungal alopecia is characteristic in almost all animals, fungal growth lesions are usually rounded, raised and centrifugal in nature that may be irritating. The fungal alopecia may localized or general all over the body surface (Fig-1). In goat most affected parts with alopecia is head and ear region (Fig-2)

Fig-1: Goat alopecia in different locations. Fig-2: Alopecia on head and ears only

Characteristic alopecia can be seen in most of the cases of parasitic infection in and on the skin surfaces. Besides autolysis of hair follicle dead tissue debris thick deposition on the skin may be seen in different parts of body Para lumbar region, hind limbs, lower abdomen, face, ear, nose, tail and back region. Alopecia due photosensitivity may be seen in some cases with clinical signs such as edematous swelling of lips, eye lid, vulva, facial area. Nutritional alopecia may be a chronic in nature and progressive and characteristic dermatological changes may be seen with different

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deficient nutrients. Traumatic alopecia may be due to damage of skin integrity cause scar tissue formation and lesion with complete damage of hair follicles.

Diagnosis

Skin lesions with alopecia may be due to vary wide ranges of etiology, therefore a thorough clinical history and physical examination are needed. Detail physical examination for types and distribution of lesions are essential. **Fibers** should be examined to determine if they are being shed from the follicle or broken off, which suggests pruritus. Breeds of goats may be studied to ascertain congenital predisposition and genetic proneness. Several diagnostic specific tests may be done for correct diagnosis of the disease.

Microscopic Skin scrapings examination- Skin scraping may be taken a fresh from the skin lesion; the scraping may be digested with 10% KOH and is examined under microscope. Mange causing parasite may be seen in the scraping may be due to parasitic infection of Sarcoptic/psorotic or else.

Screening of hair by brush- Some time it happens that several ectoparasites such as fleas, keds, mites and lice may be infested on the skin and they fed on the skin tissue and blood causing alopecia. Brushing with comb may be help to identify their presence

Fungal and bacterial isolation- May be done by detailed cultural, biochemical and staining studies. Bacteria and fungi may be isolated in different media and their identification may be done based on biochemical characterization and molecular methods.

Skin biopsy and histological study- This can be done to identify the etiology and in-depth pathogenesis for advance treatment.

Economic impact

Healthy goat skin is very valuable for the finishing stage of animals. Good skin shows the healthy status of meat and milk production. Skin disease indicates loss of production in different degrees according to area involvement. Infections that would causes reduction of sale values of goat. Skin after slaughter is a costly byproduct, each raw skin provides money amounting Rs150-180. Therefore, good skin is the index of sale value of goat that also fetches money saling salted raw skin. Alopecia causes economical loss through production loss of milk, meat, skin, fibre, morbidity and medical expenses

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Treatment

This will depend on the underlying cause of the alopecia.Successful therapy depends on the underlying cause and specific diagnosis (Corke and Matthews, 2018).Comprehensive parasite control should be started to ensure that the cause of hair loss is not pruritis associated with parasite infestation. In addition, topical antimicrobial therapy can be started to rule out concurrent bacterial, fungal or yeast infections. Antibacterial for skin infection are Novobiocin, Clindamycin, Cephalexin, Vancomycin, Piperacillin-tazobactam can be used asper nature of bacterial involvement. Antibiotic with steroidal compounds may be used for extended response of skin infection. Combination of antibiotic and corticosteroid may be used such as Bacitracin / Hydrocortisone / neomycin / polymyxin B. For fungal infection nystatin / triamcinolone, hydrocortisone / ketoconazole may be used For mange and mites several acaricidal may use such as arsenical, chlorinated hydrocarbons (lindane), organophosphorus (coumphos), formamidines (amitraz)pyrethroid (flumetrin), macrocyclic lactones (ivermectins). These acaricide may be used as dipping or spray or even injection (ivermectin).

Autoimmunity may be treated with Rogain or minoxidil 2-5% solution may be applied in the skin surface for regain of hair growth. Deficiency oriented alopecia may treated with specific causes and their supplementation.

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Pesticides Residues in Feed and their impact on Animal Health and its Product

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Abstract

In a developing country like India; to ensure the national food security and also to protect crops from deadly pests, application of pesticides and insecticides plays a very constitutive role. But simultaneously use of such substances also affects the health of ecosystem, humans and animals. Exposure to pesticides causes a wide range of health problems. It poses significant risks to the environment ranging from beneficial soil microorganisms, to insects, plants, animals, humans, birds etc. The residues not only affect the public health but also cause economic losses to the livestock industry in terms of health of livestock and resultant poor quality of animal products.

Introduction

Pesticides are chemicals used in agriculture to protect crops against insects, fungi, weeds and other pests. In addition to their use in agriculture, pesticides are also used to protect public health in controlling the vectors of tropical diseases, such as mosquitoes. As per World Health Organization (2018), pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). It covers a broad variety of compounds like insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others.

Pesticides play a significant role in food production. They protect or increase yields and the number of times per year a crop can be grown on the same land. This is particularly important in countries that face food shortages. Pesticides can prevent large crop losses and will therefore continue to play a role in agriculture. However, the effects on humans & livestock & the environment of exposure to pesticides are a continuing concern.

Owing to the use of persistent pesticides in crops, there is always a risk of low levels of their residues occurring in animal feed. Therefore, animal feed, whether in the form of commercially available materials or from natural grazing on grass or straw, provides the main exposure route for animals to environmental contaminants and pesticides. The consumption of contaminated animal feed and fodder by

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the food producing animals further leads to occurrence of their residues in animal products like milk, meat and eggs. Unless the residues are managed at the pre/post-harvest stages or during the storage of animal feeds, it is very difficult to prevent contamination of animal products.

Acc. to World Health Organization (WHO); Pesticide residue can be defined as "any substance or mixture of substances in food for man or animals resulting from the use of a pesticide and includes any specified derivatives, such as degradation and conversion products, metabolites, reaction products, and impurities that are considered to be of toxicological significance." As they are intrinsically toxic and deliberately spread in the environment, the production, distribution, and use of pesticides require strict regulation and control. Regular monitoring of residues in food and the environment is also required.

According to Gigliotti and Allievi (2001), pesticide residues in soil affect the soil microbial biodiversity. Some pesticides, particularly organochlorine, suppress symbiotic nitrogen fixation resulting in reduced crop production. Nitrification bacteria are very sensitive to pesticides and herbicides, and sulfonyl-urea herbicide has been found to inhibit this process.

Indian Underplot Pertaining To Pesticides

Approximately, 2 million tons of more than 800 different kinds of pesticides are used every year worldwide; out of which India accounts for only 3.75%. As per IAASTD global report (2009),

The consumption of pesticide in India is about 0.6kg/ hectare, while that of developed countries is touching 0.3kg/ hectare which is far lower than many other developed countries. But still the problem of pesticide residue is very high in India. A significant portion of the chemicals applied; has proved to be excessive, uneconomic or unnecessary both in industrialized and developing countries. Effects of pesticides have been reported in milk, feed, cottonseed, different fruits, vegetables and fish meal at different intervals. This can also occur through direct contact with the compound during manufacture, formulation or use.

The world's largest pesticide consumer is Japan and the largest pesticide market is in Asia. In India, the usage of pesticide started in 1948 with the application of DDT. Now, India is the 4th largest global producer of pesticides after the USA, Japan, and China. In India, the consumption pattern of pesticides is tilted more towards the use of insecticides that too organophosphates in comparison to other pesticides.

In India, only 84 out of 230 registered pesticides, are actually used in the agriculture sector, and only 25–30% of the total cultivated area of the nation, i.e. 143 million hectare is under pesticide cover.

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Classification of Pesticides

1. **Organophosphate (OP) pesticides:** It includes some of the most toxic chemicals used in agriculture. OP compounds inhibit an enzyme, acetyl cholinesterase (AchE) at cholinergic junctions of the nervous system. Most organophosphates are insecticides like diazinon, Malathion, coumaphos.

2. **Organochlorine insecticides:** It includes pesticides like DDT, chlordane, aldrin, dieldrin, heptachlor etc compounds.

3. **Carbamate pesticides:** It includes pesticides like Aldicarb, carbofuran, carbaryl, carbosulfan etc.

4. **Pyrethroid pesticides:** Some of the commonly used pyrethroid are Deltamethrin, cypermethrin, permethrin.

Source of Contamination

Apart from pesticides use for crop production, their indiscriminate usage during storage also play a role in the case of oil cakes, grains and milling products. Contamination of the soil and water sources used for drinking purpose of animals also forms another source of pesticide residue in animals. Direct contact of the animals with pesticides during control of external parasites on animals and insects and fly control in cattle yards and sheep sheds also form other source of pesticide residues in animal body.

Mannivannan (2001) reported that pesticide affect the quality of dairy products by inhibiting the metabolic activities of starter bacteria. Fodder maize and jowar retained residues even during harvesting which were sprayed during early stages of growth. Prasad and Chabra (2001) reported that concentrate to be an important source of pesticide residue intake by animals.

Methods of Pesticides Determination

Chromatographic separation techniques assumes greater significance in the quantitative and qualitative analysis of pesticide residues. Commonly used techniques are – GLC (Gas liquid chromatography), HPLC (High-performance liquid chromatography), GC-ECD (Gas chromatography-electron capture detector), GC-MS (Gas chromatography-mass spectrometry) etc.

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Impact of Pesticides on Animal and Its Product

Pesticides induces oxidative stress in body and the stress markers present in plasma leading to generation of free radicals and resulting in many debilitating chronic diseases. Exposure to DDT and its metabolites cause's eggshell thinning as a result of this; bald eagle population in the United States declined. Organophosphorus degrades slowly in the aquatic ecosystem, and are accumulated by crustacean and fish causing adverse effects. Organochlorines also greatly affects the top predators in terrestrial food chains and accumulates in adipose tissues of animals and humans, transferred to young ones through milk and act as endocrine disruptor.

Some herbicides may produce acute toxicity and sub lethal effects on fish that reduces their chances for survival. Glyphosate or glyphosate-containing products can cause sub lethal effects in fish such as erratic swimming and labored breathing. 2, 4-Dherbicides caused physiological stress responses in sockeye salmon and reduced the abilities of food-gathering in rainbow trout.

Domestic and wild animals may also have adverse effect on the health depending on how and where the compound is used and its persistence after use, but this is usually accidental. Animals can gather these substances from contaminated feed and water. Owing to the lipophilic nature of these pesticides, milk and other fat rich substances are the key items for their accumulation.

Amongst all meat products, greatest contamination observed in broiler chicken muscle followed by goat and beef. The cumulative occurrences of pesticide residues in the meat and milk are of a great concern for ensuring food safety and human health. Higher contents of organo-chlorine pesticide residues have been reported in meat and milk samples collected from different locations of the country.

Pesticides have been associated with serious adverse effects in birds, man and animals like causing carcinogenicity, teratogenicity, mutagenicity, infertility & birth defects, embryo toxicity, immunosuppression, hepatotoxicity, nephropathy, hypersensitivity etc.

Pesticides in the environment may play an important role in contributing to underlying causes of fertility problems in dairy livestock. In female animals; the pesticide exposure induced alterations which includes poor reproductive behavior, sub-fecundity, infertility, pregnancy loss, growth retardation, intra-uterine fetal demise, ovarian failure.

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Endocrine disruptors or reproductive toxicants modulate or upset reproductive hormone milieu by acting at a variety of sites including hypothalamus, pituitary and reproductive organs. Pesticide residues can be detrimental to male reproductive system by causing toxicity to sperm plasma membrane.

Conclusion

Pesticides have proved to be a useful for the farmers as well as for people all around the world through their contributions to increase agricultural yield and by providing benefits to society. The issue of hazards posed by pesticides to livestock health and the environment are of significant concern. Since animal feed plays an important part in the food chain and has implication for the composition and quality of the livestock products that people consume. Therefore proper monitoring and establishment of regulatory standards of pesticides use are the alternative ways to prevent the adverse effect of pesticide residues.

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Subinvolution of Placental Sites in bitches – An Overview

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Abstract

Subinvolution of placental sites (SIPS) occurs when normal healing doesn't take place or when there is delay in the normal uterine involution at the site where the placentas of fetuses attached to the wall of the uterus, this may lead to continuous bleeding from vagina in postpartum female dog, the underlying cause is not fully known, but affected bitches are mostly presented with persistent sanguineous vulvar discharge during postpartum period. For definitive diagnosis histological examination of surgically removed uterus is required. Spontaneous remission is common, but my take several months, hence therapeutic intervention to end the discharge is required.

Introduction

Subinvolution of Placental Sites (SIPS) or placental site vascular subinvolution or subinvolution of placental arteries - is the failure of the process of reparation of the endometrial lining of uterus (within 9 weeks endometrial changes take place and by 12-15 weeks involution is complete) in a postpartum female dog and sloughing of the superficial arteries at the placental attachment, which may result in persistent sanguineous uterine bleeding. The etiology of this type of bleeding is unknown. In order to establish the cause of bleeding, uterine biopsy or endometrial curettage is to be performed. Typical clinical feature and histology of the hysterectomy specimen or uterine biopsy.

Clinical Features and Diagnosis

Delayed postpartum bleeding, although bleeding in SIPS is reported at any time, but most commonly reported within Second week after delivery. Persistent postpartum vulvar hemorrhage. Abrupt onset of continuous and persistent bleeding prompts the owner to seek therapeutic intervention. The diagnosis is based on detailed history of the case, detailed examination of

animal which may include bodily condition, Temperature, Respiration and Pulse rate, clinical examination of the mucus membranes may provide vital information and duration nature of discharge, vaginoscopy (to determine the origin of bleeding and to rule out other reproductive diseases), mode of parturition and litter size. Clinical data which includes serial blood work, serum progesterone assay, vaginal cytology ultrasonographic evaluation of uterus.

Blood picture may reveal reduced red blood cells count, chronic vulvar discharge or bleeding reveal anemia in bitch and marked elevation in white blood cells count indicates infection. To investigate the uterine involvement or assess the size of uterus and rule out possibility of retained placentas or fetuses, transabdominal ultrasonography is to be performed. In ultrasonographic examination, there may be enlargement of uterine lumen near the placental attachment and presence of fluid within the uterine lumen. Excessive fluid within the uterus is suggestive of infection or inflammation of uterus. Tentative diagnosis of SIPS was made on the basis of signalment, history, clinical findings.

Treatment

Subinvolution of placental sites can be therapeautically managed by certain specific medications, which includes antibiotics, megesterol acetate, methylergometrine hydrogen maleate, combination of carnitine, vitamin B12, L-arginine, methionine, L aspartate and fructose. Megesterol acetate is synthetic progestogen, low dose of progestagen at 0.1 mg/kg for first week and then 0.05 mg/kg for second week is effective in stopping the continuous vaginal bleeding in bitches with SIPS. The tablets can be administered intact or crushed and mixed with food. Methylergometrine maleate is a semisynthetic ergot alkaloid, when given orally as an adjuvant therapy, it helps in control of postpartum bleeding. Amino acid and vitamin supplementation are to be prescribed as an appetite enhancer and treatment of the condition. During the course of medical treatment, closely monitor the bitch through serial examinations of hematological profile, vaginal examination, microscopic evaluation of vaginal discharge is recommended on weekly basis and ultrasonographic evaluation of uterine endothelium and uterine lumen. Surgical intervention (spaying) is recommended if bitch is not to be used for future breedings.

Discussion

Subinvolution of placental sites is a cause of delayed postpartum bleeding. As the uterine involution process in bitches is slow and usually require 12 weeks or 15 weeks for complete involution process. If the process of normal involution is delayed or failed, or failure of regression

of fetal trophoblasts, this may result in subinvolution. Subinvolution implies an idiopathic not an iatrogenic cause of delayed postpartum bleeding. Exact cause for this condition is not known, several theories related to cause of subinvolution is suggested, but not fully proven. However, uterine biopsy or histological findings of hysterectomy specimen are the main diagnostics regarding subinvolution. Presumptive diagnosis can be made based on ruling out other diseases that may cause vulvar bleeding in postpartum bitch. Therapeutic interventions include use of antibiotics, prostaglandins, progestin, and ergot alkaloids. Surgical treatment approaches include ovariohysterectomy. It is important to closely monitor the patient weekly for any signs of bleeding. Generally, the prognosis is good without affecting the future fertility.

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Cyniclomyces guttulatus - A Pathogen or Commensal?

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Abstract

Cyniclomyces guttulatus is a common inhabitant of gastrointestinal tract of rabbit. They are budding, sporogenous, symbiotic ascomycetous yeast and part of the normal microflora of the gastrointestinal tract of rabbits, guinea pigs, chinchillas, rats and mice. However, it is not a normal inhabitant of gastrointestinal tract of other animals such as dogs and cat and are known to cause severe gastrointestinal problems in these animals when ingested accidentally. In rabbits, *C. guttulatus* has been reported to worsen the disease caused by coccidia and other gastro-intestinal pathogens. Faecal sample examination is the most common method of diagnosis. Antifungals like nystatin and fluconazole are used for the treatment in canines and felines. Since *C. guttulatus* is a commensal in the gastro-intestinal tract of rabbits, primary importance must be given to the prevention of other pathogens and parasites that may cause co-infection with this yeast. Hence, regular deworming along with proper use of coccidiostats is advocated in rabbit farms.

Introduction

Cyniclomyces guttulatus is a budding, sporogenous, ascomycetous yeast and a commensal in the gastrointestinal tract of rabbits, guinea pigs, chinchillas, rats and mice. Cells are ovoid to cylindrical in shape. They occur as single, in pairs and infrequently in short chains. The yeast was initially named as *Cryptococcus guttulatus* which was then reclassified and renamed as *Cyniclomyces guttulatus*.

Cyniclomyces guttulatus was first observed in the stomach and intestinal contents of rabbit by Remak in 1845. Vegetative cells pass through the gastrointestinal tract and are excreted with the faeces, while some of them form ascospores in the large intestine of rabbits. Large number of *C. guttulatus* is passed in the faeces to the environment and they persist in the environment for long period of time due to the ability to form ascospores. Unlike rabbits, the organism has been reported to cause severe gastrointestinal signs in other animals like dogs. Chronic diarrhea was reported in dogs diagnosed with *C. guttulatus* by Mandigers *et al.* (2014) and Winston *et al.* (2016). Similar symptoms were reported in cats by Andersen *et al.* (2018). The dogs and cats usually acquire the infection by the ingestion of *C. guttulatus*

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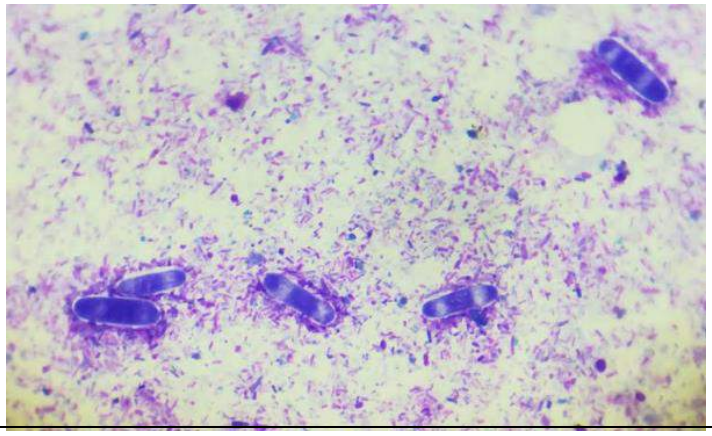
containing fecal pallets of rabbit or plants contaminated with faeces. In rabbits, though it is a commensal when present alone, it becomes pathogenic and causes gastrointestinal symptoms when present with other organisms like *Eimeria* spp. (Shi *et al*., 2021). Massive death of rabbits due to co-infection of *Cyniclomyces guttulatus* with *Passalurus ambiguus* and *Eimeria* spp. have been reported (Sioutas *et al*., 2021).

Diagnosis

The most common method employed for diagnosis is faecal sample examination. Microscopically, the vegetative cells of *C. guttulatus* are ellipsoid, double walled, colourless, approximately 20-50 µm in length and occupied by two large vacuoles in the cytoplasm. It is identified by characteristic spectacle case shape. Faecal impression smear can also be used for diagnosis. In cultures using Sabouraud's Dextrose Agar under microaerophilic conditions, the organism forms light brown glistening colonies. Molecular methods like PCR from faecal samples or the cultured colonies can also be performed. Organisms are also identified from other clinical samples such as gall bladder aspirate from dogs, urine, nasal biopsy and intestinal biopsy (Winston *et al*., 2016).



***Cyniclomyces guttulatus* in faecal smear (Giemsa, 100X)**

Treatment and control

In dogs, the specific antifungal drug used for the treatment of *C. guttulatus* is nystatin. The dosage varies from 20,000 IU/kg body weight orally once daily for three days to 1,666 IU/kg body weight orally twice a day for 4 weeks. Prednisone may be administered if eosinophilic infiltration of gastrointestinal tract is suspected. Hypoallergic diet may be advised. Fluconazole at a dose rate of 5mg/kg body weight can also be used for the treatment of *C. guttulatus* (Ferraz *et al.*, 2019). In cats, nystatin is found to be effective at a dose rate of 15,000 IU/kg body weight once daily orally for 4 days (Peters and Houwers, 2009). Regular

deworming should be performed in rabbit farms in order to prevent co-infection of *C. guttulatus* with parasites. Close proximity of pet animals with rabbit farms must be avoided.

Economic Impact

Diarrhoea is a very common condition causing more than 50% of mortalities in rabbit farms. A wide range of microbes including viruses, bacteria like *Escherichia coli*, *Clostridium welchii*, *Pasteurella multocida*, *Salmonella typhimurium*, parasites like *Eimeria* spp., *Passalurus ambiguus*, *Cryptosporidium* spp., *Giardia duodenalis* have been reported to cause diarrhoea in rabbits in association with *C. guttulatus* (Shi *et al*., 2021). These gastrointestinal pathogens lead to loss of production due to reduction in growth rate and feed conversion efficiency.

Conclusion

Cyniclomyces guttulatus is a commensal in the gastrointestinal tract of rabbits that may turn pathogenic under certain conditions causing massive loss of production. Hence utmost care must be given to the prevention of gastrointestinal pathogens and parasites by proper management practices. Considering the high prevalence of *C. guttulatus* in rabbits, the potential harm of this organism to the rabbit industry warrants attention and further investigation. The pathogenic effect of this yeast in other livestock and pets also needs exploration.

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Role of niacin supplementation in ruminants

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Abstract

Niacin has beneficial role for the metabolism of man and animals. Niacin functions metabolically as a component of the coenzymes NAD and NADP. In addition, niacin is known for its anti-lipolytic action via a hydroxycarboxylic acid-2-receptor-dependent mechanism. The anti-lipolytic effects of traditional free niacin supplementation during transition periods had been studied extensively. Many studies showed positive effect of a niacin supplementation on rumen protozoa, Improves the rumen fermentation pattern as well as no. of the rumen microbes, which stabilize the ruminal environment. Increases the digestibility of the dietary nutrient thus improves nutrient utilization by the animals which ultimately improves growth performance of the animals. It has anti-ketogenic effect, it prevents the fat mobilization and increases the blood glucose levels thus increase energy use and prevents ketosis. Climate change, with a constant increase in the earth temperature, negatively affects livestock production and health. Niacin reduces the heat stress of the animal leading to increased dry matter intake and production performance of the animals.

Key words- Niacin, rumen fermentation parameter, Growth performance, Nutrient utilization, Heat stress

INTRODUCTION

Niacin is a generic name for pyridine 3– carboxylic acids and chemically, it is one of the simplest vitamins, having the empirical formula $C_6H_5O_2N$. Niacin is present as nicotinic acid in plants and nicotinamide in animals. Both the physiologically active forms are derivatives of pyridine. Niacin is the generic term of nicotinamide and nicotinic acid, obtained by the oxidation of nicotine, one of the B complex vitamin, water soluble, white crystalline substances resistances to heat, oxidation and alkali most stable vitamin. Tryptophan is the precursor of niacin and pyridoxine is required for its action. 60 mg of tryptophan converts to 1mg niacin. Niacin mainly function in the coenzyme forms of nicotinamide i.e. NAD and NADP. Enzymes containing NAD and NADP are important links in a series of reactions associated with the metabolism of carbohydrate, protein and lipid. They are particularly important in the metabolic

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reactions that provide energy to the animal. The coenzymes act as an intermediate in most of the H^+ transfers in metabolism.

In biological oxidation-reduction systems NAD and NADP containing enzyme systems play an important role because of their capacity to serve as effective hydrogen-transfer agents. The transfer of hydrogen from the oxidizable substrate to oxygen occurs much efficiently through a chain of graded enzymatic hydrogen transfers. One such group of hydrogen transfer agents is included under different nicotinamide containing enzyme systems.

In ruminants, niacin particularly required for protein and energy metabolism, involving liver detoxification of portal blood NH_3 to urea along with metabolism of ketones in liver during ketosis. It is quite evident that niacin can enhance microbial protein synthesis (Girard 1998). This may facilitate an increased molar proportion of propionate in rumen volatile fatty acids which may cause an increased rate of flow of material through the rumen. The function of prime interest for dairy cows deals with the role of niacin in oxidation of fatty acid and glucose synthesis, particularly as a preventive and possible treatment for clinical and subclinical ketosis.

Supplementation of niacin (vitamin B_3) increased milk production, decreased incidence of ketosis and fatty liver syndrome, enhanced microbial protein synthesis and increased propionate production (Cervantes *et al*. 1996).

Funk isolated this vitamin from yeast and rice polishing. Nicotinic acid was discovered for the treatment of black tongue in dogs and pellagra in human beings (Elvehjem *et al*. 1937).

Nicotinic acid and nicotinamide (niacinamide) possess the same activity, but in lactating cows the later has slightly higher activity (Jaster and Ward 1990). Nicotinic acid is converted to nicotinamide in the rumen (Erickson *et al*. 1991). Nicotinamide functions as a component of two different coenzymes such as NAD (Nicotinamide adenine dinucleotide) and NADP (Nicotinamide adenine dinucleotide phosphate). NAD and NADP function in biological oxidation-reduction systems by virtue of their ability to serve as hydrogen transfer agents.

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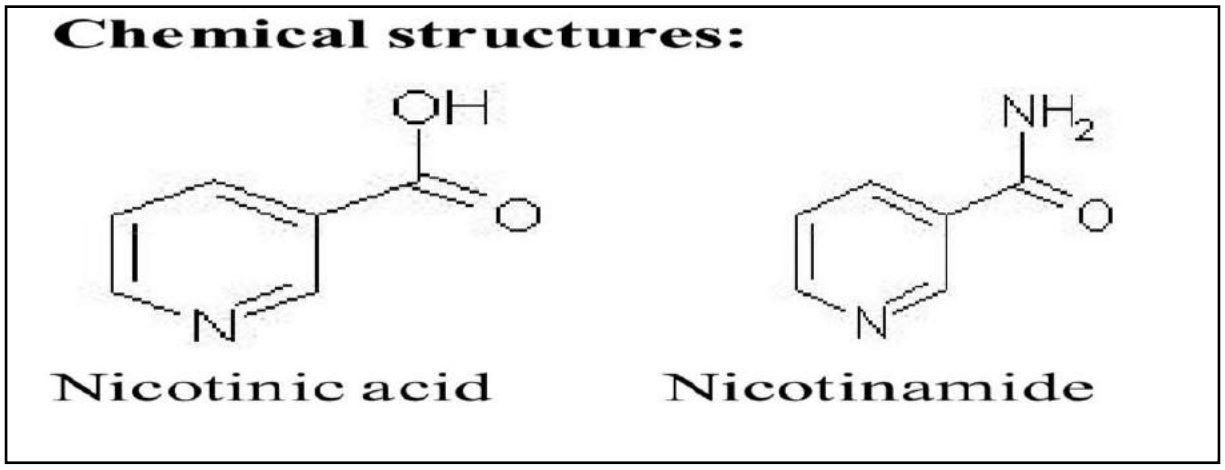


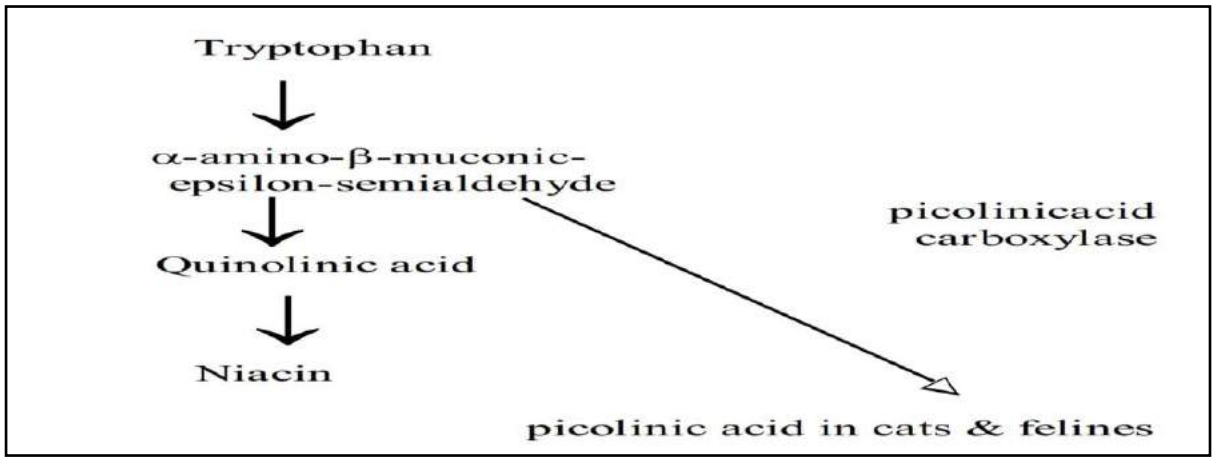
Figure 1: Chemical structure of nicotinic acid and nicotinamide

Important metabolic reactions catalyzed by NAD and NADP are listed as follows:

a. Carbohydrate metabolism: Glycolysis (anaerobic and aerobic oxidation of glucose) and TCA (Krebs) cycle

b. Lipid metabolism: Glycerol synthesis and breakdown, fatty acid oxidation and synthesis and Steroid synthesis

c. Protein metabolism: Degradation and synthesis of amino acids and oxidation of carbon chains via TCA cycle



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d. Photosynthesis

e. Rhodopsin synthesis

Conversion of tryptophan to niacin has been depicted in (Figure.2). Tryptophan is the precursor of niacin and the degree to which the metabolic requirement for niacin can be met from tryptophan will depend on the dietary availability of tryptophan and efficiency of conversion of tryptophan to niacin. At low levels of tryptophan intake, the efficiency of conversion is high. It decreases when niacin and tryptophan levels in the diet are increased. When animals have received deficient levels of tryptophan, increasing amounts of dietary tryptophan are used first to restore nitrogen balance, next to restore blood pyridine nucleotides and then to be excreted as niacin metabolites. Under starvation or energy restriction, efficiency of conversion is increased (Karkoodi *et al.* 2009)

RESPONSES TO NIACIN SUPPLEMENTATION IN RUMINANTS

(a) Effect of niacin supplementation on rumen fermentation

Khan and his coworkers (2015) have conducted an *in vitro* experiment to study the effect of supplementation of different levels of niacin (0, 300, 400, 500, 600, 700 and 800 ppm) on rumen fermentation and digestibility (Table no.1, 2, 3). The substrate comprised of concentrate mixture, maize fodder and wheat straw (40:20:40). Results revealed that TCA-ppt. N (mg/100 ml incubation media) and TVFA concentration (meq/100 ml incubation media) were significantly ($P<0.05$) higher at 600 ppm (17.56; 7.28) as compared to control (12.12; 6.38).TVFA was found higher concentration it may be because of stimulatory effect of niacin on rumen microorganisms which might have increased cellulose digestion and resulted in more TVFA production (Nangia and Sharma 1994).The molar proportion of propionate was also higher at 600 ppm (26.52%) as compared to control (25.87%). An increased propionate production might have occurred due to altered NADH/NAD ratio in microbes with niacin supplementation probably due to inhibition of methanogenesis (Flachowsky 1993; Samanta *et al.* 2000). The total gas (ml) production increased in a linear fashion whereas methane level decreased significantly ($P<0.05$) with graded levels of niacin. It may be due to the increased

utilization of ammonia for the synthesis of microbial protein (Horner *et al*. 1986 and Doreau and Ottou 1996). Methane level decreased significantly ($P<0.05$) with graded levels of niacin. This might be attributed to an increased production of propionate due to altered NADH/NAD ratio in microbes that inhibits methanogenesis (Flachowsky 1993). The acetate formation releases large amounts of CO_2 and CH_4, whereas propionate production does not release CH_4 (Stern *et al*. 1997). The IVDMD (%) and IVOMD (%) also increased from 44.04 to 48.04 and 53.91 to 57.38, at 0 and 600 ppm niacin supplementation, respectively. The three higher levels of niacin viz. 600, 700 and 800 ppm had comparable fermentation parameters viz. digestibility, total gas, methane, TCA-ppt. N, TVFA, acetate, propionate and butyrate. It was concluded that 600 ppm niacin level is comparatively better than other niacin levels.

Table 1: Effect of different levels of niacin supplementation on IVDMD (%) and IVOMD (%)

Levels of Niacin (ppm)	IVDMD (%)	IVOMD (%)
0	$44.04^{a}\pm0.25$	$53.91^{a}\pm0.56$
300	$46.06^{b}\pm0.15$	$55.13^{ab}\pm0.49$
400	$46.54^{ab}\pm0.33$	$56.93^{c}\pm0.43$
500	$46.84^{ab}\pm0.30$	$56.36^{bc}\pm0.16$
600	$48.04^{b}\pm0.56$	$57.38^{c}\pm0.40$
700	$47.96^{b}\pm0.79$	$57.01^{c}\pm0.33$
800	$47.18^{ab}\pm0.67$	$56.98^{c}\pm0.73$

^{a,b,c} Values with different superscripts in a column differs significantly ($p<0.05$)

Each value is an average of 9 observations

Table 2: Effect of different levels of Niacin supplementation on *in vitro* ruminal fermentation pattern

Levels of Niacin (ppm)	Total gas (ml)	Methane (% of total gas)	NH3-N (mg/100ml incubation media)	TCA-ppt N (mg/100ml incubation media)
0	$17.50^{a}\pm0.43$	$34.29^{d}\pm0.14$	$15.26^{d}\pm0.46$	$12.12^{a}\pm0.71$
300	$23.50^{b}\pm 0.43$	$30.84^{c}\pm0.23$	$14.35^{d}\pm0.46$	$13.43^{ab}\pm0.53$
400	$25.67^{c}\pm 0.42$	$29.57^{b}\pm0.11$	$12.95^{c}\pm0.48$	$14.84^{bc}\pm0.28$
500	$26.50^{c}\pm 0.56$	$29.14^{ab}\pm0.11$	$10.85^{ab}\pm0.53$	$15.25^{c}\pm0.22$
600	$26.67^{cd}\pm0.33$	$29.11^{ab}\pm0.12$	$10.71^{a}\pm0.24$	$17.56^{d}\pm0.35$
700	$26.83^{cd}\pm0.60$	$29.03^{a}\pm0.25$	$11.97^{abc}\pm0.31$	$16.14^{cd}\pm0.49$
800	$28.00^{d}\pm0.26$	$29.27^{ab}\pm0.21$	$12.81^{bc}\pm0.37$	$16.90^{d}\pm0.75$

^{a,b,c} Values with different superscripts in a column differs significantly ($p<0.05$)

Each value is an average of 9 observations

Table 3: Effect of different levels of niacin supplementation on TVFA*(meq/100ml incubation media) and IVFA*(molar %)

Level of Niacin (ppm)	TVFA (%)	IVFA (molar %)		
		Acetate	Propionate	Butyrate
0	$6.36^{a}\pm0.17$	68.58±0.20	$25.87^{a}\pm0.15$	$5.55^{b}\pm0.10$
300	$6.59^{a}\pm0.16$	68.90±0.10	$25.76^{a}\pm0.10$	$5.35^{b}\pm0.13$
400	$6.66^{a}\pm0.06$	68.94±0.20	$25.65^{a}\pm0.18$	$5.41^{b}\pm0.07$

500	6.73^{ab}±0.05	68.94±0.38	25.67^{a}±0.33	5.39^{b}±0.11
600	7.28^{c}±0.14	68.29±0.50	26.52^{b}±0.28	5.19^{ab}±0.26
700	7.11b^{c}±0.14	68.17±0.18	26.62^{b}±0.12	5.21^{ab}±0.13
800	7.16^{c}±0.16	68.38±0.22	26.74^{b}±0.15	4.88^{a}±0.13

^{a,b,c} Values with different superscripts in a column differs significantly (P<0.05)

(b) Effect of niacin supplementation on growth

Luo *et al*. (2019) have conducted an experiment to observe the effect of niacin supplementation on the growth performance and nutrient utilisation in Chinese Jinjiang cattle (Table no.4). They have taken 48 finishing male Jinjiang cattle were randomly divided into four groups. The cattle were fed a finishing diet having (concentrate to forage ratio of 80:20). The diets for the control, NA_{320}, NA_{480} and NA_{640} groups were supplemented with 0, 320, 480 or 640 mg/kg of niacin, respectively. Results revealed that a significant increase in the average daily weight gains (p< .05) and lower feed to gain ratios (p < .05) for the NA480 and NA640 groups than for the control group.

Table 4: Effect of niacin supplementation on the growth performance of finishing Jinjiang cattle

	Day	Control	NA_{320}	NA_{480}	NA_{640}	SEM
BW, kg	0 d	201.19	199.37	205.34	203.11	15.20
	28 d	214.63	213.93	224.10	222.43	18.49
	56 d	227.79	226.81	240.62	239.79	19.66
ADG, kg/d	1-28 d	0.48^{b}	0.52^{a,b}	0.67^{a,b}	0.69^{a}	0.14
	28-56 d	0.47	0.46	0.59	0.62	0.18
	1-56 d	0.48^{b}	0.49^{a,b}	0.63^{a}	0.66^{a}	0.18
TDMI, kg/d	1-28 d	5.28	5.32	5.45	5.20	0.16

	28-56 d	5.87	5.66	5.89	5.75	0.15
	1-56 d	5.58	5.49	5.67	5.47	0.16
F/G	1-28 d	11.01^{a}	10.24^{a}	8.14^{b}	7.53^{b}	0.78
	28-56 d	12.49^{a}	12.31^{a}	9.98^{b}	9.27^{b}	0.92
	1-56 d	11.74^{a}	11.21^{a}	$9.00^{b,c}$	8.35^{c}	0.84

In the same row, values without a common letter differ significantly ($p<0.05$)

Control=Basal diet, NA_{320}= Basal diet + 320 mg/kg niacin, NA_{480}= Basal diet + 480 mg/kg niacin, NA_{640}= Basal finishing diet + 640 mg/kg niacin, SEM=Standard error of mean, BW=Body weight, ADG=Average daily gain, TDMI=Total dry matter intake, F/G=feed to gain ratio

(c) Effect of niacin supplementation on digestibility of nutrient

Luo *et al.* (2019) have conducted an experiment to observe the effect of niacin supplementation on the growth performance and nutrient utilisation in Chinese Jinjiang cattle (Table no.5). They have taken 48 finishing male Jinjiang cattle were randomly divided into four groups. The cattle were fed a finishing diet having (concentrate to forage ratio of 80:20). The diets for the control, NA_{320}, NA_{480} and NA_{640} groups were supplemented with 0, 320, 480 or 640 mg/kg of niacin, respectively. Assessment of feed digestibility was conducted from days 52 to 56 of the study. Results revealed that a significant increased the apparent digestibility of all nutrients ($p<.05$) with supplementation of 640 mg/kg niacin. Whereas, supplementation with 480 mg/kg niacin enhanced the apparent digestibility of crude protein ($p<0.05$). Therefore, it was concluded that supplementation with 640 mg/kg niacin in a high-concentrate diet may be beneficial to growth and nutrient utilization in Chinese Jinjiang cow.

Table 5: Effects of niacin supplementation on the apparent digestibility of dietary nutrient in finishing Jinjiang cattle.

	Control	NA_{320}	NA_{480}	NA_{640}	SEM

DM	61.64[b]	62.11[b]	63.12[b]	65.33[a]	1.62
OM	65.31[b]	66.28[a,b]	67.75[a,b]	68.42[a]	1.84
CP	59.09[b]	62.96[b]	65.64[a]	63.15[a]	0.97
NDF	58.76[b]	57.04[b]	55.43[b]	62.87[a]	1.97
ADF	40.83[b]	41.68[b]	44.05[b]	49.91[a]	2.66

In the same row, values without a common letter differ significantly ($p<0.05$)

Control= Basal finishing diet, NA320= Basal finishing diet + 320 mg/kg niacin, NA480= Basal finishing diet + 480 mg/kg niacin, NA640= Basal finishing diet + 640 mg/kg niacin, SEM=Standard error of mean, DM=Dry matter, OM = Organic matter, CP=Crude protein, NDF=Neutral detergent fiber , ADF=Acid detergent fiber

(d) Effect of niacin supplementation on milk production and its composition

Al-Abbasy, (2013) has conducted an experiment to observe the effect of niacin on milk production they have taken 36 multiparous early lactating Friesian cows. Cows were assigned randomly into three main groups (Table no. 6). group 1, were received a basal diet with 0 niacin, group 2, received a basal diet with 6 g niacin and group 3 received a basal diet with 12 g niacin per day over 22 week experimental period. Results revealed that of niacin supplementation @ of 6 and 12 g/head/day in increase of weekly milk production for the months of June, July and August. The significant effect ($p<0.01$) of niacin on weekly milk production, where during the month of July, increase in milk production weekly for group 12 g niacin/cow/day is 18.27 and 40.33 kg/day compared with a 6 g niacin/cow/day and the group that did not receive any level of niacin, respectively, this improvement may be due to the role of niacin in improving energy use and increase the percentage of sugar in the blood and reduce the lipid metabolism or may be due the positive effect of niacin on milk production through the relationship between niacin and tryptophan, Horner *et al.* (1988) noted that the cows that are in the early lactation period, have decreased plasma concentration of tryptophan, rumen microbes in synthesis of niacin which has a positive effect on production or may be added niacin had

increased the level of assistants enzymes NAD and NADP who are entering in the metabolism of fats and proteins and carbohydrates and improve the utilization rate of food and encouraging protein microbial and increased of volatile fatty acids composition and increase in appetite and food intake and increased blood sugar levels so that reflected positively on milk production.

Table 6: Effect of niacin supplementation on the milk production (kg) of Holstein frisian cows

Effect of factors	No. of animals	Milk production		
		Trial-1 (June)	Trial-2 (July)	Trial-3 (August)
Control	12	49.89±3.14^{b}	42.39±2.54^{c}	33.12±1.68^{c}
6 g niacin	12	52.62±3.60^{b}	64.45±6.32^{b}	59.74±5.99^{b}
12 g niacin	12	65.12±4.28^{a}	82.72±6.44^{a}	73.70±6.43^{a}
General Mean	36	55.87±3.67	63.18±5.10	55.52±4.70

^{abc}Mean with same letters are not differ significantly otherwise they differ significantly ($p<0.01$)

Karkoodi and Tamizrad, (2009) has conducted an experiment to observe response to niacin supplementation on milk production they have taken twelve multiparous Holstein cows were divided in to four treatment group (Table no .7). The treatments were: N0 - control (no niacin supplement); N1 - control + 12 g niacin/d; N2 - control + 14 g niacin/d and N3 - control + 16 g niacin/d. Results indicated that milk yield, fat-corrected milk (FCM, 3.5%) and total solids percentage (TS) were significantly higher in the N2 compared to the other treatments. No significant differences have been seen between treatments in milk fat and milk lactose percentages, but milk fat yield was significantly higher and milk fat percentage numerically higher in the N_2 than in the other treatments. Milk protein yield and percentage were highest in N_2, but milk protein percentage was not significantly different between the N_2 and N_1 treatments. Milk solids non-fat (SNF) percentage was the highest for N_2.

Table 7: Mean milk yield (kg) and composition of the milk of dairy cows receiving different levels of niacin

	Control	Control diet			SEM
		+12 g niacin	+14 g niacin	+16 g niacin	
Milk yield(kg)	29.8^{d}	30.7^{c}	31.4^{a}	30.9^{b}	0.08
FCM 3.5%(kg)	24.5^{d}	25.4^{c}	26.9^{a}	25.5^{b}	0.07
Fat %	2.17	2.21	2.44	2.20	0.08
Protein %	2.79^{b}	2.76^{b}	2.95^{a}	2.90^{a}	0.04
Lactose %	4.96	5.17	5.22	5.23	0.06
Total solids%	10.8^{c}	10.7^{d}	11.0^{a}	10.9^{b}	0.08
SNF %	8.59^{ab}	8.53^{ab}	8.78^{a}	8.48^{b}	0.08
Fat yield (kg/d)	0.642^{c}	0.675^{b}	0.743^{a}	0.672^{b}	0.02
Protein yield (kg/d)	0.828^{d}	0.843^{d}	0.925^{a}	0.893^{b}	0.02

^{abcd} Means with no common superscript in the same row differ significantly (P <0.05)

Schwab *et al.* (2005) have conducted an experiment and observe that supplementation of 6 g of niacin per day had no effect on milk production or milk composition. whereas, 12 g of supplemental niacin per day resulted an increase in fat yield up to 26 g/d, milk protein yield 17 g/d and 3.5% fat-corrected milk increased about 1lb/d. The feeding of nicotinamide during the close-up period decreased early lactation culling and enhanced fat-corrected milk yield (FCM).

Horner *et al* (1986) found that a reduction of milk protein percentage and protein yield resulted in dairy cows because of feeding of whole cottonseed and most other dietary fat

sources to these precious animals. Diets supplemented with niacin (6 g niacin per 20.45 kg of dry matter) increased milk protein percentage in diets with 15% whole cottonseed. Milk protein depression with whole cottonseed was alleviated by niacin because of stimulation of mammary casein synthesis. In contrary to this, another study reported that supplemental niacin increased milk production by 3% with no effect on dry matter intake (Horner *et al.* 1988); though no beneficial effect of niacin on milk casein synthesis was found for cows fed whole cottonseed, which may be due to their late stage of lactation (Lanham *et al.* 1992). Another experiment suggested that feeding niacin to cows receiving heat-treated soybeans rectified a dietary oil-induced milk protein depression (Driver *et al*. 1990); however feeding cows 12 g of niacin daily increased milk protein yield and reduced plasma ketones (Erickson *et al.* 1992).

(e) Effect of niacin supplementation on ketosis

Ketosis is a metabolic disease, in which an increase in quantity of ketone bodies (acetoacetate, â-hydroxybutyrate, and acetone) and the free fatty acids in the blood plasma, while the glucose concentration decreases. More than 50% of the high yielding dairy cows suffer from sub-clinical ketosis. In case of ketosis appetite, feed-intake, live weight and milk production of the animal decreases.

Niacin has beneficial during late gestation and early lactation when ketosis may be a problem as it is involved in the breakdown of body fat and ketones. Body fat mobilization is highest during early lactation when energy demand for milk production is in peak. Recent research suggests that microbial production of niacin may not be sufficient for the requirements of high yielders. The niacin supplementation in early lactating cows may decrease the rate of fat mobilization, concentration of ketones in blood along with increase the level of blood glucose. Niacin supplementation may enhance the propionate concentration and diminish the butyrate concentration in rumen liquor.

Al-Abbasy, (2013) has conducted an experiment to observe the effect of niacin on production of ketone bodies. They have taken 36 multiparous early lactating Friesian cows (Table no. 8, 9, 10, 11). Cows were assigned randomly into three main groups. group 1, were received a basal diet with 0 niacin, group 2, received a basal diet with 6 g niacin and group 3 received a basal diet with 12 g niacin per day over 22 week experimental period. Results revealed that significant effect of niacin supplementation 6 and 12 g reduce the level of β-

Hydoxybutyrate (BHBA), acetone and triglyceride compared to the control group. This appears the niacin has improved energy use for cows postpartum, so reduced the indicators of metabolic diseases which usually infect cows in the beginning of the lactation period such as disease of ketosis.

Table 8: Effect of niacin supplementation on level of Beta-hydroxybutyrate (mmol/L)

Experimental group	No. of animals	Trial-1	Trial-2	Trial-3
Control	12	1.140 ± 0.202^{a}	1.092 ± 0.184^{a}	1.107 ± 0.185^{a}
6 g niacin	12	0.449 ± 0.029^{b}	0.446 ± 0.027^{b}	0.425 ± 0.024^{b}
12 g niacin	12	0.237 ± 0.013^{c}	0.229 ± 0.013^{c}	0.224 ± 0.024^{c}

Mean with same letters are not differ significantly otherwise they differ significantly ($p<0.01$)

Table 9: Effect of niacin supplementation on level of Acetone (mg/dl)

Experimental group	No. of animals	Trial-1	Trial-2	Trial-3
Control	12	14.17 ± 0.75^{c}	17.00 ± 0.77^{c}	18.83 ± 0.75^{c}
6 g niacin	12	11.00 ± 0.89^{b}	9.17 ± 0.60^{b}	10.50 ± 0.42^{b}
12 g niacin	12	6.16 ± 0.47^{a}	8.50 ± 0.34^{a}	5.83 ± 0.47^{a}

Mean with same letters are not differ significantly otherwise they differ significantly ($p<0.01$)

Table 10: Effect of niacin supplementation on level of triglycerides

Effect of factors	No. of animals	Mean ± standard error (mg/dL)		
		Trial-1 (June	Trial-2(July)	Trial-3(August
Control	12	18.22 ± 1.58^{b}	19.67 ± 1.70^{c}	23.67 ± 1.55^{b}
6 g niacin	12	16.16 ± 1.76^{b}	15.86 ± 0.95^{b}	16.32 ± 0.79^{a}

12 g niacin	12	13.39±1.49[a]	13.97±1.57[a]	14.48±1.30[a]
General mean	36	14.59± 1.61	15.50±1.40	18.15±1.21

Mean with same letters are not differ significantly otherwise they differ significantly ($p<0.05$)

Table 11: Effect of niacin supplementation in level of glucose

Effect of factors	**No. of animals**	**Mean ± standard error (mg/dL)**		
		Trial-1 (June)	**Trial-2(July)**	**Trial-3(August)**
Control	12	44.47 ± 1.3[c]	42.39± 0.8[c]	49.09±2.6[c]
6 g niacin	12	54.72 ± 2.3[b]	48.46 ± 1.3[b]	58.66±2.9[b]
12 g niacin	12	66.47 ± 1.6[a]	58.79 ± 1.4[a]	68.26±2.8[c]
General mean	36	55.22± 1.8	49.88± 1.3	58.67±2.0

Mean with like letters are not differ significantly otherwise they differ significantly ($p<0.01$)

Due to niacin' s involvement with fat metabolism, it may be helpful in using supplemental dietary niacin when adding fat to diets of lactating animals. Niacin is utilized both in the rumen and in the small intestine and has positive effect on rumen fermentation, N-metabolism as well as on prevention of several metabolic diseases like acidosis, ketosis.

Ruminal microbial protein synthesis was enhanced by niacin. Endogenous synthesis of niacin is slowed down by ketones and enhanced by corticosteriods, leading to the fact that a part of the beneficial effect of adrenal corticoids on ketosis is derived from increased niacin synthesis. This suggests that niacin may be a helpful adjunct to glucocorticoid therapy for ketosis. Supplemental niacin has been reported to increase plasma insulin and glucose response to beta- agonists (Chilliard and Ottou 1995).

(f) Effect of niacin supplementation on Heat Stress

Constant exposure of productive animals to high temperature and high humidity is directly concerned with the feed and water intake leading to not only reduction in growth but also decreased production potential of the animals. An experimental trial was conducted on lactating Holstein cows in which encapsulated niacin was administered to study the vitamin' s affect on heat stress (Zimbelman *et al*. 2010). Cows receiving niacin had increased dry matter intake. A comparatively higher sweating rate & lower vaginal temperature was observed in cows supplemented with niacin. Niacin, nicotinic acid, or vitamin B_3 induced skin vasodilatation and

increased heat loss at the periphery. The vasodilatory effects of niacin are the result of prostaglandin D (PGD) produced by epidermal langerhans cells acting on vascular endothelial PGD_2 receptors. Increased skin blood flow was associated with increased sweating rate and inhibiting blood flow by inhibiting nitric oxide synthase, reducing sweating rate during exercise in humans. Skin temperatures decreased during periods of mild to severe heat stress in cows supplemented with 12, 24, or 36 g of raw niacin. This may have been associated with increased sweating and evaporative heat loss from skin surface.

REQUIREMENTS OF NIACIN

The variation in the response of ruminant livestock to supplemental niacin is due to variations in:

1. Endogenous niacin synthesis from tryptophan
2. Niacin supply and bioavailability in common feedstuffs
3. Rumen niacin synthesis and degradation young, pre-ruminant calves would be expected to have a dietary requirement for niacin.

The minimum level of niacin recommended for calf milk replacers is 2.6 mg per kg (1.2 mg per lb).

Niacin supplementation in milk replacer would be of more concern when the non-milk protein sources are used as an alternative to the primary protein source in milk replacer, due to low tryptophan content (Touchette *et al.* 2003).

Recommended daily allowances for vitamin B_3 in cattle have been depicted in (Table 12)

Table 12: Recommended daily allowance for vitamin B_3 in cattle

Age	Quantity (mg)
0-6 months	2
7-12 months	4

1-3 yrs	6
4-8 yrs	8
9-13 yrs	12
Males 14 +	16
Females 14+	14

During pregnancy 18 mg; during lactation17 mg.

SOURCES OF NIACIN

The supply of niacin to the ruminant comes from three main sources: dietary niacin, conversion of tryptophan to niacin and ruminal synthesis of niacin.

Niacin is widely distributed in feedstuffs of plant as well as of animal origin have been depicted in (Table 13).

Table 13: Niacin in foods & feed stuffs (mg/kg DM)

Cereals		**Hay**	
Barley grain	94	Alfalfa hay (sun cured)	42
Rice grain	16	Alfalfa leave (sun cured)	53
Sorghum grain	43	Timothy hay (sun cured)	29
Wheat grain	64	**Animal by-products**	
Oil cakes		Liver (cattle)	269
Cottonseed meal (solvent extracted)	48	Blood meal	34
Soybean meal (solvent extracted)	31	Butter milk (cattle)	9
Linseed meal (solvent extracted)	37	Chicken broiler (whole)	230

Peanut meal (solvent extracted)	188	Fish meal, anchovy	89
By-products		**Seeds**	
Rice bran	330	Pea	36
Wheat bran	268	Soybean	24

The by-products of animal and fish origin, distiller' s grains, yeast, various distillation and fermentation soluble and certain oilseed meals are good sources. The bound form of niacin in cereal grains and their by-products is largely unavailable to monogastric species of animals. By use of a rat bioassay procedure, it was shown that in eight samples of various mature cooked cereals like corn, wheat, rice and milo, only about 35% of the total niacin was available.

Probably much of this niacin will also be unavailable to rumen microorganisms (Erickson *et al*. 1991). In calculating the niacin content of formulated diets, all niacin from cereal grain sources should be ignored or at least given a value no greater than one-third of the total niacin. Some bound forms of this vitamin are biologically available, but the niacin especially in corn is unavailable and is implicated in the etiology of pellagra in animals that consume large quantities of this grain.

BIOAVAILABILITY OF NIACIN

The bioavailability of this vitamin is 100% in soybean meal but zero in wheat and sorghum and varies from 0 to 30% in corn (Carpenter *et al.* 1988). Niacin occurs as a part of biologically available coenzymes in immature seeds which is necessary for seed metabolism.

CONCLUSION

The Following Conclusions may be drawn from the topic discussed i.e. Supplementation of niacin in ruminants

- Improves the rumen fermentation pattern as well as no. of the rumen microbes, which stabilize the ruminal environment.
- Increases the digestibility of the dietary nutrient the thus improves nutrient utilization by the animals which ultimately improves growth performance of the animals.

- Niacin supplementation improves the metabolism of carbohydrate, fat and protein which fulfills excessive demand of energy in early lactation period and thus enhances the production performance in high yielding animals. The satisfactory, level of niacin supplementation is 12 g/h/d.
- Anti-ketogenic effect of the niacin also found because it prevents the fat mobilization and increases the blood glucose levels thus increase energy use and prevents ketosis.
- Reduces the heat stress of the animal leading to increased dry matter intake and production performance of the animals.

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Measly Pork: A Public Health Concern

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Abstract

Pork, a popular meat is found to carry some of the foodborne parasites that may affect human health. Among those, the important one is the *Taenia solium*, a tapeworm whose larval form called as *Cysticercous cellulosae* was found to cause a condition in pork called as 'measly pork'. Human are the definitive host of the parasite, while pigs are intermediate one and it causes a disease condition called as *cysticercosis.* It can be control my taking measures at four levels, including farm, slaughter, post-slaughter processing and consumer level.

Introduction

Pork is one of the most popular meats consumed by the people worldwide with a good amount of nutrients in it. According to the preference of the consumers, pork may be consumed as freshly cooked or as a preserved products *viz.*, ham, bacon, sausage, smoked pork etc. Nevertheless, pork is known to carry some of the foodborne parasites *viz.*, *Taenia solium*, *T. saginata* and *T. asiatica*, of which *T. solium* is mainly responsible for causing major health problems and Food Agricultural Organisation and World Health Organization, have ranked it as the most important foodborne parasite. Foodborne zoonoses are defined as diseases naturally transmitted between animals and humans through food. Infections of the pigs occurs under conditions where the animals have access to tapeworm segments and eggs. In case of humans, consumption of inadequately cooked or raw pork containing larva of the parasite may lead to infection. Measly pork is the pork that is infected with the larval stage (*Cysticercous cellulosae*) of the tapeworm *T. solium*. Ingestion of it causes *cysticercosis* in both human and pig. However, adult tapeworms in the intestine causes *taeniosis* in human and are hardly ever life-threatening. Humans can also become infected with the other parasites i.e., *T. saginata* or *T. asiatica* when they

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consume infected beef or pork liver tissue, respectively, which has not been adequately cooked, but taeniasis due to *T. saginata* or *T. asiatica* has no major impact on human health.

Transmission Cycle

Taenia solium is a tapeworm and it can measure up to 5 metres long in its adult form. Humans are both the definitive and the intermediate hosts of *T. solium*, whereas intermediate hosts also include pigs and dogs. As definitive hosts, humans carry the adult worm attached to the small intestine from which eggs are detached and excreted with faeces which contaminates the environment. If such eggs are eaten by pigs, it will develop into the immature or larval stage of cysts in their meat (FAO, 2021). Pigs usually do not show any signs of infection, and only heavily infected pigs show cysts on their tongue. When people eat raw or undercooked pork with viable cysts, they develop the tapeworm. There is also a chance of acquiring infection by ingesting the eggs via the faecal-oral route, or by eating vegetables and drinking water contaminated with the eggs. In this case, they develop cysts in different parts of the body, including the brain (Djakovic et al., 2013).

Nature and Origin of' Measle' in the Pig

The' measle' of the pig is seen due to the larval stage of the tapeworm *T. solium*. It is found most frequently in the muscle of tongue, loin, and neck, and may also be found in any part of the body's muscle and is often seen in the muscular substance of the heart, lying between the fibres of the muscle. It occurs as an ovoid bladder formed by a thin transparent membrane, and enclosing at one extremity an opaque body of a white colour which is the worm coiled up, but when unfolded it have a head, neck, and pear-shaped vesicular tail. In the interior of the worm presents a number of microscopic corpuscles.

During the life of the pig, the bladder enclosing the worm is fully distended with a pellucid fluid, but on death a portion or whole of the contained fluid escapes into the surrounding tissues. 'Measles' are not develop in every hog that has swallowed tapeworm eggs, rather a feeble digestion and constitutional debility may especially favour their hatching in some pigs.

In case of light infection of the pig, the general health of the pig is not affected and the flesh does not differ from that of healthy pork. Such parasite are destroyed during cooking, mastication, and digestion and can be eaten. While heavily infected pig develops pale, soft, and watery muscle and the muscle fibre near the worm exhibits fatty degeneration. The health of the

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pig is much impaired with general fever, wasting, weakness and the animal loses appetite, blisters form under the swollen tongue, the skin ulcerates and death occurs amidst extreme debility and emaciation. Badly ‘measled' pork is insipid when cooked and on boiling loses more weight than healthy pork. Such meat are rejected (Fleming, 1857).

Health Impacts

Clinical signs of cysticercosis are rarely found in pigs. However, considerable economic losses are the consequences of infected pigs and are frequently detected at meat inspection in abattoirs.

Humans become infected with the adult tapeworm by eating raw or undercooked infected pork causing taeniosis which is seldom life threating while infection with the larval form of the parasite by ingesting *T. solium* eggs either from direct contact with a human tapeworm carrier or from contaminated food or water may cause human cysticercosis. Cysts developed after the infection may localize in muscle tissue, in the eye or in the brain. In the brain, the cysts cause neurocysticercosis characterized by epileptic seizures, severe headaches, and even death (Ndimubanzi et al., 2010).

Detection Methods

- Examination of stool for *T. solium* eggs is the basic diagnostic method for taeniasis, but for differential diagnosis within the Taenia species can be done by ELISA or PCR.
- Antemortem inspection of the pig by visual inspection of the tongue and cyst palpation. At the very early stage of' the disease the parasite is found in the tongue and therefore tongue should be inspected in the properly.
- Postmortem meat inspection is the routine technique used for the detection of cysticercosis in infected pork carcasses, a cut is made into the inner loin muscle at the side of' the spine and across the neck to find the worms. Cysts are mostly presents in the masseter, heart, tongue, shoulder, neck, fore and hind limbs, intercostals, diaphragm and psoas muscles, but cysts can be found throughout the body including the brain.
- Serological tests like specific serum-IgG antibody ELISA assays are now commercially available have been developed for the detection of specific cysticercosis antibodies or antigens but remain primarily as research tools.

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Prevention and Control

Generally, prevention may be carried out at four levels, including farm, slaughter, post-slaughter processing and consumer level.

- At farm level, implementation of good management measures may lead to parasite-free farms. Strategies to reduce the level of parasite infection in pigs include raising animals in controlled zoo-hygienic conditions in strictly managed intensive-type farms (rodent control, no access for cats, feed and water control, no access for pigs to refuse) and regular deworming of the animals. Treating pigs with oxfendazole (30 mg/kg), muscle cysts can be killed within 4 weeks however, visible dead cysts may persist for as long as 6 months.
- Prevention at slaughterhouse level comprises veterinary inspection at slaughter. Another way of controlling it is to condemn the heavily infected carcass with proper disposal. However, although most meat regulations require total condemnation, for more lightly infected cases there are ways to deal with contaminated carcasses. This may, however, be limited by the applicability and sensitivity of the described diagnostic methods. Training of technicians, quality assurance and accreditation are compulsory measures.
- Post-slaughter methods refer to pork pre-market processing, and include procedures such as freezing and curing to inactivate the parasites; performance of these procedures varies with the species of parasite. However, processing of pork may killed the parasites and its larve if the temperature throughout the meat reaches 60°C, or until it loses pink colour. Freezing the carcass for 4 days at −5 °C, 3 days at −15 °C, or 1 day at −24 °C will killed the cysts.
- The last front is prevention at individual consumer level, which relies on sufficient cooking of meat, i.e. until pork turns light brown. Microwave cooking is not sufficient to inactivate all parasite larvae or cysts. In conclusion, reasonably safe pork is not an unachievable goal, but to maintain this major protein source in human nutrition as safe as possible continuous monitoring and control of porkborne parasites at different levels is required in both developed and developing countries.

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Conclusion

The pork borne parasites are prevalent and may lead to severe illness. Outbreaks of foodborne disease in human transmitted via pork can be effectively controlled by adequate treatment of the meat. As well, preventive educational campaigns are essential to reduce the risk of transmission of these diseases mostly in regions where the prevalence is high or proper meat inspection is not feasible or where consumption of wild boar or home-slaughtered backyard pigs is in common practice.

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Plastination- A Technique to Preserve a Biological Specimens

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Abstract

Plastination is a specialized technique used for soft tissue preservation. It was developed by Gunther von Hagens in 1977. Plastinated specimens presents advantages over other methods of preservation because they exhibit precise anatomical features. They are clean, dry and easy to handle. Plastinated specimens are developed by using acid curing polymer technique. Plastination is a very easy method for preservation of soft parts in their dried and original form for a very long time. Plastinated specimens serves as a great aid for understanding anatomy of different organs in their original form with any disturbance of smell.

Keywords: Plastic resins, Xylene, Acetone, Melamine, Plastinated specimens

Introduction

Plastination is the process of tissue preservation by embedding tissue with synthetic polymer like Silicon, Polyester, Epoxy, Resin etc. to Produce a Dry, Durable, Handy and Natural looking Specimen useful as a unique tool for long term Educational Purpose. This method was invented in 1978 at the university of Heidelberg by Dr. Gunther von Hagens.

The Principle Behind the Technique

This technique can be used to preserve biological specimens that involve replacing water and fat in tissue with a polymer. This method produces “Plastic “Bodies or organs which are: - Non-Toxic, Odorless, Dry and Durable, can be handled very easily for Examination & Long lasting. Among the Polymers, the commonly used are Epoxy, Silicone and Polyester

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For obtaining the best plastinated specimens , the polymer used must have the following desirable properties:-

- Lower possible viscosity in uncured state.
- Its refractive index of the polymer should be different from that of tissue or else a transparent specimen would be obtained.
- Curing should not be inhibited by the tissue
- Mechanical properties of the polymer should be appropriate when cured i.e., it should be rubber like to stimulate a natural state or firm
- It should be Affordable.

Steps in Processing the Specimen for Plastination

Step 1 – Fixation

- The specimen should be fixed in 10% formalin. This stabilizes the tissues and prevent autolysis.
- To enhance color preservation cold Kaiser ling solution containing 5% formalin should be used.
- Specimen is generally fixed by injection through blood vessels (If not previously filled with coloring material)
- By infiltration i.e., injection of the solution in the muscles with the help of a syringe and needle.
- By immersion in the fixative solution.
- In case of specimen stored in solution containing glycerol, these specimens have to be rinsed off thoroughly to remove all the glycerol before being Plastinated.

Step 2- Dehydration

- Different types of polymer used for Plastination are not miscible with water hence the specimens must be absolutely dehydrated.
- Their water must be replaced by an intermediary solvent to permit penetration of the polymer with the specimens
- Dehydration can be achieved in stepwise ethanol baths, but the standard procedure is freeze substitution with acetone at -25°c.
- When immersed into cold acetone the specimens freeze immediately and shrinkage is considerably reduced.
- This procedure takes 4 to 5 weeks, with 3 changes of acetone
- The lipid rich specimen now has to be transferred in acetone at room temperature for one week to achieve Defattening and then they can be impregnated.
- Specimen prepared for Epoxy Plastination needs extra Defattening Bath in Methylene chloride to improve their transparency

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Step 3- Impregnation

- This is performed in a vacuum chamber where the acetone saturated specimens are submerged into a bath of liquid polymer.
- With a vacuum pump the pressure is slowly decreased in the chamber. The acetone is the changed from its liquid phase to vapor phase and aspirated by the vacuum pump. The extraction of the acetone creates a vacuum inside the specimen that forces the penetration of polymer into them, down to their microscopic level
- Because of the great difference between the high vapors pressure of the acetone and low vapors pressure of the polymer, only the Acetone is extracted when the vacuum is applied

Step 4- Hardening & Curing

- Finally, the polymer inside the specimen has to be Cured/Hardened
- This is achieved by exposing the impregnated specimen to a hardener which can be liquid or gases in nature
- The impregnated specimen and a bowl filled with curing agent is placed in a tightly closed chamber for several week
- To enhance the curing procedure air may be bubbled through the fluid
- For Complete curing the specimen should be kept in a plastic bag for several week

Types of Plastination

- On the basis of size, shape and nature of tissue, there are three types of plastination viz. Whole body/organ plastination, Luminal cast plastination and Sheet plastination

1. **Whole organ or a body Plastination-** in this method, Silicon (S10) and polypropylene resins are used. Using this technique, whole of the structure or organ, and its relationships can be preserved
2. **Luminal cast plastination-** is done for hollow organs like lungs, stomach, intestine, ventricles of brain, vascular pattern of heart and kidneys. Specimens are dilated/inflated during fixation, dehydration and curing. Beautiful and precise bronchial pattern can be seen by this technique.
3. **Sheet plastination -** In this method**,** thin transparent or thick opaque sections of body or an organ are preserved. These sheets are portable and shows cross sectional anatomy of organs equivalent to CT or MRI scan sections. Sheets can be taken in various planes. Thin sections (1-2mm) of organs are similar to routine histology slides. Polymers such as epoxy (E12), polyester (P35) or polypropylene (araldite) resins are used for making sheet plastinates.

Advantage of Plastination

- The Specimen are Dry, Easy to handle, Store, Transport and long lasting.
- Non-Hazardous, Non-Infectious, doesn't radiate fumes or fluids
- Can be used for imperative preparation of specimens for Museum Display.
- Can be used for Preparation of samples for Evidence
- Storage and Maintenance is easy. They can even be stored in Plastic Bags with Essential Credentials.

Disadvantage of Plastination

- Costly Procedure
- Time consuming
- Required skill technical support to carry out the procedure and in handling the equipment's.
- Prepared specimen requires handling with care.
- Chemical used such as Acetone are highly inflammable and should be used in place equipped with fire extinguishing measure.

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Heat Stress and Response of Animals to Heat Stress

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What is Heat Stress

Heat stress is defined as "inability of the animals to lose heat to the surroundings" or imbalance between heat gain and heat lose mechanisms in the body resulting in accumulated heat load.

What Causes Heat Stress

The environmental variables that contribute to heat stress are ambient temperature, relative humidity, wind and solar radiation (Fig. 1).

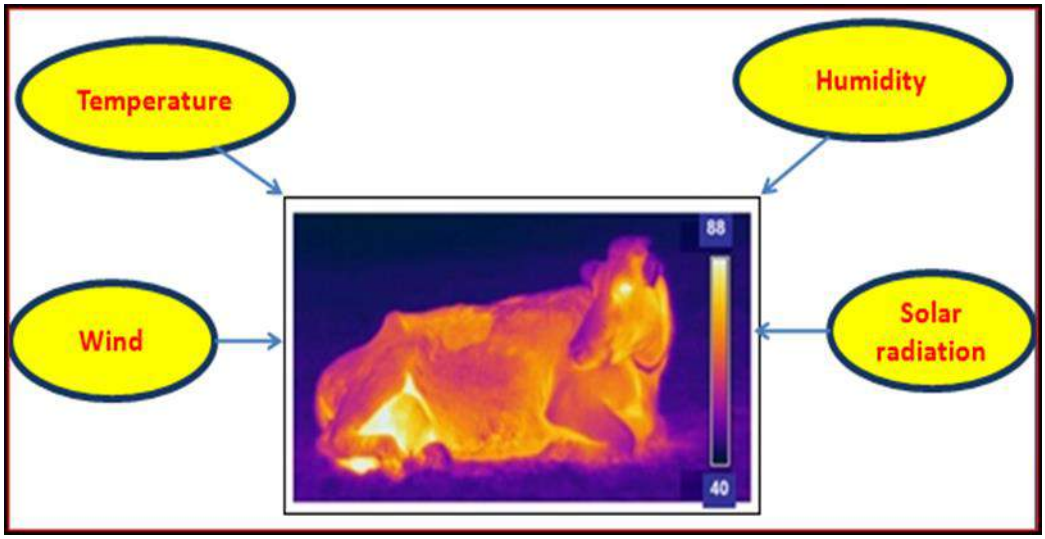

Fig.1. Factors that that contribute to heat stress

How Ruminants Respond to Changing Ambient Temperatures

Ruminants are usually homeotherms which maintain constant body temperature within a narrow range of 37.5-38 ◦C. The animals are comfortable between a temperature range of +15 to +25 which is defined as thermal comfort zone where no energy is expended for thermoregulation maintaining optimum production. When the ambient temperature begins to increase from +25 to +30 ◦C the insensible heat loss mechanisms (conduction, convection and radiation) become operated resulting in heat loss i.e animals sit/come in contact with cooler objects (conduction) and or orient their body towards wind direction (convention). Wind velocity has profound effect on heat lose via convention i.e as wind velocity increases the cool air immediately replaces the hot air surrounding the body as a result thermal gradient is once again established to lose heat to the surroundings. Any object with temperature above 0 ◦C emits radiation. Thus, animals emit radiation gained from the surroundings. Emission of solar radiation is always from high temperature to low temperature.

As the ambient temperature rises from +30 to +35 ◦C, the animals exhibit behavioral adjustments and this temperature range where animals exhibit numerous behavioral adjustments but no change in body temperature is called as zone of thermo neutrality. The behavioral adjustments operated in thermoneutrality zone are shade seeking, moving away from sunlight, reduced motor activity (to reduce metabolic heat production), wallowing, increased water intake and reduced feed intake.

When the ambient temperature (upper critical temperature, UCT) rise above the body temperature the animal tends to gain heat from the surroundings. However, the animals being homoeothermic they exhibit numerous physiological, hematological, biochemical and endocrine changes to maintain core body temperature while deviating a portion of energy for thermoregulation (Fig. 2). This temperature range is called zone of homeothermy and the animals begin to experience heat stress in this zone. If the stressor persists for longer duration the animals enter in to a state of heat stress.

Response of Animals to Heat Stress

Physiological Responses (Panting and Sweating)

In order to alleviate heat stress, the animals exhibit evaporative heat loss mechanisms called panting and sweating. In this process of panting the animals increase their respiratory rate and pant heavily with open mouth. This mechanism results in evaporation of saliva from oral mucosa resulting in heat loss. In addition to panting the heart rate and pulse rate is also elevated to deviate blood from center to the periphery to promote heat loss. Sweating cools the body by following mechanism i.e. with rise in ambient temperature the thermoreceptors of skin gets activated which stimulates the hypothalamus which inturn acts via efferent sympathetic innervations to sweat glands to enhance sweating. The environmental variable relative humidity has profound effect on sweating. As relative humidity increases the ability of the body to lose heat via sweating decreases.

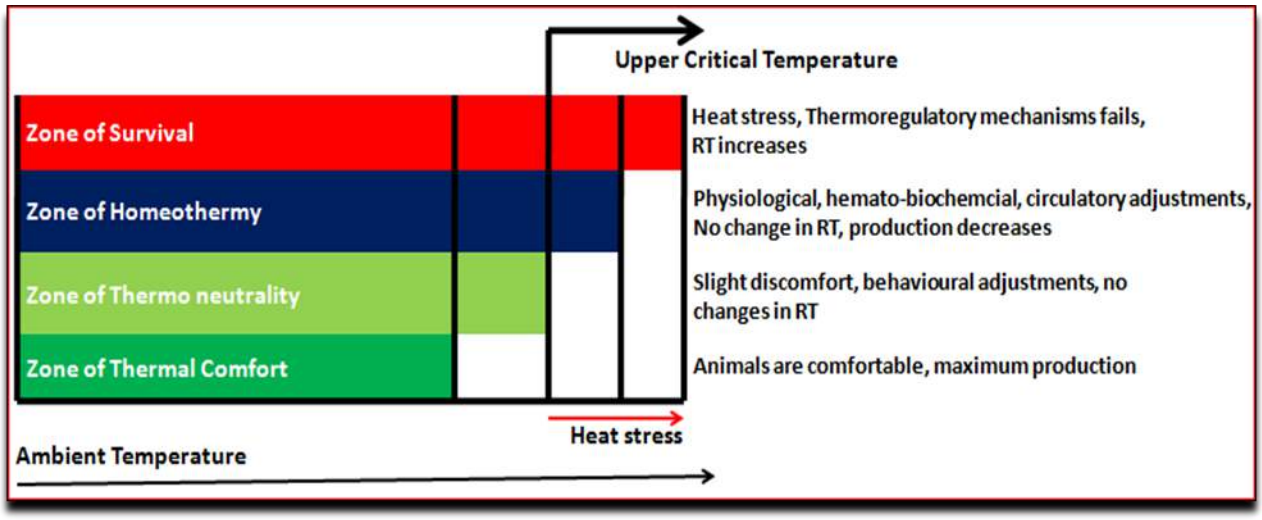

Fig.2. Different temperature zones of ruminants and response of animal to varying temperature zones (Image obtained from Silanikove & Darcan, (2015) and modified accordingly)

Reduced Dry Matter Intake

Reduction in feed intake is one the salient thermoregulatory mechanism operated by the animals to overcome the adverse effects of high ambient temperatures. This mechanism is aimed on reduction of metabolic heat production rather than loosing heat to the surroundings. As majority of heat production in ruminants is due to rumination, a decrease in rumination significantly reduced

heat production by 30%. As feed intake decreases less nutritive substrates are available intracellularly for mitochondrial oxidation and subsequent reduction in heat production. However, this mechanism has negative effect on animal growth and milk production.

Haemato-Biochemical Changes

High ambient temperatures result in significant alterations in hematological, biochemical and endocrine parameters. Initially, high temperatures cause significant dehydration, and there would be significant increase in erythrocyte, leukocytes and hemoglobin concentration relative to plasma which results in high erythrocyte, leukocyte and hemoglobin concentration. In contrast, under prolonged exposure to heat, the values of hematological and biochemical (total protein, cholesterol and triglycerides) parameters decrease due to lack of precursors for synthesis which is associated with depressed feed intake and deviation of energy for thermoregulation. Similarly, during the initial phase of heat stress, there is more of lipid peroxidation leading to oxidative stress. In order to combat oxidative stress, the antioxidant enzymes such as catalase, dismutase and glutathione peroxidase increases. If the existing stressor is for short period the antioxidant enzymes takes care of oxidative stress but under prolonged heat stress the antioxidant system fails leading to heat stress.

Endocrine Changes

The endocrine system acts as second line of defense to combat adverse temperatures. During the initial phase of heat stress the plasma glucose levels tends to decrease due to reduced feed intake and expenditure of energy for excess respiratory function. While on the other hand a low blood glucose levels are lethal to the body. Here the endocrine system plays a crucial role in maintenance of plasma glucose levels by gluconeogenesis which is primarily mediated by nor epinephrine initially and later by prolonged secretions of cortisol from adrenal gland. In order to reduce metabolic heat production, the secretions of growth hormone (GH), Insulin like growth hormone (IGF-1) and thyroid hormone (T3&T4) concentration reduces. The secretions of GnRH, follicle stimulating hormone, luteinizing hormone (LH) also reduces as production gets compromised. In addition, prolactin helps in maintenance of extracellular fluid volume and thus supports heat dissipation. Overall endocrine system is mainly involved in control of metabolism i.e homeorhesis.

Changes in Gene Expression

Gene expression acts as last mechanism to combat heat stress. As the quantum of stress increases the structural and functional proteins beings to denature or undergo misfolding. These changes are deleterious to the body. Inorder to overcome this phenomenon the mRNA expression of heat shock proteins (HSPs) are increased resulting in synthesis of HSPs which prevent misfoldings and protect the structural and functional proteins from denaturing (Fig. 3). The heat shock proteins that are exclusively involved in thermoregulation are HSP 90 & HSP 70.

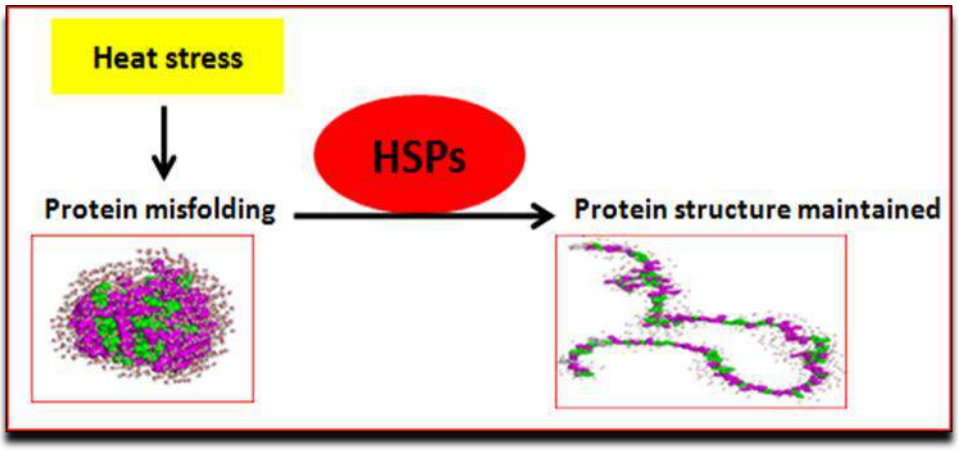

Fig. 3. Effect of HSPs on protein misfolding and maintenance of protein structure

Finally Heat stress

When the values of environmental variables (ambient temperature, relative humidity, solar radiation) far surpass the body temperature, the body is incapable of maintaining the core body temperature, the thermoregulatory mechanisms fails and the rectal temperature begins to increase and the animals enter in to a state called “heat stress” i.e inability to lose heat to the surroundings.

Signs of Heat stress

- Increased respiratory rate
- Elevated body temperature
- Panting and drooling of saliva
- Sweating
- Depressed feed intake

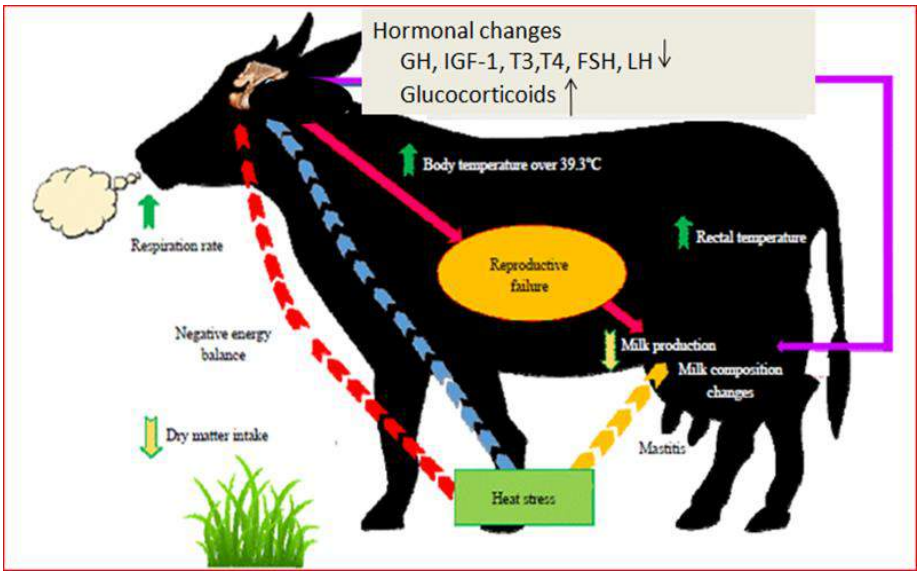

Fig. 4. Pictorial representation of physiological and endocrine responses of ruminants to heat stress (image obtained from Singh et al. (2018) and modified accordingly)

- Increased water intake
- Altered hemato-biochemical and endocrine profile
- Decline in milk production
- Low of milk quality – low fat and protein
- Decline in reproductive performance
- Reduced body weight

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Organic Meat and Meat Products: A Brief Review

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Abstract

Organic farming is an emerging area for crop and livestock production, processing, marketing, trade and consumption, and, therefore, for research all over the world. In developed countries it has made significant inroads but the developing countries especially the Asian countries are in the stage of conception only, as far as organic livestock production is concerned. Some Latin American countries have started exporting organic meat products to developed countries. In such a scenario, information needed in the area of organic livestock production has increased significantly. This paper reviews the developments so far and prospects for future for organic meat production in Asian countries.

Introduction

Food and Agricultural Organization (FAO)/World Health Organization (WHO) Codex Alimentarius Commission defines organic farming as "a unique production system which promotes and enhances agro-ecosystem health, including biodiversity, socio-economic biological cycles and soil biological activity, and this is accomplished by using on-farm agronomic, biological and mechanical methods in exclusion of all synthetic off-farm inputs". Organic system of production aims to promote sustainable production system which is environmental friendly and assures highest standards of welfare to animals. Demand for organic products is steadily increasing across the world due to increasing health consciousness among consumers. Scientific studies have also shown that organic meat has very low total lipid content and possess poly unsaturated fatty acid/saturated fatty acid and n-6/n-3 indices within the recommended values for the human diet. Keeping in mind the low input practices followed by Indian farmers especially in rural and tribal areas it is assumed that India holds tremendous potential in organic food production. This has created a business opportunity for producers too.

What is organic meat?

Organic meat comes from an animal that has not been fed anything grown with toxic or synthetic fertilizers, pesticides, herbicides, fungicides or fumigants; has not been given any kind of growth hormone, antibiotic or genetically engineered product; has been conceived by organically raised animals; and has been butchered and processed following organic regulations.

Production of organic meat is founded upon a number of basic principles, which are embodied within the Standards for Organic Production. In India, The National Standards for Organic Production developed by Ministry of Commerce and Industry, Government of India, provide guidelines for organic production. Some of those relevant to organic livestock production are given below to illustrate the concept:

Origin of animals:

- Two- day old chickens for meat production.
- 18- week old hens for egg production.
- Piglets up to six weeks and after weaning.
- Calves up to 4 weeks old that have received colostrums and have been fed a mainly milk diet.

Breeds and Breeding

Breeds should be chosen which are adapted to local conditions. Breeding goals should not be at variance with the animal's natural behaviour and should be Organic Farming directed towards good health. Breeding shall not include methods which make the farming system dependent on high technological and capital intensive methods. Artificial insemination is allowed and embryo transfer techniques are not allowed in organic agriculture.

Animal health

- Natural medicines and methods, including homeopathy, ayurvedic medicine and acupuncture, shall be emphasized.
- The use of conventional veterinary medicines are allowed when no other non-allopathic alternative is available and where these are used, the withholding period shall be twice the legally required period.
- Vaccines shall be used only when diseases are known or expected to be a problem in the region of the farm and where these diseases can't be controlled by other management techniques. However, genetically engineered vaccines are prohibited.

Packaging and Labelling of Organic Meat

Being a highly perishable product, meat needs to be packaged appropriately to maintain its quality. Packaging plays an important role to protect, contain and promote the food products. While finalizing the packaging strategy for organic food, it is essential to understand the nature of products like their physical form (minced, whole cut, pieces, etc.), characteristics (moisture level, pH, fat content etc.) and stage of processing (convenience, semi-convenience, ready to eat, etc.). Problems of preserving food and transporting it to the final consumer in the best possible condition must be addressed. Basic principle of organic system is sustainability. Hence, packages used for containing organic meat must be safe, sourced in responsible way, environmental friendly and maximize the use of renewable material. Packaging material must not release any chemicals to meat products during storage and transportation. Today, many consumers are concerned about the meat they eat; hence, accurate labelling is important to inform consumer choice. In addition, accurate labelling is important to support fair trade. While regulations enshrined in national and international law underpins mandatory label information, unfortunately, regulations are not sufficient to prevent food fraud. To ensure adherence to regulations, and to enforce punitive measures when needed, robust analytical tests are required.

Pest and Disease Control for Stored Meat and Products

A plan for pest prevention and pest control should be developed. For pest management and control the following measures shall be used in order of priority:

- Preventive methods such as disruption, elimination of habitat and access to facilities
- Mechanical, physical and biological methods
- Pesticidal substances contained in the Appendices of the national standards
- Other substances used in traps
- Irradiation is prohibited.

Marketing of Indian Organic Products: Status, Issues and Prospects.

Consumers' interest in the authenticity of the foods they purchase is increasing, especially where it concerns more expensive 'value-added' products such as organic foods, fair trade products or products with a protected designation of origin (PDO). The scope for marketing organic food in India is vast and still not yet explored to its full potential. The following points can be taken into consideration while designing the strategies

1. Literacy rate has gone up and people are more health conscious now. They think twice before buying a product. Likewise, agriculturists are now more literate and are ready to experiment with new generation of crops and improved farming methodologies.

2. Stressful lifestyle and so many diseases around have created a need for healthier and contamination free food.
3. Organic food market is still restricted primarily to metros and other major cities of the country.
4. Awareness about benefits of using organic food is very less.
5. Competitive pricing can really open a new avenue in the eatables and grocery section.

Recent Trends and Future of Organic Meats and Processing

There are several opportunities that may impact the future of organic agriculture, including organic meat and meat processing. Grazing animals in marginal land that is organic through wild collection is promising. It can increase income of livestock producers operating on these lands. Global acceptance of organic products like goat cheeses, meat and fiber is also an important opportunity for the future growth of organic markets. While energy and chemical costs are high, practicing sustainable organic meat production in general has an economic edge. Alternative medicine as a result of prohibition of restricted materials to treat diseases and illness can be promising. A number of research opportunities are also apparent, the list include: emerging health issues, welfare and production constraints; epidemiological surveillance of key production diseases; breeding studies on disease resistance and commercial traits, nutritional deficiencies in organic systems, livestock breeding, biological control and the use of novel plants and plant extracts, development of animal welfare assessment methods, and development of welfare-friendly production systems. Animal health and welfare, with a greater emphasis on disease control and eradication will likely be the main challenge for organic meat production in the future. In order to accomplish goals of disease prevention, control and eradication, monitoring and evaluating of alternative health products will gain importance. To capitalize on the opportunity of using marginal land for organic meat production, converting hill and upland systems to organic production efficiently will most likely be required in the future. In addition, the interest of consumers in organic products mainly stems from health and environmental considerations. Consumers are concerned about the safety of what they eat and about the use of pesticides, hormones and other veterinary drugs in farming practice. Most of the meats produced in India are organic by default as the animals are grown traditionally with little or no synthetic chemicals. Moreover, the country has a wide range of local animal breeds, those are tolerant to disease. However, there is no active initiative from the public sector extension organizations to promote organic meat production. It is high time to establish better partnership and cooperation among livestock farmers, NGOs, certifiers, marketing people and the programs that will support organic meat production and ultimately it

will contribute in improving the health status of population as well as agro-ecosystem. Certainly more research is needed in this field, but, at present, the advertising claims that consumption of organic meat rather than conventional meat can reduce the exposure to environmental hazards.

Conclusion

Organic meat production is important for many reasons (less environmental impact, less use of energy, and other factors); however, even if the scientific literature in this field is scarce, there is no clear scientific evidence that organic meat can better protect consumers from chemical contamination than conventional meat, even if this is one of the major forces driving consumers to buy organic products. Organic meat production can improve animal welfare, protect the environment, and sustain rewarding rural lifestyles. Traditional and alternative medicine holds the promise for alternative prevention and treatment of animal diseases. The future of organic goat production is to continue searching for alternatives that are environmentally friendly, human health conscientious and animal considerate. Understanding organic farming from economic, ecological, and animal welfare perspectives will increase the likelihood of success. Organic meat production will have to strive for a more sustainable system than the conventional one, offsetting the increased costs of organic livestock production by higher product prices, and certified organic meat products that are healthier than those conventionally produced. To get certification for meat, feeding of certified organic feed ingredients is necessary. Hence, promoting and popularizing production of organic feed ingredients is prerequisite for development of organic meat sector. Community approach in organic animal husbandry can bring about a major advance in respect of organic meat production.

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