Recent Advances in Poultry and Egg Processing and Quality Assessment of Poultry Products

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Course Director
Dr. R. P. Singh

Organizer

Division of Post-Harvest Technology
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FOREWORD

Over the last three decades, poultry production in India has emerged as the fastest growing sector of our livestock economy. With an annual output of about 45 billion eggs and over 1.6 billion broilers with a corresponding average annual growth rate of 5 and 15%, India ranks 4th in egg and 5th in chicken meat production in the world. The country has almost all the needed inputs and potential to accelerate the pace of growth by interlinking poultry production, processing and marketing activities in an efficient manner. Food processing industries in India are now recognized as a sunrise sector and poultry processing is no exception to it. However, the growth and development in both poultry and egg processing sectors have been rather slow which need to be vigorously promoted in line with the increasing demand for quality poultry products in both domestic and export markets. This calls for greater R&D efforts towards minimization of post-production losses, improvement of product quality, value-addition, product diversification, improved packaging, shelf-life extension, quality assurance, food safety, efficient processing wastes disposal to overcome environmental concern and above all creation of efficient marketing network for poultry products.

The present short-term training course on "Recent Advances in Poultry and Egg Processing and Quality Assessment of Poultry Products" is indeed timely and apt as it is in line with the need for dissemination of recent technological advances made in the diverse areas of both poultry and egg processing at this premier institute and elsewhere. This will help in the capacity building of the participants in terms of updated knowledge and skills through lectures by the expert faculty and guest speakers, lively discussions, interaction and practical demonstration of processes and product developed and quality assessment techniques.

It is indeed a matter of pleasure and satisfaction that the lectures to be delivered during 10 days training course organized from the 29th Aug. to 7th Sept., 2005 at this institute, under the aegis of ICAR, have been compiled in form of a manual which would serve as a key reference for study and use. The efforts of the organizers in producing this training manual is highly appreciated. I wish all the participants a comfortable and meaningful stay at CARI, Izatnagar.

(Rajvir Singh)
Director
CARI, Izatnagar
PREFACE

Processing is a vital link between food production and its marketing. Both egg and poultry processing sectors in many countries have undergone rapid transformation to remain competitive in the present globalised trade scenario under WTO regime. There is now more emphasis on product quality rather than mere production. The processing industry is also adapting to changing consumer needs and preferences. As overall efficiency of poultry production is greatly dependent on the efficient utilization of its produce in the present market-driven competitive environment, there is a dire need for identification, development and promotion of relevant technologies for processing, preservation, product development, packaging and evolving effective quality assurance system and efficient marketing network for sustained growth of Indian poultry industry.

The present training programme has been devised to cover the latest development in genetic and nutritional manipulation to improve both egg and poultry meat quality. Furthermore, innovative technological developments in diverse but interrelated areas of primary and further-processing, value-addition, meat tenderization, bio-preservation including hurdle processing, efficient packaging, safety against bio- and phyto- contaminants, separation technology for egg bioactive compounds, efficient waste disposal, current national and international standards and regulations for quality control of poultry products and IPR issues are covered. This apart, modern biochemical, microbiological and molecular approaches for the detection of meat adulteration and quality evaluation of poultry products have also been included. I hope that the presentation, discussion, interaction and practical demonstration of processing and quality evaluation techniques by expert faculty and guest speakers from IVRI in the aforesaid areas would be of immense benefit to the participants. The financial assistance received from the ICAR, New Delhi is thankfully acknowledged.

(R. P. Singh)
Course Director
Production, processing and marketing of perishable food like egg and poultry meat should go hand-in-hand for the benefit of producers, processors and consumers alike. This calls for adoption of an integrated approach to develop a strong interface between poultry production and processing as well as streamlining of marketing of poultry products for sustained growth of Indian poultry industry. In view of this, an attempt has been made here to present a brief account of current state of developments in poultry production and processing in India, major constraints and challenges and future thrust for interlinking poultry production and processing activities for marketing of cost-competitive, quality poultry products tailored to consumer's demand and promotion of export trade.

Current status of poultry production and processing

Over the last four decades, Indian poultry industry has witnessed a rapid growth and transformed itself from backyard venture to a dynamic fast-expanding agri-based industry. Both commercial egg and broiler production have developed at par with many developed countries during this period. India with an output of about 45 billion eggs and 1.8 million tones chicken meat in 2004, occupies 4th and 5th ranks respectively in the world. However, we should not be complacent with these remarkable achievements but continue to evolve appropriate policy, programmes and strategies for further development in poultry sector to attain the distinction of occupying top position like milk production in years ahead. The country has all the needed inputs to sustain the present growth rate of 5 to 7% in egg and 10 to 15% per annum in broiler sector, provided the processing and marketing of poultry products move in tandem with production.

At present, there are about 185 million commercial hybrid layers with an annual laying potential of over 300 eggs with feed conversion efficiency of about 1.75 kg/dozen eggs and broiler achieving an average body weight of over 1.5 kg at 5 weeks with about 1.8 feed conversion ratio. At the breeding farms, over 110 pullet chicks and nearly 160 day-old broiler chicks per dam are being produced. Indian poultry industry is today worth Rs.29,000 crore, contributing about Rs.15,000 crore per annum to our national GDP. Poultry sector provides direct or indirect employment to about 3 million people in the country. However, there is a big gap between present availability of about 44 eggs and 1.5 kg poultry meat per head per year and the recommended requirements of 180 eggs and 10 kg meat. Bridging this gap will create over 6 million jobs. As such, production of egg and meat has to increase several fold to meet the recommended consumption level.

The spectacular growth in poultry sector is attributable to several factors. These include intensive breeding and selection for developing high yielding layer and broiler stocks, better feeding management and availability of balanced feed, application of modern farm management practices and health care measures, etc. This apart, strong entrepreneurship leading to rapid development of various input industry, vertical and horizontal integration in poultry production, commercial egg and poultry processing in modern mechanized processing plants, rapid expansion of fast-food parlours, increase in export trade of dried egg products and frozen broilers, table eggs, hatching eggs, day-old chicks and above all ever-increasing demand of poultry products in the domestic markets are other contributory factors. The CARI and some other R & D institutions including State Agricultural Universities with strong Poultry Science
education and research base have contributed immensely in providing much needed highly qualified man-power and addressing problems of the poultry industry.

However, despite these remarkable breakthrough in both egg and broiler production, post-production i.e. processing sector is still in a nascent stage and marketing of poultry products is in a disorganized state. Live bird sale and on-the-spot slaughter at street meat shops in wet market are the predominant mode of disposal of broilers. Similarly, eggs are generally marketed as they are produced. Sale of clean and assorted eggs on a limited scale is a recent development in Indian metros and big cities. Most of over a dozen modern mechanized processing plants and half-a-dozen export oriented egg processing units set up in the country are running much below their installed capacity owing to less preference for frozen chicken, lack of cold-chain and negligible demand of egg powder in the domestic market. These modern processing plants are processing hardly about 5% chickens and a small fraction of total eggs produced in the country. Besides, a number of small processing units are also producing dressed whole chicken, cut-ups, boneless chicken meat and some value-added poultry products. One notable development, however, is the emergence of India as one of the major exporters of dehydrated egg products which jumped from 7000 metric tonnes in 2003 to about 10,000 metric tonnes in 2004. But this quantum jump in a year was mainly due to outbreak of avian influenza in some South-East Asian countries and high prices of eggs in the USA and Europe. Another remarkable development is the export of frozen broiler from India to Japan recently, which was due to adoption of broiler production and processing as per internationally accepted standards. However, the share of India in world’s export trade of egg and poultry meat combined together is just about 0.45% of global trade. It has to go a long way to catch up with even some developing countries like Brazil, Thailand and China in export of poultry products on a big scale.

Constraints and challenges

Indian poultry industry is characterized by a mix of small (low input – low output), medium (medium input – medium output) and large (high input – high output) farms. While layer farms are scattered, especially in rural areas, the commercial broiler farms are mainly concentrated around urban and peri-urban areas. The constraints faced by large commercial enterprises and big poultry farmers are different from those confronting the small farmers. The vertically and horizontally integrated poultry producers have set up their own modern mechanized egg and/or poultry processing plants to meet the growing demand of poultry products by urban consumers mostly through their own sale outlets and are also engaged in export trade of processed poultry products. These large poultry producers with large throughput, higher efficiency of production and better control over production, processing and marketing suffer less than the medium and small producers who are mostly dependent on middlemen for disposal of their produce and are often exploited. Escalating input cost and occasionally overproduction too give rise to non-remuneration return, forcing many small poultry farmers out of business. As a result, the big farmers are growing bigger and the small ones are forced to find their own niche market for survival. The latter does not have the economy of scale to set up their own processing units and sale outlets to compete with large producers-cum-processors to meet the growing demand of processed poultry products of consistent quality at a competitive price with efficient delivery back-up.

Marketing is the weakest chain in poultry sector. The roles of NECC and NBCC are also of limited nature in this regard. No serious efforts have been made to encourage, guide and help poultry farmers co-operative societies to set up their own small to medium size poultry processing units and sale outlets for ready-to-cook or ready-to-eat products tailored to the demand of consumers or develop linkage with local restaurants, other institutional markets and vendors for this purpose. Rural poultry production remained neglected in the process of commercial (mass) poultry production. Only in recent years, renewed efforts are being made to promote
rural poultry production in the country for co-existence of both mass production and production of the masses to generate additional employment, alleviate malnutrition and improve rural economy.

In the changing global poultry production, processing and international trade scenario under prevailing WTO regime, both poultry production and processing need to develop close interface to face an array of challenges and at the same time also grab opportunities. These include not only the production of consumer-oriented, competitive value-added products of high consistency, quality and safety against pathogenic microflora and residues of harmful substances but also increasing concern over environmental pollution arising from poultry farm, hatchery and processing plant wastes. Besides, bird’s welfare during rearing and processing is of growing concern in some economically developed countries and this issue is also being raised by animal activists and general public in India in recent years. Poultry products have to be produced and processed conforming to national and international standards by adhering to SPS regulations since quality assurance and traceability are emerging as the hallmark of trade in domestic and more particularly in export markets.

**Futuristic approach**

Poultry production and processing need to be inextricably interlinked since appropriate control over both of them is crucial to the quality and safety of poultry products. Both these sectors have to respond to the demand of market in view of a gradual shift from sale of live bird and dressed whole chicken to cut-ups, boneless chicken and further processed, value-added, convenience products in convenient packs at affordable price. Product diversification, quality improvement, quality assurance and strategic marketing are the key factors to increase demand and growth of poultry sector. Rising income and increasing quality consciousness among consumers would demand functional foods, designer eggs, nutraceuticals and pharma foods derived from egg and meat. In this respect, some thrust areas of R & D activities on poultry production and processing are highlighted below.

**Breeding**: Poultry breeders have to focus attention towards selection and breeding for low cholesterol eggs, stronger egg shell, lean broiler meat, improved muscling, especially in the breast region, low abdominal fat and reduced level of undesirable low density lipoproteins (LDL) in egg and poultry meat. Thrust should also be laid on overcoming the problem of osteoporosis and leg weakness in fast growing broilers owing to intensive genetic selection so as to minimize condemnation of carcasses due to quality defects. Biotechnology can play a crucial role in mapping quantitative trait loci (QTL) for a range of economic traits like carcass fatness, muscling, carcass composition, egg shell strength and resistance to diseases, etc. and exploitation of these QTLs by marker assisted selection (MAS) to achieve the desired goal.

**Nutrition**: Development of nutritional strategies to alter and optimize carcass composition, abdominal fat deposition, egg cholesterol reduction and egg shell quality improvement are the need of hour. Other dietary manipulation will include enriching egg yolk with highly desirable omega-3 fatty acids to produce designer eggs for health-conscious consumers. Similarly, enriching poultry muscle lipids through incorporation of 200 to 300ppm α-tocopherol acetate (Vit. E) and 0.4ppm selenium in diet two weeks before slaughter will improve oxidative stability of stored meat.

**Management and disease control**: Better managemental practices during rearing and pre-slaughter handling could minimize downgrading of poultry carcasses due to bruises, breast blisters and stress related decline in meat quality. Besides, strict bio-security measures are needed both at farms and processing units to control emerging and re-emerging diseases and improve microbial quality of poultry products.
**Processing, quality assurance and waste disposal:** Both egg and poultry processing, which is still low, should be vigorously promoted to provide products tailored to specific requirements of consumers and boost export trade. While large modern mechanized processing plants are needed to cater to the bulk demand of urban consumers and also for export trade, establishment of more number of small processing units, utilizing indigenously fabricated processing equipments, will cater to the consumer’s preference for fresh chicken and value-added products suited to demand of local people and minimize dependence of small farmers on middlemen for disposal of their produce.

Since poultry production is not evenly distributed in India, an alternative multi-stage processing and marketing approach deserve consideration. In this system, deboned meat and chicken meat emulsion or mix can be produced at centralized large poultry processing units and then supplied in frozen form to a number of secondary meat processing units which can prepare and market products as per the demand of consumers. These secondary units can further supply these semi-processed products to meat retailers/fast-food parlours, constituting the third stage unit for selling value-added products in consumer packs for home preparation or as ready-to-eat products to local consumers.

Apart from the above, thrust should be laid on surveillance, and regular serological monitoring and control of Salmonella at farm, strict bio-security measures, monitoring of residues of harmful substances like pesticides, veterinary drugs, heavy metals in feed, egg and meat to meet the sanitary and phytosanitary (SPS) regulations under WTO regime. Efficient spent hen disposal, bio-preservation, product diversification, modified/controlled atmosphere packaging, irradiation and hurdle processing are other considerations to improve product quality and enhance safety and shelf-life. Efficient disposal of farm, hatchery and poultry processing wastes as feed, fuel and fertilizer is the need of hour to overcome environmental concern. Above all, establishment of National Poultry Development Board (NPDB) on the line of NDDB and proposed National Fishery Board will go a long way in regulating and integrating production, processing and marketing of poultry products as per supply and demand for accelerating the pace of growth of Indian poultry industry.
Developments in Poultry and Egg Processing in India: An Overview

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With an output of over 45 billion eggs and 1.8 million tons chicken meat in 2004, constituting around 3.5% and 2.1% of global output, India ranks 4th largest egg and 5th largest broiler producer in the world. Poultry meat constitutes about 25% of total meat production in the country. The present per capita annual availability of eggs (44) and poultry meat (1.5kg) is, however, much lower than the world average of 102 eggs and 10 kg poultry meat respectively. There is a wide difference in consumption of poultry products between urban and rural areas. Nearly 30% of the population living in urban areas consume about 70-75% of poultry products. With the intensification in commercial poultry production, the share of desi (native) fowls to total egg production has come down to around 20%.

Commercial Poultry & Egg Processing: Despite breakthrough in poultry production as indicated above, the processing sector is still in its infancy and marketing sector is in a disorganized state. Hardly about 20% of chickens (including 5% by modern mechanized processing plants) and 5% of eggs produced in the country are marketed as processed poultry products or used as ingredient in other food products. Of the dressed chicken, about 75% are sold as hot dressed, chilled frozen whole carcass and the rest are marketed as further-processed, value-added products mainly in urban areas. The vast rural markets remain untapped for whom desi chicken, spent hens, ducks and to a limited extent broilers are the source of poultry meat.

Most of the over a dozen modern poultry processing plants (2000-6000 chickens/hr) and half-a-dozen export-oriented egg processing plants with a combined installed capacity to process 5 million eggs per day are running much below their installed capacity. This is mainly due to less preference for frozen chicken in domestic market, lack of both cold chain and organized marketing system to market it over distant deficit region, negligible domestic demand for pasteurized liquid or dehydrated egg products, inadequate development of value-added products sector and fluctuating export trade often characterized by high import duty and strict sanitary and phyto-sanitary (SPS) measures adopted by importing countries. However, after a brief slump, India has emerged as a leading exporter of both frozen liquid (16,500 tons) and dried egg products (10,000 tons) in 2004 in Asia. This apart, India has also started exporting frozen broiler and shell eggs on a modest scale. However, Indian export trade of poultry products worth about Rs.200 crores is a very small fraction of the global trade which constitutes about 8% and 2% of poultry meat and egg output in the world.

R & D on Poultry Processing: A great deal of R&D efforts have been made at this institute and some other institutions in the country to evolve cost-effective and efficient processes for processing and preservation of egg and poultry meat, tenderization of spent hen meat and development of over one-and-a half dozen value-added poultry meat products and one dozen egg products, their packaging and evaluation of storage stability, etc. Some of the processes developed have also been patented. In recent years, work on assessment of residues of some potent pesticides and veterinary drugs has also been initiated.

Several meat processes such as hot and cold deboning, thermal hydrolysis, enzymatic and blade tenderization of tough meat, massaging and tumbling for increasing myofibrillar proteins solubility and meat particle binding, flaking and dicing for restructured products, chopping and emulsification for comminuted products, marination, battering and breading for enrobe
products, canning, curing and smoking and more recently hurdle technology for shelf-stable poultry products have been successfully used to develop a variety of convenience poultry products. These include chicken essence, chicken soup mix, tandoori, barbecues, canned chicken products, chicken skin snacks, gizzard snacks, cooked chicken stock, enrobed thighs, drumsticks and wings, marinated chicken breast fillets, chicken meat spread, shelf-stable spiced chicken bits, wafers, freeze-dried chicken pulav and intermediate moisture chicken meat, etc. In addition, restructured/comminuted products like chicken sausages, patties, nuggets, rolls, loaves, balls, cutlets, steaks, kababs, croquettes and chunkalona, etc. are other premium products developed from low value spent hen/culled breeder meat, skin, gizzard, heart, ova yolk and adipose fat. The lean meat may be varied from 50% to 60 or 70% with edible offals and non-meat binders and extenders (cereal products/pulses/vegetables) to produce economy, choice and prime grade comminuted products for diverse income-group consumers in rural areas. Shelf-life of these products has also been evaluated under different packaging and storage conditions to simulate marketing conditions.

R & D on Egg Processing: A low cost, batch type immersion egg washing machine and egg washing powder were developed during late 1960's. However, in recent times, improved in-line egg spray washers are being used commercially. Besides, a small egg breaking-cum-separating machine has also been designed and fabricated indigenously at IIT, Kharagpur which can be scaled up for commercial use. The egg coating oil developed for preservation has been found to be highly efficacious in maintaining the interior quality of egg up to 4 weeks at ambient temperature (26°C). Similarly, processes have been standardized to control gelation in frozen yolk/whole egg liquid by colloid milling or addition of 0.02% pepsin, 0.5% trypsin, 5 to 10% common salt or canesugar or 5% glycerol in the egg magma prior to freezing. The manufacturing processes for about a dozen value-added egg products, such as whole egg powder, yolk granules, albumen flakes, albumen rings, canned curried egg, scrambled and omelette premixes, egg patties, egg-chicken meat patties, egg pizza, egg roll, sponge and pancakes, mayonnaise, pickled eggs and more recently biscuits and egg-milk beverage (egg nog) containing 10% and 3.5% whole egg powder, respectively have been developed within the country. These products could be commercially exploited to boost egg consumption and satisfy the growing demand of consumers for convenience egg products.

In recent years, egg drying industry has been using membrane filtration (ultrafiltration/reverse osmosis) and vacuum evaporation processes for the concentration of egg albumen prior to drying to save energy. Similarly, ultrapasteurization-cum-aseptic packaging techniques have been developed abroad to extend the refrigerated shelf-life of liquid egg from about one week to 4 and 24 weeks at 10°C and 4°C, respectively for marketing in non-frozen form. More recent development is the vacuum evaporation of liquid whole egg followed by quick freezing for the production of frozen egg pellets. Some other promising convenience egg products like scotch egg, deviled egg, and egg yogurt could be developed and marketed in the country. Scotch egg is a snack food of peeled, hard-cooked egg covered with a layer of sausage and deep fried. Deviled eggs are prepared by cutting peeled eggs longitudinally into two equal halves, removing the yolk for seasoning with salad mixture and then stuffing it back into the albumen cavity. Egg yogurt is a cultured (Lactobacilli) mixture of egg and milk.

By-product Processing: Considerable research work on processing and utilization of egg shell and poultry processing wastes as valuable by-product meals for recycling in poultry feed and also as pet foods has also been carried out in the country. However, for practical purposes, they are not being utilized properly at present. Substantial quantity of egg shell, constituting about
10% of egg weight, is being produced by egg processing industry. The conventional method of converting this value-added by-product into egg shell meal consists of centrifugation to recover residual albumen, autoclaving, oven-drying and grinding into coarse or fine powder. Recently, extrusion technology has been developed for processing of shell into extruded meal. The process, in brief, consists of centrifuging the egg shell to collect residual (technical) albumen followed by wet milling to fine particle, chilling (if not immediately used) to 2°C and blending with other ingredients (70% wet milled shell, 8% technical albumen, 5% crushed maize, 17% soybean meal and 0.15% propionic acid). The blended mass is finally fed to extruder to obtain extruded egg shell meal which contains about 9% moisture, 13% crude proteins and 24% calcium. The high extrusion temperature eliminates Salmonella and the finished product can be utilized for feeding to layers.

Similarly, a simple process of conversion of poultry processing wastes, constituting about 25-30% of live weight of poultry, into a highly palatable and nutritious pet food biscuits containing 5% moisture, 24% crude proteins, 4200 kcal ME/kg, 0.8% lysine, 0.5% methionine with high(70%) pepsin digestibility and shelf-life of 6 months at mean ambient temperature (26°C) in LDPE (300G) pouches have been developed at this institute for adult dogs. Processes have also been developed for producing intermediate moisture (semi-moist) poultry by-product based patties, using humectants, for young pups.

**Major Constraints:** Prior to the liberalization of economic policy in 1991, India’s priority was more on commercial poultry production than on processing. Thereafter, food processing in general and poultry processing in particular started gaining momentum. However, lack of strong domestic market base, conventional dietary habits of consumers, inadequate advertising and campaign to highlight the virtues of processed poultry meat products, consumer’s lack of faith in the quality of such products in the absence of stringent quality assurance programmes for domestic market, non-awareness about availability and usually high price of processed products and inadequate infrastructures like processing equipments, cold-chain including refrigerated transport and marketing network are the major bottlenecks confronting the growth of poultry processing and product development sectors. High import duty, strict sanitary and phyto-sanitary (SPS) issues and huge subsidy available to competitors in economically developed countries are the other reasons for the slow growth of Indian export of poultry products. The subsidy issue was raised by developing country led by India at WTO meet-2003 in Cancun. Besides, little or no serious effort has been made to design and develop egg and poultry processing and further processing equipments indigenously to suit the requirements of small, medium and large primary and further-processing units. The high-tech processing systems may not necessarily be relevant to the requirements of Indian conditions. Lack of a closer and dynamic linkage between R&D institutions and poultry processing industry is another impediment in this regard. In the prevailing market-driven competitive environment under WTO regime, it is pertinent to identify and address the constraints to facilitate development of processed poultry product sector.

**Future Prospects:** Diversity in tradition and culture of India has resulted in different culinary practices for the preparation of a variety of traditional poultry products, some of which (tandoori, curried chicken and kababs) have become popular abroad. However, non-availability of standard processing methods to develop products of uniform quality has met with limited success to commercialize traditional products. Hardly about a dozen chicken preparations are available to Indian consumers in sharp contrast to more than a hundred varieties of value-added poultry products being marketed in some developed countries. Hence, concerted efforts should be made to develop process for some newer products to suit the palate and pocket of the consumers.
Similarly, development of more and more comminuted, restructured, enrobred and marinated flavored poultry meat products as ready-meals, snacks, carry-home meals and full or supplementary military pack rations in accordance with the requirements of end-users would enhance the consumption of such products. In this respect, emphasis should be laid on developing shelf-stable products based on Indian cuisine for which there is an enormous potential in both domestic and export markets.

Some new processing techniques like hotshot cooking, irradiation, high pressure cooking, hydrodynamic pressure cooking, pulsed electric/magnetic field and ultrasonic wave based equipments could be used for packed ready-to-eat products to control thermo-stable pathogens. Like approval of irradiation by the Govt. of India for decontamination of meat including poultry carcasses and more particularly for control of Salmonella, ozone has also been approved recently by the FDA for food contact application to disinfect air, water, processing equipments and decontamination of carcasses/meat surface in lieu of conventional chlorinated water.

India has a booming food market and expanding economy. As per the CII-Mckinsey survey report, poultry sector has third highest growth potential after wheat-and milk-based products in India. However, future growth of poultry industry would be greatly driven by promoting processing sector, especially processed product sector and expanding domestic market base, particularly in hitherto untapped rural areas, apart from promoting export trade. Problems related to pathogenic miroflora and/or their toxins, as well as residues of harmful substances like hormones, heavy metals, packaging migrants, veterinary drugs and pesticides could be tackled by adopting GMP or more preferably HACCP system right from the production to primary and further-processing stages. The exportable poultry products must meet the quality requirements prescribed by the Codex, EC or USDA, which are internationally recognized and accepted on a large scale.

The prevailing image of value-added poultry products as domestic upmarket or foreign market products needs to be changed. This calls for production and marketing of products as per the needs, expectations and acceptance of as large population as possible at affordable price. Apart from developing sophisticated technologies for specialty products for niche market, concerted efforts should also be made to promote poultry production and develop cost-effective, intermediate technology for promoting poultry processing as a cottage industry in rural areas, where huge market potential exists, as a vehicle of rural employment and income generation.

In the changing domestic and export market scenario, a two-pronged strategy needs to undertaken. For export, applications of modern processing techniques and management approaches to produce products of international standards are vital factors, whereas adequate hygienic measures at all stages of processing and product development, clean transportation and chiller/freezer cabinets for display/storage are needed in view of the preference for freshly dressed/chilled chicken and further-processed products in the domestic markets.

It is pertinent to mention that most of the Indian Standards on egg and poultry meat products are very old which need to be revised and upgraded to the level of International Standards, taking into consideration the technological developments in egg and poultry processing and changing international trade scenario under WTO/SPS regulations. The upgradation of national standards would also prevent dumping of substandard poultry products in Indian markets. Furthermore, harmonization of Indian standard and methods of tests for quality control with ISO standards would bridge the gap to face emerging challenges in the export of
Indian poultry products. Already, the Bureau of Indian Standards (BIS) has taken up initiative in this direction in recent years. In addition, efforts are in progress to develop an integrated single food law and regulatory agency known as “Food Standards Authority” by the Ministry of Food Processing Industries, GOI so as to replace the existing multiple food standards and regulatory agencies under different Ministries to regulate production and supply of quality food products and encourage foreign direct investment in this sector.

Apart from the above, more attention needs to be given to the following R & D activities for ushering in revolution in poultry and egg processing sectors.

i) Establishment of more and more mini poultry processing units in view of preference for freshly dressed/ chilled chicken among Indian consumers.

ii) Keeping in view the growing quality consciousness among consumers and increase in disposable income, effort should also be made to produce eggs low in cholesterol, rich in ω-3 fatty acids and lean poultry meat, etc.

iii) Creation of appropriate mechanism for assessment of the hygienic status of poultry and meat processing units, energy conservation, market research/survey particularly for value-added poultry products and vigorous campaign to educate consumers about the health benefit of poultry products and removal of misconceptions about egg through mass media including EDUSAT launched recently.

iv) Shelf-life extension of poultry products through application of hurdle technology including bio-technological(microbial/enzyme) methods for storage and marketing at ambient temperature.

v) Evolving newer techniques for tenderization of meat from spent hens/culled birds and mechanical de-boning of poultry meat for comminuted products.

vi) Work on electrical stimulation and carcass conditioning for portioning and de-boning of poultry carcasses without chilling and aging to save energy, time and storage space.

vii) Evolving efficient processes for extraction of lysozyme, avidin, lecithin and other biomolecules from eggs in view of their growing commercial significance.

viii) Ultra-pasteurization-cum-aseptic packaging for shelf-life extension of liquid egg for home usage, especially in urban area and establishment of technical and hygienic requirements for the production of frozen liquid egg products for export purpose.

ix) Concerted efforts should be made to standardize uniform processing technology for traditional poultry products which are in great demand abroad and widening the range of value-added newer products to boost domestic and export market prospects of poultry products.

x) Improved packaging systems (vacuum, MAP/CAP, retort, aseptic) using eco-friendly food packaging materials need to be introduced for proper storage and distribution of poultry products.

xi) Development/standardization of quick and sensitive methods for the detection and regular monitoring of pathogenic microflora and/or their toxins as well as residue of harmful substances like pesticides, veterinary drugs and heavy metals etc. in poultry products.

xii) Application of conventional and bio-processing techniques for efficient disposal of poultry by-products need to be evolved to minimize pollution hazard and reduce the processing cost.
xiii) Design and fabrication of improved egg transport boxes to minimize egg breakage, poultry-friendly crates and lorry for transport of live birds as well as egg and poultry processing equipments for small, medium and large poultry processing units indigenously, efficient cold-chain system and establishment of sound marketing network deserve special mention in this regard.

xiv) Close and dynamic linkages between R&D institutions and egg/poultry processing sectors as well as among poultry producers, processors and traders.

The measures suggested above would go a long way in putting the hitherto neglected poultry processing sector on a sound footing for sustained growth of Indian poultry industry in years ahead.
Nutritional Manipulation for Improving Poultry Meat Quality

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There are growing public concern of the society is the quality food including the foods of animal origin. Safe food is also a slogan of the present society. The poultry meat has already gained a healthy image. The productivity of meat-type chicken has also dramatically improved due to the concomitant progress in rearing methods, nutrition, genetics, disease prevention and veterinary medicine that has resulted in reduction of slaughter age. As the latter is the main factor of sensory meat quality, it was the principal criterion of the qualitative segmentation of chicken production. Hofman (1994) briefed the meat quality as 'the totality of all properties and characteristics of meat that are important to its nutrient value, acceptability, human health and processing'. Moreover, with the increasing export potentiality, demand of frozen meat and shell of frozen meat through outlets far from the processing center, either in domestic or in international market, keeping quality of meat also assumes prime importance. Nutrition or feeding of birds influences meat qualities in terms of nutritive value, acceptability, human health and processing save guarding interest of producers, processors and consumers. These qualities are assessed through physical (pH, colour, tenderness, water holding capacity, etc.), chemical (moisture, protein, ether extract, cholesterol, triacylglycerols, fatty acids, oxidative status, residuals etc.), organoleptic (taste, flavour, juiciness, etc.), and microbiological characteristics (microbial load or contamination, etc).

Nutrition affecting nutritive value of meat

The major changes that can be brought through nutritional manipulation are increase of protein with concomitant decrease in fat, enrichment of meat with fatty acids, fat-soluble vitamins and probably certain minerals. Manipulating dietary energy to CP ratio and amino acid concentration can increase the relative concentration of protein and moisture in breast and thigh muscles. Protein accretion in the meat might be increased by chromium (Cr) supplementation (0.2 mg Cr per kg food dry matter), while fat deposition in meat could be lowered. Supplementation of Cr and Cr-yeast complex also improved sensory evaluation scores. Methionine and/or phytase did not affect carcass yield and chemical composition of meat of male Campbell ducks. Supplementation of copper (Cu) @ 35 to 126 mg/kg feed increased oleic acid content. The relatively low copper concentration of 35 mg/kg and vitamin E @ 100 mg/kg feed reduced eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). All tested copper doses (35 to 126 mg/kg feed) reduced meat cholesterol (Skrivanova et al. 2004). Probiotics containing six strains together (Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, Aspergillus oryzae, Streptococcus faecium and Toluropsis) @ 100mg/kg diet improved moisture, protein, ash and decreased fat content in leg and breast meat of control chickens (Khaksefidi and Rahimi, 2005).

Nutrition affecting acceptability of meat

An increase in the degree of un-saturation of meat may enhance the development of organoleptic problems and lead to increase susceptibility to lipid oxidation. Tocopherols in meat prevent lipid oxidation and improve sensory quality of poultry meat. The use of fish processing wastes (FPW) or fishmeal in diets of broilers or rooster diet did not add fishy flavour to meat. Similarly feeding rapeseed meal (200 g/kg) had no strong aversion of breast meat to tasting.
The addition of rosemary improved the sensory properties (taste and flavour) of chicken meat. Additions of higher vitamin A for chickens during the rearing period had no beneficial effect on physico-chemical meat traits. Beneficial effects of dietary lysine level were only evident on pH but not breast measures (height, width and length), cooking loss, and breast meat shear force (Pavan et al. 2003). Methionine and/or phytase did not affect physical characteristics of meat of male Campbell ducks (Attia, 2003).

The edible meat from broiler chickens fed Saccharomyces cerevisiae exhibited increased tenderness (Bonomi et al. 1999), water holding capacity (Lee et al. 2002) and oxidative stability of meat (Zhang, et al. 2005). There was a significant decrease in color (lightness) and increase in pH of breast muscle 5 hours after slaughtering in the probiotics treated birds. In the sensory analysis, meat flavour and general aspect 72 hours after slaughter were better when probiotics were added in both water and diet. There were no differences in water holding capacity, cooking loss and shearing force among different probiotics or the control. Thus, meat quality was better when probiotics were fed in the water and diet instead of only in the diet. Cooking loss decreased and shears force increased in the supplemental probiotics group. The number of ileum and caecum Lactobacillus spp. tended to increase in the supplemental complex probiotics group at 7 weeks of age. Chickens reared in a shed until the age of 45 days, followed by transfer to a playing field for 46-100 days or reared in a shed for 20 days, and then moved to a playing field until the age of 100 days had better meat flavour than those reared in shed through out (Liao et al. 2003)

Nutrition affecting meat quality for human health

There are growing public concern over consumption of poultry meat with more of saturated fatty acids and cholesterol. The amount and proportion of animal fat intake has been increased in many societies. Coronary heart disease and arteriosclorosis are strongly related to the dietary intake of cholesterol and saturated fatty acids. Chicken meat is relatively low in fat and cholesterol, and thus considered as healthier than other animal protein sources. Numerous including nutritional factors have been demonstrated to alter fat deposition and cholesterol contents in meat. Dietary supplementation of methionine; lysine, glycine, tryptophan and amino acid mixtures can reduce body fat deposition (Takahashi et al. 1994). Dietary fiber also reduces fat concentration and facilitates lean meat production. Fat content was low in the meat of Japanese quails that were fed fodder with standard diet. Dietary supplementation of garlic, copper and omega-3 fatty acids has been used successfully to reduce cholesterol content in poultry meat. Dehydrated alfalfa is a good source of hypocholesterolaemic compounds such as saponins. It was possible to produce chicken lean breast meat with reduced cholesterol content by feeding free choice alfalfa (Ponte et al. 2004).

Diet composition can affect the nutritive quality of poultry meat, which enables designing of fatty acid profiles in the lipids of muscular tissue. Increased omega-3 fatty acid content in the chicken meat, such as alpha-linolenic, eicosapentaenoic and docosahexaenoic fatty acids can positively influence consumer health. From a human health aspect, the fatty acid composition of meat or meat products is an important quality parameter. The fatty acids composition in body fat, that determines the quality, includes the dietary fatty acids and the fatty acids, which are synthesized in the body i.e. the fatty acid composition of chick tissues generally reflects the fatty acid profile of the diets. Dietary fat sources thus can modify mostly the fatty acid composition of liver and muscle tissues. The omega-3 group of polyunsaturated fatty acids (PUFA) has received a lot of attention from health professionals and nutritionists in recent days due to their (n-3 fatty acids) beneficial effect on heart health. Higher intake of PUFA decreases the risk of heart diseases and strokes, and also exerts immunological and neurological effects and has positive effects in the prevention of heart beat irregularities, cancers, aging and even depression, and
improving blood lipid profile and blood coagulation. The DHA is a major component of brain tissues and retina. Its intake is positively correlated in learning process and deficiency is related to impaired retinal function as has been established in rat study. The essential fatty acids linoleic (18:2 n-6) and linolenic (18:3 n-3) acids are the precursors of EPA and DHA, receptively. Broiler chicken can provide an excellent alternative source of PUFA, because poultry meat is naturally low in fat content and rich in PUFA. For this reason, many studies are directed towards the manipulation of the fatty acid composition of broiler chicks further to augment human health. Cod liver oil positively affected performance, but even at 1% supplementation rate, an off-flavour was detected. The diet supplemented with linseed oil had a negative effect both on performance and carcass quality. No significant difference was found between poultry fat and olive oil supplementation. Pumpkin seed oil resulted in significantly higher abdominal fat deposition, but improved organoleptic parameters the best. Feeding olive oil significantly increased the oleic acid; pumpkin seed oil the linoleic acid, linseed oil the linolenic acid and cod liver oil the EPA, DHA and docosapentaenoic acid (DPA) concentration in the tissues. The main sources of PUFA are fish oil, linseed, millets and sea algae The sea algae are the best sources for producing omega-3 fatty acid enriched meat or eggs. Moreover, their PUFA content is more stable and in more active form that in the plant oils. The cereals such as corn, barley and oats are also rich in linolenic acid. The combination of animal (fish oil) and vegetable oils viz. rapeseed, linseed oils is also effective. Enrichment in n-3 fatty acids results in higher rate of oxidation that could be controlled by the antioxidants such as alpha-tocopherol or vitamin E as discussed later.

In nutshell, dietary supplementation of linolenic acid showed an increase in its content in dark meat, but had little effect on white meat linolenic acid concentration. Linolenic acid is an essential component of dietary fat but relatively rare in white meat. Conversely supplementation broiler diets with EPA enhanced the levels of the fatty acid in white meat, but only to a limited extent in the dark meat. White meat rather is a far richer source of phospholipids, in which EPA and DHA are more concentrated (Mellor, 2005). The supplemental Mn affected abdominal fat percentage, pH in breast muscle, MDA content and manganese dependent super oxide dismutase (MnSOD) activities in thigh muscle, and lipoprotein lipase (LPL) activities in abdominal fat. The residuals of pesticides, insecticides, antibiotics and chemotherapeutic agents, hormones are also have significant impact on human health and need surveillance, and measure to curb their concentration much below the permissible levels. Conjugated linoleic acids (CLA) are the natural components of animal foods derived from linoleic acid, also have been proved as beneficial.

**Nutrition affecting microbial quality of meat**

Intestinal colonization of Salmonella, Campylobacter and Coliforms in the chickens is the source of carcass contamination during slaughter. Salmonella and Campylobacter cause gastro-enteritis in human beings. Nutritional manipulation that affects colonization of these bacteria may reduce their contamination in carcass. Administration of *Bacillus subtilis* C –3102 to chickens reduced the number and incidence of Salmonella and Campylobacter in the intestinal tract of broilers (Maruta et al. 1996). Carcass of broilers fed with probiotic had significantly lower Salmonella, Campylobacter and aerobic count than birds fed control diet (Frittis et al. 2000). The chicken carcasses fed diets with prebiotics had low Campylobacter and coliforms (Khaksefidi and Rahimi, 2005). The animals can express their natural behaviour more when reared outside, but they have a higher risk of microbial contamination. The prebiotics in diet and fasting hour also influenced micobiological meat quality (Mandal, et al. 2005, Elanovan, et al. 2005).
Nutrition and feeding management affecting processing

In commercial production, chickens are subjected to feed withdrawal prior to slaughter and exposed to stress during transport and handling of the animals at the slaughterhouse; this causes plasma glucose and glycogen stores in liver and muscle to decrease, which has a negative impact on meat quality. Supplementation of the energy complements, creatine and pyruvate (glucose combined with either pyruvate or creatine via the drinking water) during the fasting period appeared to have beneficial effect in delaying the postmortem pH decrease, improving water-holding capacity by increasing intracellular osmotic draw, and colour change of the meat (Young et al. 2004). Feeding the broilers before catching and transporting resulted in higher body weights (due to body wt. loss by 0.74%), increased meat quality and less stress when compared with the broilers that were fasted for 13 hr (due to body wt. loss by 5.5%) before the stress experience (Delezie et al. 2004).

The other major concern of people producing broilers is the excessive accumulation of carcass fat, particularly in the abdominal area. The abdominal fat content varies due to the type of broilers, plane of nutrition especially energy and protein concentration or energy to protein ratio, age and weight of broilers etc. This fat is generally disliked by consumer and represents a waste product to the processor. High-energy excessive diets are frequently blamed for the deposition of fat though increasing the ratio of amino acids to energy in diet reduces fat deposition but increases cost of production. Increasing the total dietary sulphur amino acids (SAA) in diets reduced the abdominal fat content.

Nutrition affecting keeping quality of meat

Poultry meat is highly susceptible to oxidative rancidity or lipid oxidation during frozen storage. Naturally occurring antioxidant - vitamin E or α-tocopherol had been reported to be effective in improving self-life of poultry meat and meat products through delaying lipid oxidation. Thigh meat is more susceptible to lipid oxidation than breast meat (about 22% of live body weight or 30% of carcass mass). The oxidative stability of poultry meat is related to tissue concentration of tocopherol. As the level of vitamin E in diet increases there is concomitant increase in its concentration in tissues and having following order of deposition in descending order: egg yolk, liver, adipose tissue and muscle. Higher levels of vitamin E in poultry not only improve the oxidative stability but also prevent excessive drip loss as it is believed that α-tocopherol, by maintaining the integrity of cellular membranes, reduces leakage of sarcoplasmic components from muscle cells thereby reduces drip. The sensory quality of meat assessed from scoring of aroma, flavour, taste, etc. is reported to be improved by adding vitamin E in diets of broilers. Even vitamin E decreased the production of fishy flavour because of fishmeal oil supplementation in diet and warmed over flavour in refrigerated cooked meat and raw frozen meat. The stability of the meat has been correlated with the tissue concentration of α-tocopherol. The dietary selenium may have a significant role on the quality of poultry meat and meat products (Tyagi et. al. 2005). The thiobarbituric acid reactive substances reduced in the breast meat of broilers in diets containing vitamin A (12 000 IU/kg diet) and E (100 IU/kg diet) and methionine 20% higher than the requirements (Lohakare, et al. 2005). The production of lipid peroxides in the carcasses of broiler chickens during storage could be delayed by astaxanthin, natural or chemical form. The antibacterial agents like lasalocid sodium salt in diet improved meat yield, stress and reduced the plasma 2-thiobarbituric acid reactant value.

References on request.
The chicken egg is nutritionally superior, unadulterated, relatively cheaper food because of its low production-cost. While considering the nutritive value, one whole egg supplies about 6.5 g of wholesome protein of high biological value with well balanced amino acids, 5.5 g of emulsified and easily digestible fat that is rich in phospholipids and needed for growth of nervous tissues including brain and supply of 90 kcal energy. The egg is also rich in all essential minerals and vitamins, but for vitamin C. However, in addition to these essential dietary components, the egg contains about 200-220 mg of cholesterol, which is considered a major source of dietary cholesterol. Therefore, many persons are restricting their habits of egg-eating, assuming that the intake of eggs will increase their blood/serum cholesterol directly. Hereunder, an attempt has been made to illustrate something about the egg cholesterol and its reduction through dietary manipulations.

Importance of Cholesterol

The cholesterol belonging to the class of molecules called “steroids” is a waxy, fat-like compound and essential constituent of our body to perform several essential functions. It forms nearly 0.2% of our body weight. A person weighing 70 kg will have about 140 g of cholesterol in his body: mostly in the brain, kidneys and liver. The cholesterol also helps in the metabolism and transportation of proteins, fatty acids and lipoproteins in the body. No animal on earth can live without cholesterol because it is the base material for several hormones synthesized in the body. As the cell membrane constituent, the cholesterol is necessary for the structure of cell walls. As the egg yolk is considered one of the richest sources of cholesterol in human diet and hypercholesterolemia is invariably linked to atherosclerosis, coronary heart disease (CHD) and hypertension in man. The cholesterol is generally present as the free (non-esterified) cholesterol or cholesterol esters (20%) in the egg yolk. The cholesterol esters are core components for triglycerides-rich lipoproteins and are much more amenable to manipulation than does non-esterified cholesterol. This may be the reason that we can’t reduce yolk cholesterol more than 20-25%. The various factors, which can alter cholesterol content in blood and egg, are summarized here as under:

Cholesterol Phobia

The cholesterol phobia has scared the people all over the world until 1990, resulting in a significant drop in egg consumption in several developed countries from 400 to 200 per year, between years 1940 to 1990. Low-density lipoprotein cholesterol (LDLC) is often called the “bad” cholesterol because it transports cholesterol from liver to other tissues. Most of the yolk lipids (over 90%) were found with in this fraction. High-density lipoprotein cholesterol (HDLIC) is referred to as “good” cholesterol because it transports cholesterol from peripheral tissues to liver.

Owing to the richness of eggs in cholesterol content, the anxiety has often been created against their use in human diets because of association of hypercholesterolaemia with CHD. To counteract these phobia of cholesterol, several works are under progress to lower cholesterol levels of chicken eggs through the manipulation of dietary energy, fiber and protein, use of drugs, herbal supplements, supplementation of the diet with poly unsaturated fatty acid. (PUFA)
and additional doses of metals and vitamins. However, various attempts to reduce the cholesterol content have met with little success.

The cholesterol is essential to the body but not dietary essential because the body can synthesize endogenously as much as 3 g of cholesterol per day whereas the daily requirement is around 1.2 g. Therefore, the exogenous dietary cholesterol is having only insignificant role on serum cholesterol levels. This is the reason that some vegetarians who are not consuming even a mg cholesterol per day will have hypercholesterolemia and CHD due to excess endogenous synthesis, low excretion and/or failure in the cholesterol metabolism.

**Recommended Cholesterol Levels**

Unlike the Americans and Europeans (300-500 mg of dietary cholesterol per day) the actual daily cholesterol intake per person in several developing countries is less than 100 mg and in India it is only 70 mg/day against the daily body requirement for cholesterol is 1-2 g. As such the average Indian diet constitutes about 5% of the daily requirement of cholesterol and the remaining 95 % is through biosynthesis. Hence, the people in these countries need not worry at all about the dietary cholesterol and they should concentrate on eliminating other risk factors. As the annual per capita consumption of eggs in India stands at a very low level of saying 40 eggs, there should not be a worrying concern about the cholesterol myth.

The Indian Council of Medical Research (ICMR) and National Institute of Nutrition (NIN) have recommended less than 20% of total calories from fat sources or 40 g of oil/fat per day (including residual oil). With this recommendations, a person who needs 2500 kcal of energy/day, can get 20% i.e. 500 calories from fat origin, which itself is low, compared to other countries and World Health Organization (WHO) recommendations. These 500 calories of energy can be supplied by 56 grams of oil or fat. But they asked to restrict the total lipid intake at 40 g only/day; which supplies 360 calories (or) 14% of the total calories only. This low oil levels, will restrict the desirable intake of mono unsaturated fatty acids (MUFA), omega 6 fatty acid (FA), omega 3 FA and fat soluble vitamins, leading to health problems.

On the other hand, the NIN has recommended that the carbohydrate (CHO) calories should not exceed 75% of total calories. Moreover, our protein consumption is very low, supplying less than 10% of calories. In such case, how it will be possible to meet the required calories within this 40 g oil. In reality, several poor people are not even eating this 40 g of oil per day in poor countries. Hence the recommendations must be revised as the CHO, lipid and protein calories in the range of 55-70, 20-25 and 10-15%, respectively, of the total calories recommended. Out of these total calories 10-15 g must be MUFA, 8-10 g PUFA and 500 mg Omega 3 FA.

**Designer Eggs**

After releasing the cardio protective and other beneficial effects of ω-3 FA, α-linolenic acid (ALN), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the poultry nutritionists have started research to incorporate more of these FA in the eggs and succeeded in developing such eggs, called as ω-3 egg. Designer egg, Diet egg or Functional egg. Such designer eggs will have nearly 7% of ω-3 FA, compared to less than 1% in ordinary egg.

The designer eggs are already available in the market in many countries, including India, with good patronage. In order to improve the quality of this functional egg, further, carotenoid pigments, selenium and vitamin E are also incorporated. Now research is going on to incorporate more health promoting components from various herbs into the eggs, which will reduce the serum LDLC and triglycerides (TGL) in humans consuming such eggs. One can assess the nutrient concentration of ordinary egg vis-à-vis designer eggs through Table 1.
Table 1. Nutrients content of ordinary and designer egg

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Ordinary egg</th>
<th>Designer egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha linolenic acid (Omega 3 FA), g</td>
<td>0.03</td>
<td>1.3</td>
</tr>
<tr>
<td>Biotin, mcg</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Carotenoid pigments, mg</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>400</td>
<td>350</td>
</tr>
<tr>
<td>Choline, mg</td>
<td>800</td>
<td>1000</td>
</tr>
<tr>
<td>EPA + DHA (Omega 3 FA), g</td>
<td>0.08</td>
<td>0.6</td>
</tr>
<tr>
<td>Folic acid, mcg</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>2.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Linoleic acid (Omega –6 FA), g</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>MUFA, g</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Niacin, mg</td>
<td>0.1</td>
<td>0.12</td>
</tr>
<tr>
<td>N-6/N-3 ratio</td>
<td>14.4</td>
<td>0.68</td>
</tr>
<tr>
<td>PUFA, g</td>
<td>1.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Thiamin, mg</td>
<td>0.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Selenium, mcg</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Total saturated FA, g</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Total unsaturated FA, g</td>
<td>6.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Unsaturated/Saturated FA ratio</td>
<td>1.75</td>
<td>2.71</td>
</tr>
<tr>
<td>Vitamin A, mcg</td>
<td>360</td>
<td>500</td>
</tr>
<tr>
<td>Vitamin B12, mcg</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin E, mg</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>1.5</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Manipulation of Egg Nutrients

In order to increase the egg consumption as a part of healthy food habits, the scientists are constantly searching for methods to nutritionally enrich the eggs. The total fat, protein and sugar contents of the egg can’t be altered much, but the concentrations of FA, minerals, vitamins and certain non-nutrient chemicals (like anti-oxidants, pigments etc) in the eggs can be manipulated dietary.

Although the egg cholesterol level can’t be reduced much; it is possible to incorporate a few ingredients (Table 2) in the diets of hen to produced designer eggs.

Table 2. Feed supplements to be incorporated in hens diet to produce designer eggs

<table>
<thead>
<tr>
<th>Feed supplements</th>
<th>Quantity/Tonne of feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa meal, kg</td>
<td>10-30</td>
</tr>
<tr>
<td>Acetyl salicylic acid, kg</td>
<td>0.5-1</td>
</tr>
<tr>
<td>Activated charcoal, kg</td>
<td>1-2</td>
</tr>
<tr>
<td>B-complex vitamins</td>
<td>Double requirement</td>
</tr>
<tr>
<td>BASIL leaves, kg</td>
<td>1-5</td>
</tr>
<tr>
<td>BHA/BHT/Ethoxiquin, g</td>
<td>100-250</td>
</tr>
<tr>
<td>Capsicum or red chilli (pepper) powder, kg</td>
<td>1-2</td>
</tr>
<tr>
<td>Choline chloride, kg</td>
<td>1-2</td>
</tr>
<tr>
<td>Corn glutten meal, kg</td>
<td>50-80</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Amount</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Fish oil (stabilized or micro encapsulated), kg</td>
<td>10-30</td>
</tr>
<tr>
<td>Full fat fish meal, kg</td>
<td>50-100</td>
</tr>
<tr>
<td>Full fat flax seeds, kg</td>
<td>100-150</td>
</tr>
<tr>
<td>Garlic powder, kg</td>
<td>1-2</td>
</tr>
<tr>
<td>Glandless cotton seed protein, kg</td>
<td>10-30</td>
</tr>
<tr>
<td>Grape seed meal, kg</td>
<td>5-10</td>
</tr>
<tr>
<td>Herbs which will reduce LDLC, g</td>
<td>1-5</td>
</tr>
<tr>
<td>Rapeseed/canola oil, kg</td>
<td>5-10</td>
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<tr>
<td>Manganese sulphate, g</td>
<td>100-300</td>
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<tr>
<td>Marigold petal meal, kg</td>
<td>3-5</td>
</tr>
<tr>
<td>Organic chromium/selenium, g</td>
<td>50-100</td>
</tr>
<tr>
<td>Sodium selenite, g</td>
<td>0.2-0.5</td>
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<tr>
<td>Tomato pomace, kg</td>
<td>5-10</td>
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<tr>
<td>Turmeric powder, kg</td>
<td>0.5-1</td>
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<tr>
<td>Vitamin E, g</td>
<td>100-400</td>
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<tr>
<td>Zinc sulphate, g</td>
<td>100-300</td>
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**Egg Cholesterol Content Modulation**

Around the year 1930, the poultry nutritionists have discovered that the FA composition of the egg can be altered by diet. However, this discovery was not utilized until 1990 for production of 0-3 FA rich eggs. Between the years 1980 to 1990, with the discovery of the importance of omega-3 FA, ALN, EPA and DHA in human health and their role in reducing the harmful TGL, LDLC and very low density lipoprotein cholesterol (VLDLC) levels, many research workers have started incorporating the omega-3 FA in the egg yolk, by modifying the diet of the hens. They have included omega-3 FA rich feedstuffs like full fat flaxseed (linseed), fish oils, fatty fish, canola oil or full fat rape (canola or mustard) seeds in layer feeds.

The supplementation of chromium (800 ppb) in the form of chromium picolinate and feeding 125 mg Cu/kg diet (11.7 and 8.6 mg/g) in laying hen diet caused a reduction in serum and yolk cholesterol content with no adverse effect on egg production. Daily injection of D-isomer of thyroxine or by incorporation into the diet of hens, markedly reduced blood plasma cholesterol and resulted in a 26% increase in egg yolk cholesterol levels without a significant change in egg size. The addition of probiotic significantly increased the egg production, shell thickness and serum calcium, and reduced the concentration of cholesterol in the serum and yolk. The egg cholesterol contents have been maximally reduced by 46% and 22% in hens fed the 0.06% level of atorvastatin and simvastatin respectively. Supplementation of Lactobacillus spp. to laying hen through drinking water (2 x 105 and 1 x 106 cfu/ml) lead to significant reduction in egg yolk cholesterol and tended to have better egg weight, egg specific gravity and feed efficiency than the control group. Digitonin at 0.025% in laying hen diet significantly reduced egg weight, egg white weight and egg cholesterol content, while there was significant increase in HDLC and TGL in serum.

Among the dietary herbs, the garlic has been shown to have potent hypocholesterolemic effect by several workers. Supplementation of laying hen diet with 0, 8, 12, 16 and 20% *Perilla frutescens* seeds resulted in increased content of omega-3 FA, while content of omega-6 FA decreased in egg yolk. Serum total cholesterol, TGL, VLDL and total lipid decreased while HDLC increased with addition of seeds as compared to control. The cholesterol lowering effect of dietary supplementation of herbs (*Guggul*-Commiphora mukul, Cinnamon-*Cinnamomum verum*, Amla-*Emblica officinalis*) in diet of hens was almost similar in moulded and normal hens. The long-term supplementation of herbs in combination for 8 wks in laying hen diets might be
helpful in lowering the blood and egg cholesterol contents. The feed of hens producing designer eggs must be free from antibiotics, drugs and any other harmful and banned substances. The researchers have identified the egg as the best vehicle to deliver all these health promoting components to the humans at a low cost along with other nutrients present in the egg. These eggs contain several folds more of omega 3 FA, carotenoids, selenium and vitamin E than ordinary table eggs, thereby making it a health promoting food.

Further Reading
Naharari, D, 2003 Egg Cholesterol Fat and Healthy Diet. Pixie Publication, India (P), Karnal-132001 (Haryana).
Heat Stress and Egg Quality

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High ambient temperature in the tropics, like that of ours in India accompanied by high relative humidity is one of the most important stressor. Birds are more susceptible to high environmental temperature than low due to absence of sweat glands in the feathered body, fatty nature and high body temperature (40.1°C to 41.6°C). The degree of susceptibility to tropical heat stress is higher in broilers than layers. Among broilers males are more susceptible to heat stress than females (Marin, et.al.2002). Good layers housed in cages are more susceptible than poor layers reared on deep litter. Birds, being homeothermic like mammals can maintain a relatively constant deep-body temperature of 41.7°C (107.1°F). The temperature of newly hatched chicks is about 39.7°C (103.5°F). Deep body temperature of higher mammals ranges from 36 to 40°C which is lower than bird. The permissible range of body temperature variation is only for a few degrees in birds. Birds are capable of generating a considerable amount of heat within their tissues (endotherm). A caged layer, eating a normal ration and laying at a rate of 80%, will produce about 180 kilocalories of heat every day. Thus 10,000 layers will produce as much heat in a day as a furnace burning 231 litres of fuel oil. This means that on a warm summer day, a ventilation failure could result in a rise in the temperature, within the building, of 16°C in one hour (Bird,2005) To establish the relationship of poultry with various temperatures few general guidelines are presented in Table 1. The upper lethal temperature in bird in found around 47°C (116.8°F). In this paper, a general guide to reaction of birds to various temperatures, pattern of heat loss by the bird, effect of heat stress on egg quality and preventive measure of heat stress in birds are discussed.

<table>
<thead>
<tr>
<th>Table 1. Thermo-neutral zone and heat stress</th>
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<tr>
<td><strong>55° to 75°F</strong></td>
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<td><strong>65° to 75°F</strong></td>
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<td><strong>95° to 100°F</strong></td>
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<td><strong>Over 100°F</strong></td>
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The heat production in the body and heat loss from the body should be equal. The bird is continuously producing heat through metabolic processes and muscular activity. The heat produced in the body is lost through a) radiation, b) conduction, c) convection, d) evaporation (painting) e) faecal excretion and through egg production in hen. Overall, the total heat lost by the bird is divided into two main categories: I) sensible heat loss, II) evaporative heat loss (Kundu, 2000).

I : Sensible heat loss :

When the hen is in a comfortable environmental temperature (21-25°C) she will lose most of that heat by sensible means. During sensible heat loss (zone of thermal neutral: 55-75° F) bird maintain optimum growth rates, hatchability, egg size, egg shell quality and egg production. Sensible heat loss by the bird is done by employing three pathways:

1. Conduction, whereby she touches a surface cooler than her own surface, for example the floor of the cage or the sides of a cool water trough;
2. Convection, whereby a cool breeze carries heat from her body; and
3. Radiation, the electromagnetic process whereby heat moves from a warmer to a cooler surface without using a medium or without touches of surface (heat flows to the earth from the sun by radiation).

The proportion of heat lost through radiation, conduction, and convection depends upon the temperature difference between the bird and its environment. The bird loses heat from surfaces such as wattles, shanks, and unfeathered areas under wings. To maintain body temperature by sensible heat loss, the bird does not need to drastically alter its normal behavioral patterns, feed intake, or metabolism. The purpose of poultry house ventilation is to maintain a high enough air velocity or a low enough temperature in the house that the birds can maintain body temperature by sensible heat loss (Anderson, 2005).

II: Evaporative heat loss (insensible heat loss or panting):

Once the environmental temperature reaches approximately 80°F, the method of heat loss begins shifting from sensible to evaporative heat loss. Infact, heat stress begins when the ambient temperature climbs above 80 °F and it readily apparent above 85 °F. Dissipation of body heat by the evaporative process requires the bird to expend energy by panting (hyperventilation), which begins to occur at around 80-85°F (Anderson 2005., Bird, 2005).

One reason of panting is that, as the environmental temperature increases, the difference between the temperature of the hen's body (41°C) and the surrounding air, equipment and walls becomes very small. Thus she cannot readily lose heat by sensible means and must do so by evaporation. A second reason is that the evaporation of water uses a lot of heat - it is an effective way for her to keep cool (Bird, 2005).
As the bird does not have sweat glands therefore, she reduces the body temperature by panting, similar to the dog. The hen can easily increase her respiration rate to 10 times normal. Birds also reduce the heat by raising and spreading her wings and separating herself from other birds if possible. Birds have tendency to keep body in touch with cool surface.

**Physiological effects of heat stress (panting) on egg shell quality:**

High environmental temperature tremendously damages the egg quality and causes the loss of millions of dollars to the egg industry (Bell, 1998). The main quality characteristics of egg as influenced by stressor are egg size, freshness, shell thickness, yolk colour, and thickness of albumen protein, nutritive value, and sensory quality. Among them shell quality is the prominent (nearly 90%) attribute of egg quality. Etches (1996) advocated approximately 500 million dollars loss to the farmer due to poor egg shell quality. Very little is known about the molecular mechanisms that transfer Ca²⁺ from blood to the surface of the shell where it precipitates with the carbonate ion. It is recognized, however that the laying hen possesses remarkable physiological adaptations which facilitate the absorption, deposition and storage of Ca²⁺ from feed, and which utilize CO₂ dissolved in blood to synthesize the carbonate ion in the eggshell (Etches, 1996).

Panting removes heat by the evaporation of water from the moist lining of the respiratory tract. However, panting itself generates body heat, and it causes poultry to eliminate water from the body. It can induce respiratory alkalosis, which occurs because the bird "blows off" excessive carbon dioxide (CO₂) when it pants. As a result, body fluids become more alkaline, causing the kidneys to excrete excessive amounts of several electrolytes. As the shift in body fluid pH occurs, feed intake is increasingly depressed, adversely affecting growth, production, and overall performance of the bird. During the hot summer months, evaporative heat loss typically becomes the primary method by which birds regulate their body temperature unless proper ventilation is provided and other steps are taken to reduce heat stress (Etches, 1996).

Perhaps the most frequent result of heat stress is a decline in egg shell quality due to excessive loss of CO₂ as discussed earlier. Occasionally, this may be due to inadequate calcium intake, but this is rare, since most commercial layer feeds provide enough calcium to support shell formation even at low feed intakes. The maximum daily calcium intake recommended for most layers is 4.0 g. This would be obtained if the hen consumed 90 g of a feed containing 4.4% calcium, or a feed with 3.6% calcium plus 20 kg oyster shell supplement per tonne. Most of the shell quality problems arising from heat stress are not the result of dietary calcium deficiency. Rather, they result from extremely complex metabolic changes within the hen. As the bird pants to keep cool, excess carbon dioxide is exhaled. This causes the blood to become more alkaline, and reduces its ability to hold and carry calcium for shell formation. Such a situation cannot be remedied by increasing dietary calcium. Under these condition, egg shell quality can be improved by providing high concentrations of CO₂ in inhaled air, although this is not a practically possible to improve shell quality in commercial poultry houses (Etches, 1996 and Bird, 2005).

**Prevention of heat stress and improvement of egg quality**

Following measures should be adopted to make the bird free from heat stress and thereby for improvement of egg quality.
1. **Feed Intake:** Hens continually adjust their feed intake according to environmental temperature. Up to about 27°C, this fluctuation in feed intake does not affect production unless an important nutrient is marginally deficient. Above 27°C, the bird's body temperature rises and a much more dramatic reduction in feed intake can be anticipated. Subsequently, weight of the egg, and body weight of birds declined. As temperatures rise above 32-35°C, egg production as well as egg quality may also decline, as total nutrient intake is insufficient to support normal rates of lay (Bird, 2005).

Any management technique that increases nutrient intake during heat stress will minimize drop in egg production and egg quality. To ensure an adequate intake of nutrients it is extremely important to feed the birds early in the morning which will stimulates feed consumption during the cooler hours of the day. To increase the nutrient density of the ration also help to combat against the heat stress but it should be avoided when survivability is under question.

Withdrawal of feed during extreme heat (103°F and 50% humidity) to help poultry survive under heat waves. Birds that are digesting feed produce more heat. The extra heat from metabolizing that feed can raise their body temperatures another 1-2°C. Withdrawal of feed should be done about six hours before the temperature is expected to reach 90°F. The hotter and more humid the weather, the more beneficial feed withdrawal will be (Froning, 2005). Special balancing of amino acid (arginine, leucine, sulphur containing amino acid) is essential under heat stress. Lysine deficiency increase the body temperature.

2. **Lighting system:** It should be switched on in the early morning (cooler hours) so that bird starts feeding early. Light 14 hour/day is considered an ideal for poultry production.

3. **Calcium:** Calcium is the prominent mineral component utilized by bird for egg shell formation. To maintain the normal egg shell quality under heat stress, its requirement around 3.5 to 4.0% in layer ration. Main source of calcium are marble powder (37%), calcium grit (37%), oster shell (38%), and DCP (dicalcium phosphate-30%). Fishmeal is also a good source of calcium.

4. **Phosphorous:** Various other blood minerals are affected by heat stress. One of the most important is phosphorus, and the requirement for this element is increased at high temperatures. Marginal phosphorus levels, when combined with heat stress, can lead to increased mortality rates, particularly among older birds (Bird, 2005). Main source of phosphorous in ration is fish meal and DCP.

5. **Water:** Monitor water consumption. Ten thousand layers in full production will consume 2,000 L per day during normal environmental temperatures. Above 32°C water consumption can increase up to 50%. Further, it has been investigated the providing 50°F cool drinking water for the bird can lower broiler body temperature by about 2°F (Anderson, 2005, Froning, 2005).

6. **Vitamins:** High temperatures may also result in less efficient utilization of vitamins, and certain vitamins in feed are themselves less stable in these circumstances. A series of studies showed consistent benefits from feeding additional vitamin E to laying hens experiencing heat stress. Dose response works suggested that 250 mg vitamin E is optimal for alleviating at least, in part, the adverse effects of chronic heat stress in laying hens (Chung, et.al. 2005). The decline
in egg shell quality is not only affected by the decreased intake of calcium and phosphorus but also by the depletion of ascorbic acid required for the conversion of 25 hydroxyvitamin D3 produced by the liver into the hormone calcitriol produced in kidney, which is essential for regulation of calcium metabolism and egg shell calcification (Brake, 1988). Chung et. al.(2005) recommended 200 mg vitamin C/kg b.wt. for heat stressed hen. Vit. C is also synthesized by the birds but that is inadequate to meet all physiological requirements under heat stress.

7. Hormone: Exogenous estrogen and high level of dietary vit. D of both before heat stress are effective in alleviating at least some of the effects of the heat stress (Hansen et.al.2004). Forman et.al.(1996) found higher plasma estradiol and greater Ca2+ transport in hens that had received exogenous estrogen before 12 hours of heat stress. Lower level of T3 and T4 ratio also support the bird to survive under heat stress.

8. Density: The effects of heat stress will all be aggravated by other environmental factors such as increased bird density, feed and/or water deprivation, inadequate ventilation, vaccine reaction and the presence of diseases or parasites.

9. Ventilation: It will bring in an equal amount of outside air and uniformly direct the inlet air in all the areas of shed. Ventilation will remove the harmful gases from the poultry houses.

10. Grass cover and shade tree: A grass cover on the grounds surrounding the poultry house will reduce the reflection of sunlight into the house. Vegetation should be kept trimmed to avoid blocking air movement and to help reduce rodent problems. Shade trees should be located where they do not restrict air movement.


12. Feeding of catecholamine antagonist: Chemical agents which block the catecholamine synthesis in the body reduced the heat stress of the birds and improve the feed intake, quality and quantity of egg of the bird. Some commonly known agent are: zinc sulphate, diazepam, metyrapone, α-MPT, clonidine and magnesium aspartate (decrease corticosterone also).

13. Humidity: Optimum humidity for poultry production is 55 to 65% (RH). Below 40% RH it will cause dust problem whereas above 70% it may cause micotoxins, coccidiosis, wet litter and ammonia formation in shed.

14. Ammonia and other gases: Ammonia is the prominent gas found in the poultry houses. This gas is found heavier than air and other gases present in shed. Therefore, it remain in close contact with the birds and damage the health. Higher ammonia formation cause respiratory tract problems in the birds, eye burning, irritation, decrease feed intake and growth and reduction of egg production including quality of egg.

Fresh litter pH was recorded 5.0- 6.5 and under storage (old) pH of litter reaches between 7.0-8.5 (increase uricolytic bacteria in litter). Old wet litter at this pH produce tremendous ammonia gas. Therefore, to reduce the pH of litter below 7 or to neutralize alkaline pH, acidic agents should be required. Some chemical agents such as ammonia paraformaldehyde (1kg/5M ²)
for 21 days or super phosphate 0.5 to 1kg/M² for 15 days or zeolite and phosphoric acid should be used. Care should be taken for other gases that are H₂S, CO, CO₂, nitric oxide etc.

15. Immune system: There is considerable evidence that the immune system of the bird is adversely affected by heat stress. Therefore, administration of vaccines to pullets or layers is not usually recommended during very hot weather (Bird, 2005).

References


The production of poultry meat and meat products represents one of the world's leading economic activities. The dynamic sector of poultry meat production occupies second place in volume in the world just behind pork. Genetic improvement through conventional selection of broilers has successfully increased growth rate, feed efficiency and breast muscle percentage. However, physiological disorders are occurring such as increased obesity and decreased skeletal integrity (Deeb and Lamont, 2002).

Through world consumers are concerned with value for money- but there is increasing pressure for broiler production systems that provide safer meat, better animal welfare, less artificial inputs, less environmental impact and improved product quality.

**Meat and Meat Product quality:**

The important meat and meat product quality attributes include appearance texture, juiciness, flavor and functionality (Fletcher, 2002).

- The two most important quality attributes for meat are appearance and texture.
- Appearance is the most critical for the selection of many food commodities including meat and meat products.
- Consumers most often select or reject a product based solely on its appearance.
- Appearance is also critical for final product evaluation since it often affects other sensory properties.
- One of the major contributing components of appearance is colour.
- Colour has long been known to be a major selection criterion for fresh meat and meat products as well as for final product satisfaction.
- Texture is another single most important sensory property affecting final assessment.
- With the increasing trends in further processing meat functionality and all of the sensory quality attributes have increased in relative importance.
- The needs of the meat industry have changed and will continue to change as we move further into 21st Century. These changes are being driven by both the changing economic and social environment in which meat production operates and by changes in technology.
- The consumer will be king and dictate higher ethical standards and accountability.
- Therefore, in future, the focus must be increasingly on meeting consumer specifications for the right meat and product at the right time, in the right place and at the right price.

Where are we in terms of understanding the molecular genetics of meat quality, what kind of research in underway or planned and what are the prospects for finding molecular information that will be useful in genetic improvement programmes. My objectives today are to review the recent state of research in this area and suggest important issues for future research.

**Breeding for Poultry Meat quality:**

As we have observed that traditionally broiler meat quality attributes have emphasized mainly on the appearance of both the carcass and meat and the tenderness of the meat. In future
highly diversified broiler meat industry will focus on cut up, deboned meat and ready-to-eat further processed products.

- Broiler breeding is a specialized applied science in which principles from quantitative, population and molecular genetics and quantitative sciences such as statistics and computing combine to provide the tools needed for genetic changes in population.

- Selective breeding has produced enormous improvement in broiler traits (growth rate, feed efficiency, breast muscle etc) in the latter part of the twentieth century. This progress has largely been achieved through selection on phenotype – the identification of genetically elite birds through their own performance and physical characteristics and those of their relatives. Random environment factors (temperature, disease, feed quality, peck order etc) are a hindrance to breeding superior genetic stock.

- Even greater problems are sex limited traits which can only be measured in one sex and still there are traits which cannot be measured on either sex, such as meat quality in live birds.

- The desire of poultry breeders has always been to get directly at the underlying genetic worth of the bird, free from environmental effects, and on all animals regardless of sex or ability to measure the phenotype. Now molecular techniques allow breeders to provide directly into the genetic code of life.

- The explosion in our understanding of the poultry genome and accompanying technological innovations are opening up possibilities for direct identification and selection of poultry stocks carrying the best genes – selection on “Genotype”.

- Since carcass and meat quality traits are measured post mortem, the broiler industry would like to test birds before slaughter to determine if they would make valuable breeding stock. These genes can be identified using molecular biological techniques on blood samples taken from live birds early in the life.

Chicken genome and map:

- The genome of poultry species consists of 38 pairs of autosomes and pair of sex chromosomes. Into these chromosomes is packed a DNA sequence of an estimated 1 billion (approximately) base pairs in length in chicken.

- Last year geneticists have succeeded in assembling the entire chicken genome sequence, following pioneering work on the human genome.

- Chicken is the first Agricultural animal in which this has been achieved. Further, the precise content and order of the chicken genome is almost complete.

- In the paper published in nature (December, 2004) members of the international Chicken Genome Sequencing Consortium, report that the Chicken genome contains significantly less DNA than the human genome but approximately the same number of genes. Researchers estimate that the chicken has about 20,000-23,000 genes in its 1 billion DNA base pairs compared with the human count of 20,000 – 25,000 genes in 2.8 billion DNA base pairs. The difference in total amount of DNA reflects a substantial reduction in DNA repeats and duplications, as well as fewer pseudo genes, in the chicken genome.

- DNA sequencing, the process of determining the exact order of the 1 billion chemical building blocks (called bases and abbreviated A, T, C and G) that make up the DNA of the 39 different chicken chromosomes was the greatest technical challenge in the chicken Genome Project. The resulting DNA sequence maps are being used by the 21st century scientists to explore chicken biology and other complex phenomena.

- Researchers used a “shotgun” approach to assemble information on the genome.
• Chickens and human have a similar number of genes for olfactory receptors, suggesting the chicken’s sense of smell is much greater than previously thought.

• The recent genetic variation map has reported 2.8 million single nucleotide polymorphisms (SNPs) for the chicken genome and this is based on a comparison of the sequences of three domestic chicken breeds (a broiler, a layer and a Chinese Silkie) with that of their wild ancestor Red Jungle fowl.

• The map indicates that at least 90% of variant sites are true SNPs and at least 70% are common SNPs that segregate in many domestic breeds.

• About 60% of chicken genes correspond to a similar human gene. Chicken genes involved in the cell’s basic structure and function showed more sequence similarity with human genes than did those implicated in reproduction immune response and adaptation to environment.

• Chickens have an expanded gene family coding for a type of keratin protein used to produce scales, claws and feathers, while mammalian genomes possess more genes coding for another type of keratin involved in hair formation.

• Chickens are missing the genes involved in production of milk proteins, tooth enamel and the detection of hormonal substances called pheromones, which may mirror the evolution of the mammary glands and the nose in mammals and the loss of teeth in birds.

• When compared with mammals, chickens have much smaller family of genes coding for taste receptor, particularly those involved in detecting in bitter sensations.

• Alignment of chicken and human genes indicate that 2000 human genes may actually start at different sites and this may have implication for the understanding of human disease and the design of new therapies.

• Chicken genes that code for eggshell specific proteins, such as ovocleidin –116, have mammalian counterparts that play a role in bone calcification.

• In contrast to chickens, mammals are missing key genes coding for proteins involved in egg production, such as egg white and yolk storage.

• Chickens have a gene that code for interleukin –26(1L-26) a protein involved in immune response, as in humans. Now the chicken may serve as a model organism in which researchers can investigate the function of 1L-26.

• Chickens possess genes coding for certain light dependent enzymes, while mammal have lost those genes.

• The avian genome contains a gene that codes for an enzyme involved in generating blue colour pigments while mammals are lacking that gene.

• Less than 11 percent of the chicken genome consists of interspersed segments of short, repetitive DNA sequences, compared with 40 to 50% of mammalian genomes. With genes comprising another 4 percent of the chicken genome, that leaves that there is no explanation for the function of more than 85 percent of the chicken genome. Scientists have hypothesized this genetic “dark matter” may contain previously unrecognized regulatory elements, but also may include ancient DNA repetitive elements that have mutated beyond recognition.

• In chicken genome, 571 non-coding RNA “genes” were identified, may use different dupication / translocation mechanisms than do regular protein coding genes. This would be new area of scientific enquiry.

• Chicken genome may help to understand the genetic variation in susceptibility of different strains to the Avian flu disease.
The genome will also serve as a resource to enhance the nutritional value of poultry and egg products.

Chicken is the first of 9,600 species of birds to have its genome fully sequenced and analyzed, and this will further help to understand avian genomics and biology in general.

This new genome map will guide the search for meat quality traits— a night - day difference.

The map has improved overnight from having 2000 genetic markers to potentially 3 millions.

Now it is evident that this chicken genome sequence will be an immense help in finding the meat quality and resistance genes using integrated functional genomic approaches that combine DNA, RNA, and protein methods of molecular tools.

Deriving meaningful knowledge from DNA sequences will define biological research through the coming decades and require the expertise and creativity of teams of biologists, chemists, engineers, and computational scientists, among others. A sampling follows of some research challenges in genetics—what we still won’t know even with the full chicken sequence in hand.

- Gene number, exact locations and functions
- Gene regulation
- DNA sequence organization
- Chromosomal structure and organization
- Non-coding DNA types, amount distribution, information contact and functions
- Coordination of gene expression, protein synthesis and post – translational events
- Interaction of proteins in complex molecule machines
- Predicted vs. experimentally determined gene function
- Evolutionary conservation among organisms
- Protein conservation (structure and function)
- Proteomes (total protein content and function)
- Correlation of SNPs with meat quality
- Gene involved in complex traits (meat quality)

Molecular markers for improving meat and carcass traits:

Since carcass and meat quality are measured post-mortem, the broiler industry would like to test birds before slaughter to determine if they would make valuable breeding stocks. These genes can be identified using molecular biological techniques on blood samples.

- Broiler meat quality comprises a set of key fresh meat quality, processing, and sensory characteristics that are important for the further profitability and competitiveness of the broiler industry. These include body fat, cholesterol, ultimate pH, colour, water holding capacity, tenderness, cooking loss, and sensory traits involving taste. In the recent past, leanness was considered one of the most important traits. As a result, improvement in the body composition of broiler have been made to some limited extent. However, it has been shown that lean meat is not always associated with good meat quality. Improving meat quality genetically is difficult by conventional selection methods, but possible if the genes responsible for meat quality are identified and mapped.

- A limited number of studies have attempted to map QTL for meat quality traits. In most poultry breeding programmes, crossbreds are used for commercial production to capitalize on heterosis and complementarity and the aim of selection within pure lines is to maximize crossbred performance. Selection is however, within pure lines and primarily based on
purebred data. In some poultry breeding programmes reciprocal recurrent selection or modifications thereof are also given more importance to make use of the whole additive genetic variance (general combining ability) and non-additive genetic variance (heterosis) like dominance, over-dominance and epistasis.

- With the development of QTL mapping, specific favorable loci can be observed and selected directly, with presumed benefits of increased rate of genetic gain (Dekkers and Chakraborty, 2001). Recent results using molecular genetic tools have clearly shown that genes function in complex ways. The dominance and epistatic components of quantitative genetic theory are more biologically realistic.

- Prior to recent mapping of chicken genome, several studies have identified QTL regions in the poultry genome affecting growth and carcass traits; on chromosome 1 for body weight, feed intake, and carcass percentage, on chromosomes 2 for body weight, feed intake and meat colour, and on chromosome 4 and 23 for feed intake (Groenen et al., 1997; Van Kaam et al., 1998, 1999a, 1999b; Tatsuda et al., 2000; Tatsuda and Fujinaka, 2001).

- Breast meat is the most economically valuable part of the chicken and makes up about 50% of total muscle weight. McElroy et al. (2002) reported a QTL effecting breast meat yield at 125 cM on chromosome 2.

- In broilers, excessive adiposity is a major drawback for production, reducing feed efficiency and lean meat yield. Bourneuf et al. (2002) in their study indicated that CYP2C gene and cb-2c product could be involved in the regulation due to differential expression and polymorphism, respectively. These results could be helpful for the development of new selection programmes in the chicken. Pitel et al. (2002) revealed a significant QTL for fatness on chicken chromosome 5 and this is the first localization of a fat/lean QTL in poultry.

- In poultry most QTL mapping studies have been performed on crosses between genetically and phenotypically divergent lines (broiler x layer or crosses between two extreme broiler lines). A region of chicken chromosome 4, affecting body weight and feed intake in experimental chicken population has been shown to explain a significant proportion of genetic variance within a commercial broiler line. Other effects were found for the weight of the thigh muscle and a subjective score of fleshiness. This important information that the same chromosome regions that explain variation within lines that have been under selection for these traits for over 50 generations. This QTL could be used to make broiler breeding programmes more efficient (de Koning et al. 2005).

- Ledur et al. (2005) have reported an F3 resource population to identify QTL affecting the functional properties of chicken meat, including PSE (Pale soft and exudative meat).

- Zhou et al. (2003) reported that several beneficial effects (improved growth, increased breast muscle weight, decreased abdominal fat and enhanced skeleton integrity) are associated with insulin like growth factor I (with one allele) and this indicate the presence of one or more loci (QTL) near IGFI-SNPI.

- Fumihiko et al. (2005) have reported the linkage mapping of four chicken calpain genes. Calpins are intracellular Ca^{2+} - dependent proteases and enzymes that contribute to growth and meat quality. They identified polymorphism in four calpain gene (CAPN1, CAPN2, CAPN3 and CAPN1.5).

- Li et al. (2005) have reported that a SNP marker in the apo VLDL-II gene was associated with growth rate, skeletal development, and muscle weight and yield in chicken growing to market weight and is, therefore, a potential marker for use in molecular MAS programme.
Knowing the genotype sequence takes us a significant step closer to being able to select mainly on genetic information. The locations for genes for specific traits such as body weight or carcass traits can be identified using markers and then they can be selected directly. This technique is known as marker assisted selection (MAS). As a result of the genome sequence project, new genome map will guide the search for genes and this makes a night-day difference. We have reached overnight from having 2000 genetic markers to having potentially 3 million markers, opening the way for genome wide MAS or GMAS, selection.

To detect all genes for all traits is probably not easy and thus most effective approach to breeding will be a combination of technologies.

Several computer based simulations have been completed comparing MAS with phenotypic selection for populations in linkage equilibrium utilizing within family LD. Meuwissen and Goddard (1996) showed that if the marker QTL explain 33% of the genetic variance, and selection is before or after recording the traits, MAS increased genetic gain by 9% to 38% and for sex limited traits 38% and for carcass traits 64%. When selection combine GMAS with BLUP, this approach should significantly outperform the current best method using BLUP.

It has now been well demonstrated that QTL with considerable effects can be identified in broiler populations with investment, skill and some good fortune and could be used to make broiler breeding programmes more efficient, but before this is attempted further scrutiny of the effects on all relative traits and a refinement of the location the QTL are required.

Future Research issues:

- Limitations to the improvement of muscle quality through traditional methods suggest the need for more refined molecular techniques. Success in locating QTL with important effects on these characteristics that can be used to assist selection will depend on appropriately designed resource families of commercial broiler populations.
- The broiler meat science research activity covers a broad area from gene to meat product, aiming to understanding of biological aspects of cell development and fundamental physics, biochemical and molecular-biological aspect, processes and mechanisms of importance for the quality of fresh meat and its quality as a raw material for further processing and meat functionality aspects in relation to the quality of meat products.
- The availability of chicken genome sequence has enabled the exploration and exploitation of the chicken genome and proteome. Proteomes is the study of the entire protein compliment of an organism. The proteomes will focus on obtaining a better understanding of the basic biochemical mechanism in the muscle that has an influence on meat quality by investigating the post mortem changes in the muscle proteome such as protein expression and degradation, but also the effect of changes in protein modifications caused by storage and processing.
- In future, the cell biology and microscopy will focus on the use of cell cultures as model systems for the whole bird in relation to muscle growth and physiology for better understanding to obtains meat of high quality.
- Future studies on muscle physiology will focus on biochemical, biophysical and mechanical properties of the main components of meat in relation to muscle development, muscle growth and meat quality.
- Muscle and meat biochemistry will focus on increasing the understanding of the effect of ante and post-mortem metabolic processes on meat quality, characteristics including texture, water holding, chemical composition and flavor.
More studies on meat functionality will be taken up to study meat as a raw material for further processing with focus on physical, chemical and micro structural properties in relation to the quality of meat products.

Further, it is hoped that Nanoscience and molecular imaging studies of muscle and meat will get more attention in the future.

In summary, it is considered that future commercial broiler breeding programmes will become more complex and challenging because so many objectives need to be simultaneously considered to reduce production costs, maintain health, and improve product quality. References on request
Geneticists have long been concerned with identifying key genes responsible for variation in multifactorial production traits in animals. The genes that are part of the somatotropic axis play a crucial role in the regulation of growth and development of chickens. The identification of genetic polymorphisms in these genes will enable the scientist to evaluate the biological relevance of such polymorphisms and to gain a better understanding of quantitative traits like growth in order to augment the production. The improvement in production can be viewed in terms of quality or quantity. The conventional breeding approaches however have brought desired genetic gains in body weights through mass selection as body weight has been a highly heritable trait. As a result poultry stands 2nd in the world meat production. In India, the rapid growth of 15% in broiler and 10% in eggs has put the country at 4th place in egg and 6th in broiler production in the world. In spite of such a tremendous growth in poultry production the per capita availability is 40 eggs and 1 kg meat against recommended level of 180 eggs and 10–11 kg meat per annum in the India, which warrant further improvement in poultry production. On the other hand selection for faster and higher growth rates and muscling have resulted into musculoskeletal deformities and meat quality defects like tibial dyschondroplasia, obesity, ascites, pale soft and exudative (PSE) meat, focal myopathy, deep pectoral myopathy etc. Further, the production stress in high yielding stock also acts as a predisposing factor for precipitating the disease problems. The qualitative assessment of poultry meat conventionally is being done by external appearance and carcass grading is based mainly on bruising and discoloration however, diversification of poultry industry demands emphasis on meat quality aspects due to competitiveness in global market, consumer health awareness towards eating quality (quality product e.g. meat tenderness and juiciness) nutritional value (low fat and protein rich) and food safety (disease free birds). In addition the long term selection programmes have resulted into exhaustion of genetic variability to a large extent in high yielding stocks. The situation therefore warrants further investigations in these areas to improve meat quality vis a vis yield. The newer biological tools opens new horizons by providing the means for analyzing the genes involved in the process of growth and for modulating the potent genes in a desired way.

Molecular approaches: The exploration of poultry genome using molecular markers like microsatellite, AFLP, SNPs, SSCP etc. could provide an assessment of existing genetic variability in poultry germplasm for their characterization and better utilization of poultry genetic resources. Certain microsatellite markers have found associated with growth traits. Recently, a total of 283 SNP were discovered in 31 897 base pairs (bp) from 12 genes of the growth hormone (GH), growth hormone receptor (GHR), ghrelin, growth hormone secretagogue receptor (GHSR), insulin-like growth factor I and II (IGF-I and -II), insulin-like growth factor binding protein 2 (IGFBP-2), insulin, leptin receptor (LEPR), pituitary-specific transcription factor-1 (PIT-1), somatostatin (SS), thyroid-stimulating hormone beta subunit (TSH β). Fifteen non-synonymous SNP altered the translated precursors or mature proteins of GH, GHR, ghrelin, IGFBP-2, PIT-I and SS. Fifty-nine PCR-RFLP markers were found in 11 genes. The SNP discovered may provided suitable markers for association studies of candidate genes for growth related traits in chickens.
Candidate genes for production: The genes which are directly associated with production traits like TGF betas, Growth hormone, Myostatin, IGF-1, Calpain genes etc. may serve as the candidate genes and analyzed using different molecular techniques to identify suitable molecular marker for optimization of production.

a. Chicken Growth hormone (cGH) gene: One of the most important genes involved in somatic growth and substrate metabolism causing increased skeletal muscle accretion, cGH is located on chromosome 1 and is 3.5 kb in size consisting of 5 exons & 4 introns. It has effects on satellite cells proliferation and differentiation also. cGH gene are associated with various production traits e.g. body weight, egg production, carcass traits fatness and leanness immuneresponsiveness etc. Intron I (756 bp) and IV (1164 bp) are highly polymorphic thus can be easily studied by PCR-RFLP for identification of best genotypes.

b. Myostatin Gene: Also named as growth and differentiation factor-8 (GDF-8) this gene is 8.0 Kb in size and comprised of 3 exons and 2 introns and expressed at higher levels in skeletal muscle. This gene inhibits myoblast cell proliferation and differentiation by targeting myogenin and blocking MyoD activity and expression. Also maintains quiescent status of satellite cells. Mutations in myostatin alleles results in double muscle cattle 'breeds e.g. Belgian blue and Piedmontese. This gene is also supposed to be associated with meat tenderness. SNPs of chicken Myostatin gene were found associated with skeletal muscle growth. This gene also shows high level of polymorphism which can be used in association studies of economically important traits in poultry. The ontogeny of expression of myostatin gene at different embryonic stages (E0 to E18) was studied in coloured broiler stock developed at CARI, Izatnagar. At all the stages highest expression was observed in breast and leg muscles whereas liver and brain showed lower expression and heart showed no expression till 7th day

c. IGF-I: Insulin like growth factor –I (IGF-I) is an important component gene of GH-GHRH – IGF-I axis. IGFs play central role in main growth signaling system of vertebrates. IGF components include IGF-I and IGF-II, type I and II receptor and family of six secreted IGF binding protein (IGFBs). IGFs are ubiquitously expressed. During embryonic and post-natal growth. In addition to their endocrine effects locally produced IGFs produce autocrine/paracrine effect on cell differentiation and proliferation. IGF-1 knock out mice have birth weight approx. 60% of normal with defective ossification. Deletion of type -I IGF-I receptor yielded hozygous animals that were 45% of normal birth weight and have very short life span (few minutes).

d. TGF-βeta: The transforming growth factors β (TGF-β) are multifunctional cytokines with diverse effects on cell growth, differentiation and function. The chicken TGF-β subfamily consists of four currently identified members. TGF-β1, TGF-β2, TGF-β3 and TGF-β4. The TGF-β super family members play pivotal roles in tissue homeostasis, and development. Biological effects of TGF-β isoform are broad, including effects of cell growth and immune function. The TGF-β genes influence the growth and differentiation of many cell types and play an important role in processes such as myogenesis-chondrogenesis, osteogenesis, hematopoiesis, epithelial cell differentiation and adipogenesis. The first report on TGF-β relating to muscle formation and growth reveals that it blocks differentiation of satellite cells prepared from adult rat skeletal muscle, as well as that of primary myoblast. Association of TGF-β gene with growth and body composition has been detected by using molecular and breeding techniques in chicken. Temporal and spatial expression analysis of TGFβ 2 in our coloured stock revealed that TGFβ2 started expressing from day2 embryo and it expressed at highest level in skeletal muscles.

e. Calpains: Calpains are intracellular Ca²⁺-dependent proteases and enzymes that contribute to growth and meat quality. So far two isoforms-μ (calpain I- requires micromolar Ca++ conc. for
activity) and m (calpain II requires millimolar Ca++ con. for activity) have been identified. Both the calpains have been reported to cleave the most myofibrilar proteins (except actin myosin) in restricted manner to modify their properties rather breaking down in sub units. This proteolytic system plays a key role in tenderization process of meat that occur after post mortem during refrigeration. Polymorphisms have been reported for four calpain genes (CAPN1, CAPN2, CAPN3, and CAPN1.5) expressed ubiquitously in chicken by PCR-RFLP. CAPN2 and CAPN1.5 mapped to two locations on chromosome 3 about 30 cM apart, while CAPN3 mapped to chromosome 5. CAPN1 was linked to a previously unlinked microsatellite marker LEI0140 to form a new linkage group called E66.

**Candidate gene analysis for Osteoporosis:** Osteoporosis is a significant problem in egg-laying chickens because of welfare concerns and lost markets for spent hens due to splintered bones. Single nucleotide polymorphisms (SNP) in candidate genes for osteoporosis were identified in a chicken resource population. Candidate genes included Vitamin D receptor, calcitonin, insulin, and TGF-beta2. Human and chicken sequences were used to design PCR primers for the candidate genes, and SNP were identified by comparing sequences of PCR products from layer (White Leghorn) and broiler (Cobb) lines. At least one SNP was identified for each candidate gene, and PCR-RFLP assays were developed to genotype SNP within the calcitonin, insulin, and TGF-beta2 genes. Frequencies of the predominant allele of the TGF-beta2 and calcitonin SNP were 1.0 and .7 in the layer and broiler grandparents, respectively.

**Candidate gene analysis for fatness traits in chicken:** Uncoupling proteins (UCPs) are integral membrane proteins of the mitochondrial respiration from oxidative phosphorylation, diminishing the resulting production of ATP and instead yielding dissipative heat. UCPs provide new clue to the causes to obesity therefore was chosen as the candidate gene for studying fatness trait in chicken. Primers for the 3'-untranslator region in UCP were designed from database of chicken genomic sequence. Polymorphisms were detected by PCR-SSCP and DNA sequencing. The results showed that there was significant difference (P < 0.01) in the frequency of genotype among breeds except few cases. A A/C mutation at base position 1197 was found among individuals in broiler line and the least square analysis showed that BB birds had significant lower (P<0.01) abdominal fat weight and percentage of abdominal fat than AB or AA birds. UCP gene were found to be the major gene to affect the fatness traits or it links with the major gene.

**QTL linked to production traits:** A QTL that explained a large proportion of the phenotypic difference between broiler and layer chickens in an experimental cross was evaluated in a commercial broiler line. A three-generation design, consisting of 15 grandsires, 608 half-sib hens, and more than 50,000 third-generation offspring, was implemented within the existing breeding scheme of a broiler breeding company. Four markers from a candidate region on chicken chromosome 4 were selected for their in formativeness in the grandsires and used to genotype the first two generations. Using half-sib analyses, linkage was studied between these markers and 13 growth and carcass traits. The QTL analyses confirmed the presence of significant QTL for body weight (P < 0.01) and residual feed intake (P < 0.05) on chicken chromosome 4. Furthermore, evidence was found for QTL affecting the relative weight of bone and muscle in the thigh. Four more markers were added to increase resolution of the QTL positions. This increased the significance of the QTL for body weight (P < 0.001) and residual feed intake (P < 0.01) and showed evidence (P < 0.05) for additional QTL affecting carcass weight and conformation score. This study showed for the first time that a QTL that explains differences between broilers and layers was segregating in lines that have been selected for body weight over 50 generations. A possible explanation could be a pleiotropic or closely linked effect on fitness-related traits that are not part of the present study. The results demonstrate the
feasibility of QTL detection and the potential for marker-assisted selection within a commercial broiler line without altering the existing breeding scheme.

**Candidate gene marker for reproductive traits:** Three physiological candidate genes i.e. genes for growth hormone (GH) and gonadotropin releasing hormone receptor (GnRHR) and neuropeptide Y (NPY) were analysed to find out their association with egg production, number of double yolk egg and age at first egg (AFE) in broiler breeder pedigreed dam line. SNP and deletions were detected in these genes PCR-RFLP was done to determine genotype frequency. Dominance effect of NPY on AFE and additive effect of GNHR on double yolk egg were found.

**Conclusion:** The growth is multifactorial and multidimensional phenomenon. There are many important genes, which are involved in growth as well as immune functions. The molecular analysis of important candidate genes having major role in growth and differentiation of cells may help in identification of suitable markers to be used in marker assisted selection (MAS). Fine molecular tools may further lead to identification of QTL marker or the positional candidate gene to assist in selection programme. Further the modulation of one or more of these genes may also be viable approach for augmenting the production.
Poultry processing industry has seen a rapid increase in automation over the past few decades, especially in countries with highly mechanized operations. However, the growing and transport of live birds, slaughtering and selling of poultry products are still small scale operations in many countries including India. In India just about 5% of total broilers produced are processed in automated or Semi-automated plants, about 10-15% are processed in crude and generally unhygienic facilities by wholesalers and the rest are sold live or fresh dressed before the customer in the retail markets.

Chicken purchasing habits are slowly but surely changing, particularly in the large cities and towns and an acceptance of fresh chilled or frozen chicken is gradually developing. However, the process is slow and requires to be stimulated.

The technological developments in the primary processing of poultry are a continuous process. The rate of introducing new operations depends on several factors such as labour costs, product quality, new regulations and profitability. The advancement made in almost all departments of poultry processing is briefly discussed.

Pre-slaughter operations

The main operations of pre-slaughter handling of live birds include fasting, catching, transport/holding, hanging and stunning/killing. The aspects involved are visual quality defects, downgrading, dead on arrival, weight losses, labour requirement, human and animal welfare.

**Fasting:** A period of fasting before slaughtering poultry is initiated to avoid contamination with feces during processing, especially during evisceration of carcass. Normally it starts some hours before catching with withdrawal of feed and at the beginning of catching with withdrawal of water supply. In the beginning of fasting period (2-3 hours) the carcass is still growing by converting feed to body-mass and energy for the physiological process. The contents of digestive tract cannot deliver any energy at 5-6 hours after withdrawal of feed.

The energy required for the physiological process for keeping the body temperature at 41°C and the activity of the birds is delivered by using body components for these reactions. This introduces weight loss of the birds related with the basal metabolic heat production.

Based on the results of available literature, the following conclusions can be drawn on the recommended length of fasting period for optimum yields of broilers:

- All feed passes the digestive tract in about 5-6 hours,
- Weight loss because of metabolic heat production during starvation period is at 0.22% to 0.35% per hour,
- To achieve the highest yield, broilers should be slaughtered about 2-3 hours after withdrawal of feed Development of new evisceration methods will be worthwhile which should make it possible to slaughter all birds after a starvation period of two hours without contamination. It can increase the total yield of poultry industry by attest 1-2%.
Catching, loading and transport: In recent years, although several systems have been developed to improve catching and transportation, the broilers are generally still caught by hand and loaded in crates or container modules in the poultry houses and the crates/containers are lifted on the transport vehicle by hand or a forklift. The catching crew should be well trained and great care should be taken during catching, loading and unloading to avoid bruises and broken legs and wings which adversely affect the quality of dressed carcass and lead to downgrading. Typical hemorrhages in the thigh can also be the result of catching and holding birds on one leg.

The handling of container modules is only possible with forklifts and has reduced the number of downgraded birds compared to crates which are often manually handled and loaded on the transport vehicles. Container modules are being used more in poultry industry for reasons of labour efficiency and product quality. For product quality they are considered more bird friendly than crates.

**Automatic catching system** has been in development for more than 15 years. Although this system is more humane than manual catching and reduces the stress level and catching damage to birds, it is not generally applicable due to its high initial investment (US $ 110,000 to 150,000) and running costs.

Transport and holding of birds are very important steps and are responsible for most downgrading of birds. If the birds are to be transported by open-sided vehicles, the critical aspects are exposure of birds to the prevailing climate and to high wind speed, from which they must be protected. Attempts should be made to reduce the degree of exposure in adverse weather conditions by sheeting on the sides of vehicles. When the vehicles arrive in premises of processing plant, the birds should be protected from the direct rays of Sun and from adverse weather and are provided with adequate ventilation. In warm weathers, the number of birds placed in each crate or module should be reduced.

Reception, unloading and hanging: The reception or arrival area is one where the live birds are brought into the processing plant for unloading. This area should be under cover and of sufficient size to contain all the transport vehicles awaiting unloading. To avoid the cross contamination the ideal way is that the vehicles enter the building at one side and leave at the other after washing. In warm weather, good ventilation and control of relative humidity in reception area are essential as regards the welfare of birds. The relative humidity should not be allowed to rise above 70%.

Crates are unloaded from the vehicle one by one and placed on a conveyor system which carries them to hanging station. Here the crates are opened, the birds removed and hung on the killing line. The empty crates are washed and brought back to the vehicle. Modules are unloaded without taking them off the transport vehicles. For this, a system comprising two vertically moving platforms is usually employed. The vehicle is driven between the platforms. The killing line overhead conveyor extends along these platforms. The hangers standing on the platforms open the modules, take out the birds and hang them on the killing line.

In plants where hanging of birds on shackles is a manual operation, it should be done carefully as this operation can also cause damage to the birds.

**Stunning:** Stunning prior to killing is universally practiced by the American and European poultry industries to render the birds unconscious so that they do not experience the panic and stress associated with killing and death process. Such panic and stress can cause struggle which damages the carcass and an erratic heart beat which results in poor bleeding.
Electrical stunning is the most common method of stunning. It is carried out in an electrically charged water bath by dragging the head of the birds through water in which an electrode is submerged. The shackles of the killing line simultaneously touch an earth electrode, causing an electric current to run through the whole body of the bird. Research showed that the minimum current required in the water bath stunner to induce cardiac arrest is 120 mA for broilers and 150 mA for turkeys. Evidently, electrical stunning introduces hemorrhages in breast and thigh meat. This resulted in consumer complaints, downgrading and loss of yield of most valuable parts of the carcass. Further research were carried out to develop alternative stunning methods.

Gas or modified atmosphere stunning is an alternate method that exposes the birds to a gas to render them unconscious just before throat cut. In this system the gas is administered inside a tunnel to birds on shackles. This system is in commercial use. As regards the use of gas or gas mixture, the results of behavioral studies indicated that the first choice of gas would be 90% argon in air (leaving 2% oxygen and 8% nitrogen from air) that induced anoxia. The second choice would be using a mixture of 30% CO₂ and 60% argon in air.

Gas stunning is generally more gentle than electrical stunning and reduces the incidence of muscle hemorrhages. Gas stunning using mostly argon accelerate rigor mortis development which reduces aging time before deboning.

The investment cost of gaseous stunning equipment and running costs are expected to be high respectively about US$ 250,000 to $ 350,000 and US$ 0.01 to $ 0.02 per broiler. The highly sophisticated equipment requires well trained people to maintain.

Slaughter and Post-slaughter operations

Killing: Killing is performed either manually by cutting the jugular vein and carotid artery, or mechanically by guiding the bird's head across a single revolving circular blade or between a pair of revolving blades. The minimum time permitted for bleeding is 90 seconds in case of chickens and 120 seconds for turkeys. The blood is collected in a tiled bleeding tunnel or stainless steel trough and pumped to a holding tank at regular interval.

Scalding: After bleeding the birds are scalded by immersion in hot water to attain feather release. Birds are immersed for up to 3.5 minutes in scald tank depending upon the water temperature. Choice of water temperature depends on how the birds are to be packed and sold. For a fresh chilled market, a soft or semi-scald at 50-52°C is required. This permits the retention of cuticle which is essential to avoid severe discoloration and drying of skin during air chilling. For frozen market, a sub or hard scald at 56-60°C is used since retention of epidermal layer is not necessary for a water chilled product. At higher temperature, the birds only need to retain in the scald tank for 2-2.5 minutes to facilitate the feather removal.

Further research has been conducted on the immersion scalding system that lead to the development of Multi-stage counter current scalding. Experiments on semi-technical and commercial scale proved the advantages of this process. This system improves the hygienic condition of the product with reduced energy and water consumption.

Defeathering: Feather removal is achieved mechanically in plucking machine by rotating rubber fingers beating on the body surface to rub the feathers free of the follicles. Constant improvement is being made in type of machines used for different species and types of birds and specific parts of carcass to be defeathered. Since increasing the duration or severity of beating action increases the degree of toughness in young chickens, manufacturers have developed lighter weight fingers for picking breast and thigh portions of carcass so as to do minimum
damage to meat tenderness. Pin feathers are usually removed by hand. Then the carcass is passed through an arc flame to singe the remaining fine hairs (filoplumes) and pin feathers.

**Washing:** The final process on the killing line is a thorough washing of the external surface of the carcass. Pasteurized water jets are most often used, frequently with soft rubber fingers to assure a complete removal of residual feathers or other materials from the skin surface. Then the carcasses are transferred to evisceration line to complete the evisceration process.

**Evisceration and Inspection:** On the evisceration line the carcasses are hanged by the feet in shackles. The head, upper esophagus and crop are removed from the front of the carcass. An incision is made through the abdominal wall under the tail. The cut is continued around the vent so that intestines are free of any connection to the skin or abdominal wall muscles. Through this opening all organs of body cavity are removed. The edible organs like heart, gizzard and liver are separated, washed and saved as giblets. Gizzard is cleaned mechanically by a gizzard harvester. The lungs and other remaining materials within the carcass are removed by suction using a lung gun. Kidneys are removed by a special shaped vacuum nozzle. The feet and hock are then cut from the carcass at the end.

After the abdomen is opened and partially cleared of intestinal organs but before any part of carcass separated, the meat inspector inspects each carcass for wholesomeness. When the carcass is passed by the inspector, the inedible viscera is pulled out of the carcass and dropped into carry-off system.

**Automatic evisceration line** for chicken industry has been a major technological development. This line can handle up to 6000 broilers per hour and comprises of a vent cutter, an opening machine, an eviscerator, a neck cracker and a final inspection machine which removes residual debris by vacuum.

Another recent development has been the automatic cropping machine, which removes the crop, trachea and neck glands from the eviscerated carcass.

**Washing:** Complete washing of inside and outside of the carcass is the final operation on the evisceration line. A number of mechanical inside outside washers is available which thoroughly clean the body cavity and also the outside of the carcass simultaneously.

**Chilling:** Chilling of poultry is essential to control microbiological growth on the surface. As cooling the product takes time, the onset and resolution of rigor mortis may occur during chilling process. Slush ice, crushed ice and circulated slush ice are of equal value in the rate of cooling poultry carcass. However, In-line evaporative air chilling developed in Netherlands has almost completely replaced the immersion chilling operation in Netherlands and Germany at this moment. Advantages of this system include no excessive water uptake, product more uniform, a better eating quality of the product, reduced water use and increased productivity by automatic transfer of carcass.

**Packaging:** After weighing and grading the dressed whole chicken is trussed prior to packaging. The wings are folded behind the back and drumsticks are tucked into the opening of body cavity. Elastic bands are sometimes used to secure the legs. An automatic trussing machine has been developed for broiler carcasses, which folds the legs into the sleeve of skin. Then the carcasses are packed in polyethylene bags and sealed with the help of a clip or tape.

**Marketing of processed chicken:** Today in India, the marketing of processed chicken is mainly conducted through existing retail cold store outlets, supermarkets and institutional bulk consumers. Bulk consumers like hotels, restaurant, industrial canteens and armed forces etc.
form an important part of the customer base. The infrastructure for refrigerated transportation and storage and dedicated retail outlets has not yet been developed.

**Application of HACCP in poultry processing**

HACCP system is a scientific approach to process control. It is designed to prevent the occurrence of problems by assuring that controls are applied at any point in a food production system where hazardous or critical situations could occur. Hazards include biological, chemical or physical contamination of food products. Hazard analysis (HA) involves investigation through examination that identify, estimate and calculate the risk of all factors associated with processing and marketing of a given product i.e. materials, processes, practices, products, premises, equipments, utensils and personnel. Critical control points (CCP) involve control by monitoring the points mentioned above. In new regulations a CCP is a point or step or procedure in the food process where control can be applied and as a result a food safety hazard can be prevented, eliminated or reduced to acceptable level. In addition, HACCP Plan also includes establishing critical limit for each CCP, continuous compliance with established critical limits, corrective action plan to prevent recurrence when there is deviation from the established critical limit, effective record keeping procedures for documentation of HACCP system and procedures for verification that HACCP system is working correctly.
Newer Techniques in Tenderization of Poultry Meat

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Tenderness is rated as one of the most important factors for an average consumer among the different attributes of eating quality of meat. It is related to structure and biochemical properties of skeletal muscle fibers especially myofibrils and intra-muscular connective tissue. Meat texture which ultimately affects meat tenderness, is a function of the size of bundles of fibers and the perimysial septa of connective tissue which divides the muscle longitudinally.

Improvement in tenderness of meat specially spent layers and culled birds has attracted the attention of the meat scientists for long. Number of methods like electrical stimulation, treatment with enzymes, electrotypes, phosphate, inorganic salts etc. have been developed but each method has its own limitations. Storage of meat at refrigeration temperature for sufficiently long period i.e. conditioning, is reported to be one of the best methods for tenderization but to reduced time for conditioning and save energy, alternative methods have been tried.

**Application of enzymes**

Treatment by proteolytic enzymes is one of the popular methods for meat tenderization. Proteolytic enzymes of plant origin such as papain, bromelin and ficin have been widely used as meat tenderizers in most part of the world. The tenderization of meat with plant enzymes is more on muscular fibers than the collagen. Antemortem injection of plant enzymes to poultry is more effective than postmortem use due to quicker distribution of enzymes through the vascular system. 75ppm of papain or bromelin was ideal for optimum tenderization of poultry meat. However, with 100ppm injection, it had an undesirable effect on flavour resulting in lower overall acceptability. Breast muscles of fowl soaked in 0.03% Bromelin or 0.02% papain were equally effective as tenderizer. Enzymes alone or in combination with sodium chloride and phosphate solution have been used successfully for tenderizing spent hen and culled fowl meat. Enzymes with pressure treatment have also been used as a tenderizing method for hot deboned prerigor meat.

Recently other plant proteoses like ‘cucumin’ from Cucumis trigonus Roxb (Kachri) and ‘Zingibain’ from Zingiber officinale roscoe (Ginger rhizome) have been tried in buffalo meat for tenderization and results are comparable to papain treatment. Its proteolytic activity on both collagen and actomyosin was reported to produce more tender meat. However, in poultry meat ginger extract used at 3% for 24 hours, was found optimum for tenderization. This was due to the action of zingibain on both myofibrillar and connective tissue component of muscle.

**Use of Electrical stimulus**

Electrical stimulus (ES) has been used as a means of decreasing the aging period to tenderize early harvested fillets by increasing myofibrillar fragmentation due to intense muscle contraction associated with pulsed electrical current passing through the carcass. White fibers have been found to be affected more by ES treatment than red fibers. Hence the red fibers of duck meat have less potential for tenderization due to ES than do the white fibers of broiler.
Use of weak acids

Lactic acid along with acetic acid used in foods, is generally considered as safe. These are mostly recognized as decontaminants and hence used in preservation. However, during the recent years weak acids have been used as tenderizing agents. Marinating spent fowl muscle in 1.5% lactic acid or acetic acid for 72 hrs. reduced the shear force value and were comparable to broiler drumstick. Tenderness of chicken breast meat was improved by dipping in Lactic acid for 10-30 minutes. It has been suggested that organic acids (Citric/acetic) changed the structure of collagen. Collagen consists of two main soluble fractions. Tropocollagon is dissolved in neutral salt solutions and procollagen in weak organic acids. The mature collagen is more cross-linked than that of young birds and has become more resistant to mechanical stress. Significant tenderization of spent layer drumstick meat could be achieved via marination with mild lactic/acetic acids. Vacuum tumbling of drumsticks in lactic/acetic acid with 2% NaCl for 2 hrs. reduced the shear value of drumstick by 50%. A significant reduction in thermal resistance and degradation of collagen in spent hen drumstick was observed during marination in 1.5% lactic acid/acetic acid.

Mechanism of tenderization

Research indicated that acid labile bonds on collagen might be cleaved by the action of lactic acid. Improvement in tenderness was due to swelling of collagen-rich skin covering of muscle and muscle mass, enhancing the fragmentation of and Z line and degrading myosin heavy chains and actin.

Treatment with calcium chloride

In recent years the use of calcium chloride as a tenderizer, has attracted the attention of researchers. Numbers of studies have been undertaken with the aim of reducing the aging time and variability in meat tenderness. The use of CaCl₂ in poultry as a tenderizer is of recent past. Calcium chloride injection (100 to 300mM) to spent hen muscle immediately postmortem improved tenderness. Combination of CaCl₂ with electrical stimulation offered an additional advantage. Although CaCl₂ injection in spent fowl meat without aging reduced shear force value but with aging, additional tenderization also occurred.

Besides injection marination of meat pieces in 0.15 to 0.2M and 50 to 200mM CaCl₂ solution in broiler breast were found effective in tenderization. Freezing prior to marination intensified tenderizing effect of CaCl₂. Even 48 hrs. marination was more effective that 24 hrs. Marination with CaCl₂ at post-riger state was more effective than pre-riger stage. Injection and infusion of CaCl₂ can be attempted by the processor/retailer and marination can be practiced at household/resturant level.

Mechanism of tenderization with CaCl₂

During postmortem aging of carcass, numerous changes take place in myofibrillar and connective tissue component. The loss in integrity of tissue during aging resulted in improvement of meat tenderness. These changes are mostly brought about by either calpain proteolytic system and/or catheptic enzymes. The role of catheptic enzymes during postmortem proteolysis is negligible.

The calpain proteolytic system consists of n-calpain, m-calpain and calpastatin and is susceptible to calcium induced proteolysis. Although the exact mechanism of hydrolysis is not very clear, the elevated calcium concentration due to postmortem release from mitochondria and
sarcoplasmic reticulum is considered to be responsible for the activity of \( n \)-calpains. The use of CaCl\(_2\) as a tenderizing agent is based on the activation of \( n \)-calpain after slaughter. Proteolytic activity of calpain require 10mM calcium and that of \( m \)-calpain 200-300mM calcium respectively, which is much higher than the free calcium concentration level in living and postmortem cells. The third component of calpain proteolytic system is calpastatin (their specific endogenous inhibitor) The ratio of calpain: \( n \)-calpain + \( m \) calpain has a bearing on the tenderizing action. Reports available indicated that calpain proteolytic system was primarily responsible for p.m. proteolysis and calcium activated tenderization through use of exogenous calcium.

**Advantages of Calcium activated tenderization**

1. Maximum tenderness values obtained within the first 24 hrs. of p.m. storage.
2. Consistently uniform tender meat production.
3. Because of unique built-in-control (autoproteolysis of calpain in the presence of Ca) meat is never over-tendered as with other proteases i.e. papain.
4. Process can easily be applied to hot boning, thus considered as means of decreasing energy and labour costs associated with chilling fabricating carcass.
5. From nutritional point of view, this process can be used as a method of calcium fortification of meat. The conc. used is within the limit approved by regulation (FDA).

**Tenderization with salts and phosphates**

Phosphates have been used successfully in tenderizing poultry meat due to their ability to increase charge, \( \text{pH} \) and subsequent hydration of proteins. Inorganic salts had a positive response on the tenderness of spent hen muscle. This was possibly due to increased charge of muscle proteins, introduced by the salts.

**Tumbling/massaging for tenderization**

The basic goals of tumbling and massaging are to produce enough protein exudates (mainly actin and myosin) to effectively promote cohesion during thermal processing, to enhance tenderness and to develop desirable slicing characteristics and enhance curing process with attractive and uniformly developed cured meat colour. However, firm tissues such as beef, mutton and turkey are subjected to impact tumbling whereas soft tissues such as pork and chicken are subjected to massaging.

Tumbling involves the influence of impact energy on the muscle tissue, which occurs when meat falls from the upper part of a rotating drum or can be caused by striking the meat with paddle or baffles. On the other hand, massaging is a less physically rigorous process and involves frictional energy rendering from rubbing of one meat surface on another and on the smooth surface of a container. Vacuum tumbling along with injection of 0.3 M CaCl\(_2\) was essential for maximum tenderization of spent fowl meat. Marination or tumbling with 0.3M CaCl\(_2\) + 0.5% lactic acid for 24 hrs caused significant reduction in shear force value and improvement in over-all acceptability scores.

**Conclusion**

For texture improvement of culled and spent chicken, a satisfactory tenderization technology has to be standardized which would achieve maximum tenderization in a shortest time. Concerted efforts must be made for practical application of this technology under our condition to ensure desirable tenderness and improved palatability of poultry meat. Once the technology is standardized, it would help in effective utilization of tough meat from spent hens and culled poultry and this can provide a big boost to the Indian Poultry Industry.
Hen egg is not only a rich source of nutrients but also possesses polyfunctional properties. Egg white has excellent foaming, gelling and coagulation properties which are of specific values in bakery for making cakes, confectioneries and custards, etc. The excellent emulsifying and emulsion stabilization properties of yolk is utilized in mayonnaise and shortened (fat-rich) cakes. Apart from this, both these components of egg find application in a variety of non-food industries. It also contains a number of biomolecules of commercial importance for use in food preservation, quality improvement and pharmaceutical industries. Separation technologies and usage of some commercially important bioactive compounds of egg are, briefly, described below.

Lysozyme: Egg white lysozyme (1,4 β-N-acetyl muramidase) is a natural enzyme with well known antimicrobial activity. Its antibacterial activity is exhibited by the hydrolysis of β-glycosidic bond formed between N-acetylmuramic acid and N-acetyl glucosamine in the bacterial cell wall. It exhibits more antibacterial property towards Gram-positive than Gram-negative bacteria due to differences in the structure of their cell membranes. In case of latter group of bacteria, an additional barrier for lysozyme action is the outer cell membrane composed of protein, phospholipids and glycolipids. The monomer form of lysozyme in native egg albumen is mainly effective against Gram-positive bacteria. Attempts are being made to polymerize this enzyme into dimers, trimers by chemical and thermal treatments (pH 5-6; 70-80°C; 10% H₂O₂) for wide antibacterial activity against Gram-negative bacteria including E. coli.

Because of its high thermal stability, lysozyme can retain its hydrolytic activity at food pasteurizing and even boiling temperature in acidic pH (4-5) range but it gets inactivated in alkaline (pH>9) medium.

Lysozyme has been extracted from egg white by salting-out process using 5% NaCl at pH 9.5 which is close to its isoelectric point and chromatographic methods. However, in recent years, it is being extracted commercially by cation-exchange column chromatography. using carboxymethyl cellulose (CMC) resin or cyclodextrin. The elute is dialysed and spray-dried. As lysozyme and avidin have similar charge properties, they can be isolated together more economically and efficiently by cation-exchange resin technique. Lysozyme is first eluted in high salt buffer and purified by crystallization, leaving behind avidin in spent salt solution because of its higher solubility than that of lysozyme at alkaline pH. Secondary purification of crude avidin is done by affinity chromatography, using CMC as the medium of purification.

Lysozyme is primarily used as a natural preservative for a variety of food products like pasteurized milk, cheese milk (20-35 ppm) for making fermented cheese in which its concentration reaches to a level of about 400 ppm, processed meats, seafoods and fresh water fish products, tofu bean curd from soy milk, fresh fruits surface coating, sauces and salads, etc. It is also used as a clarifying medium for wine at 20ppm level. More recently, lysozyme is being tested to inhibit biofilm formation in food processing and drinking water supply pipelines.

Avidin: It is a glycoprotein with strong binding affinity with biotin. About 3 moles of biotin are bound to one mole of avidin. Avidin is another natural defensive agent present in egg albumen.
The widespread developments in fast food sector have seen a rapid growth in ready-to-eat/ready-to-prepare egg-based foods. This trend is expected to continue to grow worldwide. Changes in consumer's lifestyle and eating habits have immensely contributed to this growing demand. Although formulating convenience egg products for the Indian market is new development, their demand will continue to grow for the reasons of popularity, availability and preference. Further, the large Indian population creates a big potential market for processed egg products. Thus, there are ample opportunities to develop and improve our technology to satisfy the demand for quality egg products of greater variety.

The projected per capita annual consumption in India is 180 eggs by 2015 as against the present level of merely 40 eggs. This vast gap indicates tremendous growth potential of egg and egg products industry in India. Hence, the availability of variety of egg products in convenient form is bound to play a significant role in achieving the target of egg consumption by creating new market outlets for eggs. Although the traditional egg products such as hard boiled, poached, omelet, scrambled and egg curry are still very popular, it is imperative to develop newer egg products to meet the growing demand of egg-based fast food in the country.

Technologies developed at this institute for preparing some innovative and improved egg products are discussed briefly. These products can be commercially exploited to boost the growth of egg products sector in the country.

**Egg Patty:** Formulation and processing methodology of whole egg patty have been standardized through a series of trials. A sensorily highly acceptable egg patty can be prepared with 70 percent liquid whole egg, 20 percent mashed potato and 10 percent texturized soy flour mixed with spices and condiments, non-fat dry milk and salt. Patties are formed by transferring the patty mix into stainless steel molds of 9 cm diameter, streaming at 98 KN m⁻² pressure for 4 minutes followed by frying for 2 minutes on each side in refined vegetable oil. The ready-to-eat patty contains 16.5 percent protein and 11.8 percent fat and retained 76.8 percent of its water after cooking and frying, which gave 88.8 percent cook yield. Physico-chemical, microbiological and sensory analysis of the product under different packaging and storage conditions indicate a refrigerated (5°±1°C) shelf-life of 14 days in vacuum and 12 days in aerobic packaging, while at frozen temperature (-18°±1°C) the product is stable for 90 days in vacuum and 80 days in aerobic packaging. Based on the existing market price of ingredients, the formulation cost of egg patties is calculated to Rs. 25.70/kg.

**Egg-Meat Patty:** Inclusion of meat in egg product formulations is not new to the egg product industry. Earlier, poultry meat has successfully been used in the manufacture of omelettes in USA and the product was popular in both household and institutional market settings. On similar line, as well as, in view of strong demands from meat loving consumers, opportunities were identified to develop egg-meat patty incorporating minced chicken lean in egg patty formulation. Our studies have shown that the egg-meat patties produced from 70 percent liquid whole egg, 20 percent minced chicken lean and 10 percent texturized soy flour with spice mix, fresh onion and ginger paste and salt was much more acceptable. The ready-to-eat-meat patties contain 19.5 percent protein and 11.1 percent fat with a cook yield of 91.6 percent. The product is stable for
up to 14 days at refrigerated (5°±1°C) and 90 days at frozen (-18°±1°C) temperatures without development of oxidative rancidity. The formulation cost of the product is estimated to Rs. 55.80/kg.

**Low Fat, Egg Patty:** Today, there is increasing awareness of the importance of diet in human health. Health conscious consumers are looking for healthy, nutritious and convenient foods, which contain no-or low fat. This preference creates challenges for the food industry and researchers. Although several studies have shown that reducing or increasing egg consumption does not significantly affect the blood cholesterol levels in normal people, consumers’ attitudes towards yolk lipids have not yet changed. Keeping these facts in view, we have attempted to develop a low fat, convenience patty type product from egg albumen. Our experiments have shown that the patty made from 70 percent liquid egg albumen, 20 percent mashed potato and 10 percent texturized soy flour alongwith non-fat dry milk, salt, spice mix and fresh onion and ginger paste had highest cook yield (92.3%), greatest moisture retention (86.5%) and were organoleptically most acceptable. The ready-to-eat fried patty contained 13.6 percent protein and merely 2.5 percent fat and thus could be a product of choice for health conscious consumers. Storage studies revealed a refrigerated (5°±1°C) shelf-life of 14 days and frozen (-18°±1°C) storage life of 90 days in vacuum packaging. The formulation cost of producing one kg of low fat egg patties was calculated to Rs. 33.50/kg.

**Egg Pancake:** Egg pancake is a convenience egg-rich product which can be popularized as a complete breakfast meal at homes as well as at growing fast food outlets. Attempts were made to reformulate the existing recipe of pancake to make it egg-rich product with traditional characteristics and local taste. Our studies revealed that the pancake produced from 35 percent liquid whole egg was lighter, more fluffy, more cohesive and spongy and had highest overall acceptability ratings. Other ingredients of pancake batter included liquid whole milk, refined wheat flour, wheat flour, sugar and baking powder with a few drops of vanilla essence. A pancake of 12 cm diameter and 2 cm height could be prepared by cooking in a pan heated at 60°C in 4-5 minutes. The ready-to-eat pancake contained 10.3 percent protein and 4.5 percent fat with a cook yield of 92.2 percent and had a refrigerated shelf-life of 12 days in vacuum packaging without any detectable deteriorative changes. It is one of the cheapest egg product. The cost of formulating one kg of pancakes was estimated to Rs. 19.06.

**Egg Pizza:** Pizza is one of the most popular fast food throughout the world and so is the egg pizza. If the egg pizza could be popularized as a meal or snack at fast food outlets, it would create new market outlets for egg and would help achieving the target of egg consumption. We have evaluated 9 egg-crust formulations consisting of either various albumen: yolk ratios, whole egg, foamed all-albumen or albumen with various texture improving ingredients like refined wheat flour, skin milk solids or refined vegetable oil spread on a crispy pizza base prepared earlier with 50% refined wheat flour, 25% egg albumen, 10% refined vegetable oil, 10% yogurt, 2% each of dry yeast and sugar and 1% salt and baked at 180°C for 5 minutes. Results have shown that egg-based pizza prepared with either 98% foamed all-albumen, albumen-flour-oil (80,13 and 5%) or albumen-skim milk solids-oil (81, 7 and 10%) were perceived as best in overall acceptability and had a refrigerated shelf-life of 6 days in vacuum and 4 days in aerobic packaging.

**Egg Roll:** It is a nutritious, tasty and convenience egg product suitable for meals or as snack foods. This product offers a potential market at growing fast food outlets. Egg filling formulations were standardized through several trial. The filling consisting of 80% scrambled egg and 20% chicken meat mixture (Shallow pan fried) was rated best in flavour texture and overall acceptability. For encasement of egg filling, a dough prepared with 32% refined wheat
flour, 22% whole wheat flour, 6% refined vegetable oil, 7% skim milk powder, 23% water, 1% salt, 1.5% yeast and 7.5% yogurt was most suitable because of its best baked appearance and crispiness. For preparing rolls, about 70g portion of dough was rolled into a 8x4 inch rectangular shape, 80g portion of egg filling was spread on the rolled surface, rolled length wise and baked in an oven at 190°C for 20 minutes. The most acceptable blend contained 14.8% protein and 25.7% fat and had a refrigerated shelf-life of 8 days in vacuum and 6 days in aerobic pack without any detectable deteriorative change. The cost of formulating one egg roll weighing approximately 150g is calculated to Rs. 8.85.

Enrobed Egg: Enrobed egg, primarily, is a hard cooked peeled egg enrobed with chicken meat mixture around its plain surface and deep fat fried. This product offers quality and convenience and suitable as single item breakfast entree at fast food outlets. Enrobing mixture consisting of 62.5% minced chicken meat, 7.5% textured soy flour and 5% bread combs, among other ingredients was most acceptable. Small chicken eggs weighing around 45-48 g are most suitable for enrobing. About 80 g portion of meat mixture was flattened into an oval shape of approximately 6” long and 3” wide. Slightly damp hard cooked peeled egg was rolled on rice starch before placing it is the center of flattened meat mixture and the mixture was folded around the egg to give an egg shape form. The enrobed egg was then dipped in egg-wash (wheat flour 45% in water), rolled in bread crumbs and deep-fried at 175°C for 7-8 minutes. The most acceptable blend contained 16.9% protein and 10% fat and was found microbiologically satisfactory and organoleptically acceptable upto 12 days of refrigerated storage. The cost of formulating one enrobed egg weighing about 120 g comes to about Rs. 14.50.

Albumen Rings: Albumen rings are egg snack food prepared by cooking blended egg albumen in ring molds, then battering and breading the coagulated albumen and deep fat frying. It can be popularized as egg snacks at growing fast food outlets. Albumen rings of 3.8 cm diameter and 0.5 cm thickness can be prepared by blending chicken egg albumen, placing about 8 ml of blended albumen in ring molds and steam cooking at ambient pressure for 5-6 minutes. For battering the rings, cereal flours like wheat flour, rice flour or corn flour alone or in combination with legume flours like black gram flour or gram flour were compared. The composite batter containing 25% wheat flour and 15% black gram flour was found best among all other batters in terms of coating adhesion and sensory quality of the product. The batter-breaded albumen rings contained 11.5% protein and merely 3.2% fat and had a refrigerated shelf-life of 18 days in vacuum and 12 days in aerobic packing. The formulation cost of preparing batter-breaded albumen rings was calculated to Rs. 68.60 per kg. The formulation cost of one batter-breaded albumen ring weighing about 8.7 g was estimated to 60 paise.

Egg Rings: Like albumen rings, the egg rings are prepared by steam cooking of blended whole egg liquid in ring molds, the coagulated rings are batter-breaded and then deep fried. This product too has great potential as egg snack food at fast food outlets and eating establishments. For battering the egg rings, the composite batter containing 25% rice flour and 15% black gram flour was found best among all other coatings tested in respect to coating adhesion and sensory quality. The batter-breaded egg rings contained 12.25% protein and 11.2% fat and had a refrigerated shelf-life of 12 days in aerobic pack with satisfactory microbiological and sensory quality. The cost of formulating batter-breaded egg rings was calculated to Rs. 39.00 per kg, while the cost of one ring weighing about 8.2 g was estimated to 32 paise.

Egg Crepe: Egg crepe is a thin, flat, circular egg-rich product prepared from thick batter of liquid whole egg and mixture of soaked and blended whole white rice (WR) and dehusked black gram splits (DBGs). Crepes may be filled with meat or vegetables and rolled or folded. It can be popularized as a convenience egg rich item at fast food outlets and at homes. Trials conducted is
our laboratory revealed that the crepes prepared from batter containing 60% whole egg liquid and 40% mixture of WR and DBGS (in ratio of 2.5:1) had best flavour and texture and were most acceptable. An egg crepe of 25 cm diameter and 1.0 mm thickness can be prepared by spreading 75 g portion of thick batter into a circular shape on a non-stick pan heated at 60°C and then raising the temperature to 95°C to complete the cooking in 4.5 minutes. Egg crepes contained 15.6% protein and 9.6% fat and could safely be stored for 22 day in vacuum and 20 days in aerobic packs at refrigeration temperature and for up to 60 days at -18°C in both packs. Further research is underway in our laboratories to develop cost-effective technologies for more and more new convenient egg products, which can be popularized at fast food outlets. Some of the technologies discussed above can also conveniently be exploited by the cottage industry in rural areas and popularized among rural people. This will not only help improving the nutritional status of rural population but also improve their socio-economic status, provide them supplementary income and generate more jobs.
Parallel with the phenomenal increase in both egg and broiler production to about 45 billion and 1.5 billions respectively, concerted efforts have been made towards development of further processed value-added convenience poultry products for catering to the growing demand of consumers. Poultry meat which constitutes about 25% of total meat productions in the country is going to become the major source of animal protein supply.

However, the commercial processing of poultry meat is still in its infancy. The bulk of chickens are sold as live birds, 15% are dressed in small processing units and only 5% are slaughtered in modern mechanized processing plants. Large human population with over 300 million middle class people, rising income, changing food habits, increasing urbanization have laid to a great demand for value-added convenient poultry products in view of the convenience to consumers with little or no kitchen time required. In view of the above, an attempt has been made here to present the innovative technologies for value-added convenience poultry meat products developed in India.

Convenience poultry products

The concept of convenience poultry products incorporates convenience to consumers and economic advantages to producers and processors alike. Main features of these products are to be tailored to individual market requirements and responsive to consumer’s demand. Moreover the added value to a particular product in terms of either convenience, cost reduction, improvement in nutritional and organoleptic quality, improved packaging and shelf-life extension etc. should not exceed the cost of adding the value to maintain marketability of the product. Successful development of such products involves the following sequential steps.

i) Idea generation
ii) Screening and evaluation based on consumer’s needs
iii) Product formulations
iv) Processing technology development
v) Testing
vi) Packaging
vii) Shelf-life evaluation
viii) Cost analysis
ix) Pilot plant scale-up
x) Market testing
xi) Commercial production.

Product Development

Before going for the main task of product development, physico-chemical and functioned properties of raw materials like meat from spent hen and culled breeder stock, less preferred parts like skin, giblet, wing and back and their yield have to be evaluated. Efforts have been made to utilize the separable abdominal fat of the carcass and other edible by products in the
formulation. A variety of processes such as portioning, deboning, grinding, massaging and tumbling to increase myofibrillar protein solubility and meat particle binding, flaking and dicing for restructured meat products, chopping and emulsification for comminuted products, marinating, battering and breading for enrobed fried products have been successfully attempted to manufacture a variety of semi-convenience, value-added ready to cook/ready to eat poultry meat products. These include chicken tandoor and barbecue, chicken canned in brine, gizzard pickle, fried liver and gizzard, and comminuted products like chicken sausage, patties, nuggets, rolls, loaves, balls, sticks and croquettes. Besides, meat from other avian species like duck, turkey, guineafowl have also been utilized for the preparation of sausages, patties, nuggets and pickles. Tandoori quail, quail meat pickle and battered fried quail are other novelties to the range of meat products developed. Various edible by-products from spent hen carcass such as skin, gizzard, heart, ova yolk and separable fat along with non-meat binders and extenders (textured soy, refined wheat flour, milk solids, potato, peas, gram flours, whole egg liquid or egg albumen etc.) have been incorporated in emulsified meat products for improving the quality and reducing cost of production.

Packaging

Development of cost effective, efficient and attractive package is an important consideration in product development and marketing. A number of food grade packaging materials based on thermoplastics, paper, metal and glass are available indigenously. However, efficient packaging for many of the value-added products developed, has not received much attention. Scaling-up of the processes/products developed and their familiarization to processing industry and consumers are the need of the hour for commercial exploitation and popularization among the masses.

Conclusion

Poultry processing sector particularly value-added segment needs to be vigorously promoted for boosting production by increasing the domestic consumption of processed products and also for promoting their export. Family dynamics, rising income, increasing exposure to various mass media, changing food habits with preference for fast foods and rapid industrialization have greatly enhanced the demand for fresh or frozen value-added products. Efforts must be made by all concerned to transform this sector of poultry industry into a more dynamic and liberal enterprise. Apart from developing sophisticated technology, attempts should also be made to develop cost-effective, intermediate technology for production of processed poultry products on a small scale in rural areas. The products should be prepared and marketed to the needs, expectations and acceptance of as large a population as possible at affordable price.

At present, we have more than a dozen poultry meat products available to the consumers of this country where as there are 100 of such preparations in developed countries. As such efforts must be made to develop more restructured, comminuted, enrobed or flavoured value-added poultry meat products as ready meals, snacks and easy-to-prepare items to add to the range of poultry products. There is also an imperative need to fabricate processing equipments indigenously, establish cold chain facilities, develop shelf-stable products for marketing under ambient conditions, improve packaging and to adopt strict quality control measures for ensuring consumer safety and wider distribution of the products.
Packaging is the scientific method of enclosing a food product in a condition to provide optimum protective properties for its anticipated shelf life. It has evolved from art to science and then from science to technology in the last few decades. Besides it has been bestowed with a significant role in the successful marketing of a product. Modern marketing has a visual appeal and can markedly influence the consumer behaviour.

Poultry products have different shape, sizes, physico-chemical properties, sensory quality and microbial flora. The selection of packaging material and techniques is done very carefully to protect these qualities and present the product to the consumers in the most attractive manner. Actually poultry products are perishable in nature and unless proper consideration is given to the specific characteristics of raw products and the processing undergone, it becomes difficult to select the right packaging material and technique. In this lecture, we shall discuss the packaging of some of the important poultry products – whole eggs, broken-out eggs, egg powder, dressed and cut up poultry, processed poultry products etc.

1. Packaging of Shell Eggs

Inspite of strength provided by shell, egg still remains a fragile commodity and needs protection against breakage. Eggs are susceptible to loss of moisture, deterioration from ageing and attack by bacteria and fungi. Eggs are also susceptible to the absorption of foreign odours.

Packaging Materials and Techniques:

i. Moulded pulp filler flats.
ii. Plastic filler flats.
iii. Proper board cartons with dividers.
iv. Folded paper board cartons with shrink film overlap of PE, PVC or PVDC.
v. Expanded polystyrene foam egg cartons or trays.
vi. Bulk packaging for export: All white paper board shipping cartons.

2. Packaging of Broken-Out Eggs

Broken-out eggs are generally sold frozen – either whites or yolks separately or whole after homogenization. Before freezing, salt or sugar (10% by weight) is added to prevent rubberiness due to coagulation. Broken-out eggs are very much susceptible to bacterial contamination.

Packaging Materials and Techniques:

i. Polyethylene pouches.
ii. Paper canisters.
iii. Cans.
3. Packaging of Whole Egg Powder

Commercially, spray drying is the most widely used process for the manufacture of whole egg powder, wherein de-sugaring is usually done by yeast fermentation. Moisture content of the product is restricted to a maximum of 2%. The spray dried whole egg powder is highly hygroscopic and its shelf life is influenced by storage conditions.

Packaging Materials and Techniques:

i. MST cellophane (300 gauge) pouches placed in laminate pouches of Brown casing (BC) paper / Alu foil (0.04 mm) / PE (150 gauge).

ii. Egg powder blocks of 30 g each compressed into blocks of 4 cm x 4 cm in MST cellophane over-wrapped in BC paper / Alu foil (0.02 mm) / PE (150 gauge).

iii. Thirty gram each of egg powder packed in plain sanitary cans under nitrogen gas.

iv. Bulk packaging in 2 lb and 14 lb capacity plain sanitary cans hermetically sealed under vacuum or 100% nitrogen.

4. Packaging of Dressed and Cut-up Poultry

Poultry meat has low fat content. The fat mostly runs beneath the skin, only very little being inside the muscle tissues. By nature, poultry fat is unsaturated and is very prone to the development of rancidity. Evisceration during dressing operations exposes meat to many microorganisms. Among them, Pseudomonas and Salmonella are comparatively more conspicuous by their presence in raw meat.

Packaging of poultry meat should be undertaken immediately after the dressing operations are over. Unpackaged refrigerated storage may result in surface dehydration whereas frozen storage may give rise to freezer burn.

Packaging Materials and Techniques:

Short Term Storage:

i. Overwrap – PE, PP, PVDC, RH or Nylon-6 (200 gauge).

ii. Polystyrene foam tray with overwrap of PE, PP (200 gauge).

iii. Biaxially oriented PE, PP, PVDC, RH shrink film – Ideal for contour tight package. Also recommended for frozen storage.

Long Term Storage:

iv. Vacuum packaging
   - Polyester / PE
   - Polyamide / PE
   - PVDC copolymer film
   - Polymer Coated Cellulose / PE
   - Nylon / EVA

v. Modified Atmosphere Packaging
   - Gaseous mix
     - 30% carbon dioxide + 70% nitrogen
     - 40% carbon dioxide + 60% nitrogen

vi. Bulk Packaging
    - Wire baskets
    - Plastic crates
5. Packaging of Mechanically Deboned Poultry Meat
Mechanically deboned meat is obtained as finally ground paste in which myofibrils are in a highly fragmented form. It contains considerable quantities of lipid and heme component. Hence pigments act as a catalyst in the auto-oxidation of lipids and may cause flavour problems.

Mechanical deboning causes maceration of tissue blending even microbial contamination. Further, heat evolved during deboning process may also enhance bacterial growth. Psuedomonas, Achrombactor and Flavobacterium are the predominant flora. MDPM is susceptible to soft texture, discolouration and rapid deterioration during storage.

Packaging Materials and Techniques:
   i. Vacuum packaging.
   ii. Modified atmosphere packaging.
      Gaseous mix
         20% carbon dioxide + 10% oxygen + 70% nitrogen
         20% carbon dioxide + 5% oxygen + 75% nitrogen
         30% carbon dioxide + 70% nitrogen

6. Packaging of Thermo-processed Poultry Meat Products
Most chicken meat products are cooked to an internal temperature of 65-70°C, to kill most of the micro-organism. Thermal processing over 100°C is done to prepare commercially sterile meat products. In cooked products, packaging should preserve typical appearance, delicate flavour and desired texture.

Packaging Materials and Techniques:
   i. Short Term Storage of chicken meat patties, sausages, nuggets, meat balls etc. can be done in the pouches of PE, PP, PVDC, RH etc.
   ii. Long Term Storage
      a. Tin plate cans for chicken curry, boneless chicken, soup etc. with hermatic sealing and retorting.
      b. Retort pouches for sausages, patties etc. with any of the following laminate pouches under vacuum:
         Polyester / Alufoil / PE
         Polyamide / Alufoil / PP
         Oriented PP / Alufoil / PE
         Polyester / Alufoil / PE
      c. Modified atmosphere packaging
         30% carbon dioxide + 70% nitrogen
         50% carbon dioxide + 50% nitrogen

Thus, packaging is an effective means of ensuring safe delivery of the product to the ultimate consumer in the sound condition. It should be selected with utmost care. Choice of a wrong packaging material or technique for a particular product can do it more harm than good. It should also be emphasized that modern packaging is also a part of marketing process. It should be suitably decorated on the exterior and properly labeled to attract and persuade the consumers to purchase the product.
In this competitive world of trade and service, main thrust of the manufacturers and business promoters has been to market cost efficient products having moderate durability. Growing demand for fast foods supported by the well-managed marketing channels has not been exception to this sector. To meet out such challenges, the sensory compatibility of the food products plays decisive role in successful launch and acceptability of eatables in the market. Virtually, sensory or organoleptic evaluation relates the physico-chemical properties of food to the behavioral responses depicted by the testing human. In other words, it deals with interdisciplinary approach between psychology and food science to investigate basic issues in the human perception of taste, smell and flavor.

If we go back to the history of sensory evaluation, in the decade of forties of last century, US Army Quartermaster Food Container Institute felt difficulty in contenting acceptability of food by army personnel irrespective of the quality or menu of the food served. Responding to such needs, flavor profile method was developed and initially, the help of expert brew-masters, tea tasters etc. was availed to serve tasty foods. Gradually, the specific sensory techniques were developed to record human perceptions for physicochemical properties of food. On November 1, 2000, Ohio State University Sensory Science Group was established in USA to advance and promote Sensory Science through research, education and application of knowledge for the benefit of consumers and ultimately the Food Industry.

To be leader in Science of Sensory Evaluation, one has to be committed to reliably fulfill commitments, meet deadlines and consistently produce high quality work without being influenced by the hypotheses or desired results. Learn and anticipate stakeholders’ needs, assist stakeholders by incorporating appropriate methods and techniques to conduct sensory analysis matching to the objectives particularly for those without expertise. So, the utility of sensory evaluation has mainly been for-

- Quality control of raw and finished products,
- Innovative approach in product development, and
- Devising formulation changes for improvement in product qualities.

However, the limitations of sensory analysis, appended below have also to be looked into very diligently-

- Schematic study of variables and interrelationship is complicated and needs lot of time and money,
- Preference and acceptance values are not static, rather dynamic
- Lot of assumptions are made and accepted about the relevance of perceived variables and their relationships purely on apparent face value.

Requirements of sensory evaluation

To organize the sensory evaluation programme, aimed at identification of problems, testing / adoption of new techniques and development of new skills, following aspects are necessary to be looked into:-
1. Management goals and objectives- Prior to conductance of a sensory evaluation (SE) programme, the manager should be given full freedom of movement to operate without interference and should be fully aware of the goals already set.

2. Adequate planning – Involves proper planning at the level of -
   (i) Experimenter – should have clear objectives, products and priorities.
   (ii) Organization- to be equipped with review requests and infrastructure for managing sensory evaluation programmes and necessary database.
   (iii) Implementation- involves conductance of SE programmes and analysis of interpretations.
   (iv) Recommendation- through authorized channel.

3. Standardize subject screening procedures- Judges called for sensory evaluation act as subjects or as Early Warning System for which certain guidelines are quite necessary-
   (i) Subjects should be encouraged to volunteer and take interest in learning sensory test procedures.
   (ii) Successful testing programme involves employees from all cadres and all parts of the institute.
   (iii) Subjects should be frequently involved so that the skill acquired is not forgotten.
   (iv) An individual should not participate more than 4-5 times in a week.
   (v) Subjects should be rewarded through appreciation (Not through payments) for their participation.
   (vi) Subject information should be treated as confidential.

4. Professional staff- Selection of professional staff/sensory panel individuals facilitates smooth operation of sensory evaluation. After acquiring willingness of the staff to cooperate for this programme, they should be screened through product aptitude survey (PAS) and monitoring of performance, for delineation of skills.

5. Pool of qualified subjects.

6. Test facilities- Include design and lay out of sensory booth. Floor space requirements of the building having- Sensory Reception, Sensory booths (6), Preparation, Holding and storage room, Panel discussion room and Experimenter's office with record/data processing facilities are recommended as 50, 100, 300, 350, 75 and 125 sq. ft. respectively.

7. Programme strategy-It is based on management of test issues, determination of test products, policy on working with problems, scales applied and market research.

8. Scope for online data processing.

Sensory techniques

Prior to use of sensory techniques, it is pertinent to ensure that the-
- Sensory panel judges understand the meaning of technical words being used,
- They should be vigilant to define their words they use to describe perceptions about the product.
Various descriptive sensory techniques could be classified as-

1. Qualitative methods viz. flavor profile method
2. Quantitative methods including Texture profile test and Quantitative descriptive analysis (QDA)

**Flavor profile method** - In this, taste experts viz. tea tasters, brew masters or perfume masters are involved for the relevant products. Selected judges (sensory subjects) are asked to taste the product and after their discussions, the panel leader summarizes the result in report form.

**Texture profile method** – This is a kind of descriptive analysis on the basis of structure of the food particles. In this method, behavioral issues are not considered. Texture profile means mechanical, geometrical, fat and moisture characteristics, the degree of their availability in the food and order in which they appear from first bite through complete mastication. The results are compared with the known materials and provide relationship with instrumental methods.

### Relationship between textural parameters and popular nomenclatures

#### Mechanical characteristics

<table>
<thead>
<tr>
<th>Primary parameters</th>
<th>Secondary parameters</th>
<th>Popular terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>-</td>
<td>Soft, firm, hard</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>Brittleness</td>
<td>Crumbly, crunchy, brittle</td>
</tr>
<tr>
<td>Viscosity</td>
<td>-</td>
<td>Thin, viscous</td>
</tr>
<tr>
<td>Elasticity</td>
<td>-</td>
<td>Plastic, elastic</td>
</tr>
<tr>
<td>Adhesiveness</td>
<td>-</td>
<td>Sticky, tacky,</td>
</tr>
</tbody>
</table>

#### Geometrical characteristics

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size and shape</td>
<td>Gritty, grainy, coarse</td>
</tr>
<tr>
<td>Particle shape and orientation</td>
<td>Fibrous, cellular, crystalline</td>
</tr>
</tbody>
</table>

#### Other characteristics

<table>
<thead>
<tr>
<th>Primary parameters</th>
<th>Secondary parameters</th>
<th>Popular terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>-</td>
<td>Dry, moist, watery, wet</td>
</tr>
<tr>
<td>Fat content</td>
<td>Oiliness, greasiness</td>
<td>Oily, greasy</td>
</tr>
</tbody>
</table>

For each parameter a scale of 5, 7 or 9 categories is developed. Limitations of this test embark upon proper training of the subjects so that the variability is minimized for each category. The difficulty of separating texture from other sensory properties is to be overcome.

**Quantitative descriptive analysis (QDA)**

Any method in which subjects provide responses to product attributes along a continuum that can be assigned numerical values may be considered as quantitative descriptive method. It is intended to record the competitive sensory properties of the products for their success in the market. It also helps in launch of new products. This test is based on following precautions-

1. Be responsive to all sensory characters
2. Multi product testing should be conducted
3. Use limited number (10 to 12) subjects
4. Perceptions are quantified through numbers
5. Involve data processing capabilities

The subjects who can not write their perceptions through numericals, QDA could be conducted through drawing a line scale measuring 6” with two ends bearing extreme characters. The subjects may pin point their perception on the line through a mark.

<table>
<thead>
<tr>
<th>Like very much</th>
<th>perception recorded</th>
<th>Dislike very much</th>
</tr>
</thead>
</table>

For each sensory character, such lines can be drawn and perceptions could be recorded accordingly. By joining the perception points a web would be formed. The relative intensity of results increases as it goes farther from the center point. Generally, wider the web, high rated is the product. This is practiced to attain visual display of the SE results.

Application of descriptive analysis

1. Market competition – in order to know competitive difference of the product this method helps in anticipating changes and identifying product weakness.
2. Storage testing – to evaluate storage quality changes in the product, the QDA eliminated need for control samples.
3. Product development- these tests indicate whether the product formulation matches to targets fixed. It helps in setting quality control specifications.

**Discrimination testing** - It based on the comparison of perceived differences of two different products.

Qualities of discrimination testing –
- a) Sensitive
- b) Subject must make a choice
- c) Normally performed prior to other testing
- d) Sample perceived different can be equally liked for different reasons
- e) Not necessary for all products

Components of testing are –
- Clear objectives,
- Test requests and report forms,
- Management of test- include product screening, subject selection criteria, selection of experimental designs, guidelines on test procedure, product code and amount of serving / timing and lighting etc., motivational efforts for subjects.
- Test procedures- are mentioned as under
1. **Paired comparison test** or two sample test or directional paired comparison test
The subjects are required to compare two products through discriminative preference for the better one.

Another way of paired comparison test is ‘A not A’ method. In this first sample is served prior to the serving of second sample. Subject decides whether it was same sample or different.

2. **Duo-trio test** – It is conducted for the products with relatively intense taste, odor and or kinesthetic effect i.e. low sensitivity. Here three samples are served. One sample acts as control or reference and the subjects are asked to differentiate one of the remaining two samples nearer to the reference. But difficulty with this test is that it requires more quantity of the product.

3. **Triangle test** – It is more commonly used. In this three samples are coded and subject are required to determine which two are the same and third one is different. But difficulty in this type of testing is that subject is compelled to recall the properties of the samples to make the authentic discrimination.

4. **Just noticeable difference (jnd)** – It is a method to identify the level of change that could be tolerated by the subjects. It is used for a particular variable.

5. **Constant stimulus method** is most commonly used to express the relationship of results. In this, series of paired comparison tests are conducted and subjects are asked to find the pair which is more intense. Equations for the line of best fit can be computed from proportional judgments.

- Data analysis and interpretation
- Magnitude and description of difference

Guidelines to minimize problems-
1. Test subjects should be selected on the basis of specific performance including their previous experience.
2. Replications are strongly recommended.
3. Avoid/ minimize product carriers, as far it is possible.
4. Results of tests should be expressed in probabilistic terms.

**Components of sensory measurement** - For proper use of components of sensory evaluation i.e. sensory scales, it is imperative to be sure of the test objectives and the type of information desired. Basically, four scales were postulated-

A. **Nominal scale** or Low order scale – are used to classify the respondents as per their age, sex, income, product use and behaviors.

**Advantages**-
- i. This offers basic information for which subjects generally do not have any difficulty in responding to the questions particularly when problem has large number of alternatives.
- ii. More responses can be obtained from large number of respondents.
- iii. Requires limited test time.
- iv. Offers rapid response with limited computation.
B. Ordinal scale

These are considered as ‘First or most basic’ scale. Here no assumptions are made about the product quality. The subjects may allocate direct rankings to the product or may do it through paired comparison or other discriminative tests. Ratings are done between two extreme characters viz. like very much and dislike very much.

Advantages-
   i. Rankings do not depend upon memory.
   ii. Method requires uncomplicated statistical assumptions.

Limitations
   i. There is limited use of rankings of product.
   ii. Sensory fatigue could be noticed due to lingering taste of the large number of samples.
   iii. There is no indication of the magnitude of difference between products.

C. Interval Scale

It is truly quantitative scale. Scale has interval or distance between points on the scale. Its advantage is that numerical data can be further processed and suitable statistical tests can be applied.

D. Ratio Scale

It is based on formulating ratio between stimulus concentration and perceived intensity e.g. ratio between salt level 2.5% and acceptability. It helps in comparing different products and establishing their goodness ratio. These are more frequently used in magnitude estimation.

E. Hedonic scale

It was developed to assess the acceptability of several hundred-food items. It is most commonly used in SE programmes. Its reliability is very good. However some researchers held the view that bipolarity of scale between subjects’ viewpoint and mathematical treatment are not directional.

F. Face scale

These are used particularly for infant foods where the subjects are unable to write their observations. Here facial expressions are tallied with the standard chart.

G. Just about right scale

This is also bipolar in nature. Here a cut off point/ level is fixed and products ranked below that are not generally considered as approved or fit for marketing.
Hurdle Technology for Shelf-Stable Meat Products

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Hurdles in foods are substances or processes inhibiting deteriorative changes. Hurdle technology (HT) is the use of two or more factors/hurdles, none of which is independently capable of sufficiently inhibiting the spoilage or pathogenic microbes to extend shelf life of foods. The HT concept was unconsciously used in many traditional foods especially in developing countries over centuries. A treasure of knowledge of food preservation methods which fulfill the requirement of traditional food has been accumulated consequently hurdle concept was evolved at Federal Centre for Meat Research, Kumlbach, Germany by Leistner and Rodel in 1976. Shelf stable products (SSP) are those food products which have been mildly heated (70-100°C core temp.) in a sealed container but sufficient to inactivate all bacteria but not sporulated by adjusting hurdles like aw, pH, Eh etc. and can be stored without refrigeration.

Importance of HT:
The microbial stability and safety of most foods is due to combined action of several preservative factors (hurdles), which should not be overcome by the microorganisms present. The hurdle effect is the basis for the prevention of microbial spoilage, food poisoning and to have the desired fermentation process (Leistner, 1994). Hurdle technology allows improvements in the safety and the nutritive as well as the economic properties (i.e. amount of water in a product compatible with its stability) of foods by an intelligent combination of hurdles. As the main objective is to inhibit the growth and proliferation of undesirable organisms, several hurdles are used minimally in optimum combination thereby contributing improvement in sensory qualities and stability (Leistner and Gorris, 1995) as well as saving of money and energy (Berwal, 1994). Products can be stored at ambient temperature. If the meat products are processed according to the outlined Hazard Analysis Critical Control Points (HACCP) concept, they are safe, stable and of high sensory qualities. This successful technology is also known as combined processes, combination preservation/techniques or barrier technology.

Hurdle effect and Homeostasis:
Hurdle concept illustrates the complex interactions of temperature, water activity (aw), pH, redox. potential (Eh), etc for the microbial stability of food. For every stable and safe food a certain set of hurdles is inherent, which differs in quality and intensity for the particular product, the hurdles must keep the normal microbial population in the food under control. The microorganisms present at the start in a food product should not be able to overcome (jump over) the hurdle present, otherwise the food will spoil or even cause food-poisoning. If the homeostasis of microorganisms, i.e. their internal equilibrium, is disturbed by hurdles in food, they will not multiply, remain in the lag-phase or even die, before their homeostasis is reestablished. Thus, food preservation is achieved by disturbing the homeostasis of microorganisms in food temporarily or permanently.

The different hurdles in a food have a additive effect on stability and could also act synergistically by hitting multi targets (cell membrane, DNA, enzyme systems, pH, aw, Eh) within the cell and disturb the homeostasis of the microorganisms. (Leistner, 1994). Thus it is more effective to use different preservatives/hurdles in a small amounts in a food than only one preservative in larger amounts.
Hurdles and quality of foods:

The important hurdles generally used in food preservation are high temperature (F value), low temperature (t value), water activity (aw), activity (pH), redox potential (Eh), preservatives (nitric, sorbate), packaging (vacuum, modified atmosphere), radiation (UV, microwave, irradiation), competitive microorganisms (Lactic acid bacteria) and various other preservatives etc. Any of them may not commonly applied for the same food product. Some hurdles (Maillard reaction products- melanoidins) affect the safety as well as the quality of foods because they have antimicrobial properties and at the same time improve the flavour of the food. Certain hurdles in meat might influence the stability, sensory, nutritive, technological and economic properties of a product. (e.g. nitrites, polyphosphate). Some hurdles could have a positive or negative effect for securing the desired total quality of foods depending on its intensity like the pH of fermented sausages, which should be low enough to inhibit pathogenic bacteria, but not so low as to impair taste the safety and quality of hurdles should be kept in the optimal range in order to secure the total quality of a food (Leistner 1994).

Application of Hurdles:

The most important hurdles used for safety and stability of meat products are classified as physical, physico-chemical and microbially derived hurdles (Bogh-Sorenson, 1994).

I. Heat Processing: In addition to pure cooking and changing food, the main object of heat processing of foods is to inactivate microorganisms and/or enzymes. It is also essential to protect the food product against recontamination by means of hermetically sealed containers/packages. Three types of heat processing techniques such as sterilization, pasteurization and blanching are commonly used in food manufacture.

A. Sterilization: The food is heated to a temperature of 100°C or above at the coldest point to destroy bacterial spores in order to make the canned foods shelf stable at least for one year. The sterilization process is evaluated by means of the F value, defined as the period of exposure, in minutes at 121.1°C, which will have a sterilizing effect equivalent to that of the process. The symbol F0 is used when the sterilizing effect is calculated based on 2=10°C.

For low acid food (pH>4.5), F0 should be at least 2.5 to secure a reduction of Cl. botulinum spores by a factor 10^{12} or more. It is also known as botulinum cook or 12D cook. When ingredients in food have an inhibitory effect, such as salt and sodium nitrite in canned cured meat, lower F values will be sufficient. Higher F0 values may be necessary to ensure stability in low acid foods.

B. Pasteurization: The food is normally heated to a core temperature of 65-75°C depending on food product in order to inactivate nearly all enzymes and vegetative microorganisms but bacterial spores will survive. Packaging in hermetically sealed containers is necessary to protect against recontamination after pasteurization process. It is always combined with other hurdles especially chilling.

C. Blanching: It is heat processing at 70-100°C, mainly used for inactivation of enzymes in fruits and vegetables before drying or freezing.

2. Storage temperature: Normally, chill storage temperature for perishable foods i.e. meat, fish and poultry and dairy products is between -1°C and maximally 7°C. Chilling is the only preservation method/hurdle normally used for several perishable foods. Shelf-life is enhanced by lowering storage temperature. Chilling is used in combination with packaging, pasteurizing, curing etc.

Freezing of meat involves lowering the temperature to and storage at -18°C or below temperature. There is no microbial growth normally below -8°C. Quality degradation processes, often caused by enzymes can take place down to -30°C or even colder (Bogh-Sorenson, 1994).
Freezing is often used as the only hurdle, but in most cases freezing is combined with packaging, blanching etc.

3. **Irradiation:** Ionizing radiation (β radiation and γ radiation) is characterized by a very high energy content, which can destroy microorganisms, depending on dose. Ionizing radiation with doses of about 5 kGy is sufficient to kill or inactivate most pathogenic and spoilage organisms. Thus enhancing the safety and stability of foods. Irradiation of spices with doses about 10 kGy, can significantly reduce the bacterial number in spices; The irradiation of spices and few other foods is permitted in most of the countries. The advantage is that no (very little) heat is involved in destruction of microorganisms, so the product has the characteristics of fresh food stuff after irradiation. The disadvantages are that off- flavours and off-taste occur in some irradiated foods and most consumers are skeptical of this method. A maximum dose of about 10 kGy ionizing radiation is prescribed. It must be combined with packaging to prevent recontamination and chilling to increase safety and stability of food.

4. **Microwave energy:** It is used to heat food products fast by internal heating resulting from molecular friction between vibrating components (polar molecules in foods often water) excited by the absorption of the microwave energy. It is employed for pasteurization, drying, thawing and blanching but not for sterilization in food industry. The main problem in microwave cooking is the non-uniform heat distribution throughout a food product resulting hot and cold spots due to which localized survival of bacteria may occur. Microwave pasteurization or blanching is used in combination with packaging and chilling or freezing.

5. **Packaging:** It is essential to preserve the quality and protect against damage during storage, distribution and marketing. Packaging acts as a barrier to prevent entry of microbes, insects, dirt etc. transfer/passage of water vapour, gases and aroma. The selection of optimal packaging materials and method depend on the food product.

A. **Vacuum packaging:** The packages are evacuated and sealed leaving a very low amount of air, especially oxygen (O₂) in contact with the product. The carbon dioxide (CO₂) concentration in the package increases considerably in may cases which slows down many processes and also affect the type of microorganisms that are able to grow on the food. A max temperature of 3.3°C is advisable for vacuum packed chilled foods, especially for pasteurized products since Clbotulinum and some other pathogen grow well in the absence of O₂.

B. **Modified atmosphere packaging (MAP):** In the package an atmosphere with a gas composition different from that of atmospheric air is created. The volume of products is about the same as the volume of air in the package which must have very low permeability to O₂, CO₂ and other gases. In MAP with high CO₂ concentration, the storage temperature should be kept low (<5°C), as the effect of CO₂ increases with lower temperature. More than 21% O₂ is advised for fresh meat, beef and lean fish.

C. **Controlled atmosphere (CA) storage:** The products are stored in airtight chilled storage rooms, wherein a modified atmosphere is created, continuously controlled and regulated. CA can be maintained in the containers during transport. It is used in chill storage of fruits and vegetables, especially of apples and pears. The composition of the atmosphere in the storage room is kept constant, eg. about 3% O₂ and 3% CO₂ which slows down most quality degrading processes.

D. **Aseptic packaging:** Food after heat processing are transferred to sterile and hermetically sealed containers under aseptic conditions in a clean room so that no re-contamination takes place. It is practiced for liquid products such as UHT milk, fruit juices. Aseptic packaging is a combination of heat processing and packaging and may also be combined with chilling.

6. **Water activity (a_w):** It is the ratio of water vapour pressure of the food to that of pure water at the same temperature. Water activity affects the growth, resistance and survival of microorganisms and the reaction rate of most quality degrading processes. Bacteria are less
tolerant to a reduced $a_w$ than yeasts and moulds. $a_w$ may be decreased by dehydration, or by addition of solutes such as salt, sugar etc and by lowering of temperature. To make the food shelf stable $a_w$ should be about 0.6 or lower. Very few microbes and no pathogens grow at $a_w$ less than 0.7. In dry/dried foods $a_w$ may be the only hurdle. A packaging acting as a barrier against vapour is necessary. It is often combined with chilling.

7. **pH:** The use of pH decrease or an increase of acidity of food has enhanced microbial stability. This has been done naturally by fermentation or by the addition of acidulants (weak acids). Most-microorganisms do not grow below on specified minimum pH, the limit being 4.6 for Cl botulinum. pH is often combined with packaging, additives such as NaCl, organic acids, chilling or heating.

8. **Redox potential (Eh):** Eh (oxidation-reduction potential) indicates the oxidizing or reducing potential of food system and is expressed in mV. (in the range of +300 to 200 mV) and measured with potentiometer. The Eh of food is influenced by the removal of air (oxygen), the exclusion of light, addition of reducing substances (ascorbic acid, sucrose etc.), presence of nitrite, the temperature, pH and growth of bacteria. The Eh determines whether aerobic or anaerobic microorganisms will grow in a food and it influences considerably the color and flavour of the food. It is used in combination with curing, chilling, packaging.

9. **Salt:** Most consumers prefer foods with a less salty taste today. It reduces the $a_w$ and has some bacteriostatic effect. A stable product must contain at least 27g salt per 100g water ($a_w<0.7$). Curing is often combined with chilling, smoking, packaging.

10. **Sodium Nitrite (NaNo2):** Nitrite imparts cured meat products a pink colour, improves flavour and prevent WOF. Nitrite at the permissible level used commercially inhibits the growth of many microbes, especially spore forming bacteria (clostridia). The effect of nitrite is greater when it is heated together with meat, a specific anti-botulinal compound (the perigo-factor) seems to be formed.

11. **Phosphates:** Pyrophosphates or tripolyphosphates are extensively used in processing of most meat products. They improve the functional properties of meat and enhance the yield and sensory attributes of the product. The have antioxidant effect.

12. **Ascorbates:** Sodium ascorbates or isoascorbates may increase the anti-clostridial effect of nitrite in canned cured meats. They stabilize the colour and chelate pro-oxidants or act as synergist in the presence of other anti-oxidants. Ascorbic acid is also used to lower the pH.

13. **Glucono δ-Lactone (GDL):** It is used in cured meat products. GDL slowly hydrolysis to gluconic acid and lower the pH and thus contributes to safety and stability.

**Smoking:** Presently it is primarily used to impart flavour and colour to many cured and fish products. Smoking process inhibits the microbial growth and rancidity since natural smoke contained a variety of organic compounds, especially phenolic compounds. Use of liquid smoke has no preservative effect. It is always combined with curing, packaging, chilling.

Other important hurdles include phenolic antioxidants (BHA, BHT, TBHQ); organic acids (lactic acid, lactate; acetic acid, acetate), chelators (citrates, lactates, pyrophosphates, EDTA); humectants (ethanol, propylene glycol), spices, and microbially derived hurdles (starter cultures, bacteriocins, antibiotics), etc.

**Some shelf-stable Meat products**

1. **Canned cured meat products**

   This category includes hams, shoulders, luncheon meat, chopped pork containing about 2.5% NaCl. Heat treatment (F value) is the important hurdle for safety and stability of these shelf stable products (F.SSP). NaCl, NaNa2, phosphates and often ascorbate are added during manufacture. The product after packaging in hermetically sealed cans/ retort pouches is heat
processed to a F-value a little below 1. The mild heat treatment (max-core temp about \(104^\circ C\)) is given to prevent the deterioration of sensory properties with that of severe heat processing. Heat processing to F-value less than 1 is not sufficient to kill Cl. botulinum spores, as it is well established that it requires a F-value of at least 2.5. The safety of such products is demonstrated by the fact that millions of cans have been produced without food poisoning caused by Cl botulinum surviving the heat process. The safety is based on a combination of three factors: heat treatment, inhibition and low bacterial count. (Bogh-Sorensen, 1994).

2. Keep refrigerated canned cured meat:

The process is similar to that used for shelf stable canned cured meat, but keep refrigerated products are pasteurized at 72-75\(^\circ\)C in order to get a core temp. of 70\(^\circ\)C or above. The heat process kills most vegetative bacteria, but does not kill spores so the products be kept refrigerated at 5\(^\circ\)C or below. The safety of these products depends on the storage temperature, heat treatment and hermetically sealed container, as well as inhibitory effect of NaCl, NaNO\(_2\), pH etc.

3. Sliced cured Meat products:

Ham, bologna type sausages etc are sliced and sold from chilled cabinets (max 5\(^\circ\)C) in supermarkets: Normally, vegetative bacteria are not present before slicing. The colour of heat processed cured meats fades if oxygen is present. Hence, these products are often vacuum packed (in PA/PE laminates or MAP is used with 50% O\(_2\) and 50% N\(_2\)). The safety of such products is due to storage temperature, as recontamination often occurs during slicing process. As pathogenic bacteria (e.g. salmonella and L.monocytogenes) could be introduced during slicing and as some of them can grow at 1\(^\circ\)C or below, so other hurdles such as NaCl and NaNO\(_2\) acts as inhibitors to ensure the safety and stability of products. Use of starter culture or GDL for lowering pH is also beneficial. Hence large number of hurdles are involved in ensuring the safety and stability of these products: heat treatment (pasteurization), good hygiene during slicing and packaging, vacuum packaging or MAP, NaCl (aw), NaNO\(_2\), pH, chill storage and smoking is used for some meat products. (Bogh-Sorensen, 1994).

4. Goat meat Keema:

The traditional goat meat ‘Keema’ using hurdle technology for extending the shelf life was standardized. (Karthikeyan et al., 2000). Minced goat meat blended with humectants, preservatives, calculated amount of lactic acid and spices was fried to produce keema with a water activity of 0.90 (aw). Excess oil decanted and the fried product was vacuum packed in nylon-cpp pouches and pressure cooked for 7 min. The hurdle treated keema had shelf life of 3 days (Control 1 day) at 35±1\(^\circ\)C and 18 days (Control 6 days) at refrigerated storage (4±1\(^\circ\)C). The hurdle treated keema had comparable sensory attributes with that of control. The hurdles include, heat treatment, pH, aw, preservatives, vacuum package, chilling.

Other meat products:

Several traditional intermediate-moisture meats and dehydrated meats (Huang and Nip 2001), shelf stable meat products (Leistner, 1994b, 1999), buffalo meat chunks (Malik, 1999) and mutton curry (Himanish and Radha Krishna, 2001) meat and chicken pickles etc. were developed by application of hurdle technology. It offers vast potential for manufacture of novel and modified products with safety, shelf stability and better sensory attributes for the benefit of consumers.
Guidelines for making a shelf stable food products using hurdle technology, predictive microbiology and HACCP

1. Define the sensory attributes and expected shelf life the novel or modified food products.
   → Plan a processing technology to be followed
   → Manufacture the food product
   → Analyse the $a_w$, pH, preservative (levels), processing temperature, storage temperature, shelf life etc.

2. Predictive microbiology should be used for testing preliminary stability
   → Challenge test the food product with food poisoning and spoilage microorganisms
      (use higher/doses of inoculum and storage temperatures than normal so as to take care of abusive conditions)

3. If necessary, as indicated by the finding of the challenge test, the hurdles be modified (increased/decreased, added or deleted) but the sensory quality should not be compromised.
   → Modified food product is again challenge tested
   → If necessary hurdles are modified-again
   → Predictive microbiology is done to assess the safety of the food
   → Established hurdles of the modified or novel food are exactly defined, including tolerances
      → Pilot plant studies carried out
      → Scale up process must be validated
      → For the industrial process the critical control points (CCPs) and their monitoring be established
      → Manufacturing process should be controlled by HACCP

The success lies in the combination of the processes by a team work of experts

Source: Leistner (1999) and Berwal (2000)

References on request

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Biofilm: Significance, Formation And Control in Poultry Processing Plant

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Biofilms, or in more conventional terms biofouling and slime layers, are matrix-enclosed bacterial populations (viable and non-viable) adherent to each other and/or to surfaces or interfaces. The microorganisms are embedded in polyanionic extracellular polymeric substances. Extracellular polymeric substances (EPS) contain polysaccharides, proteins, phospholipids, teichoic and nucleic acids, and other polymeric substances hydrated to 85 to 90% water. EPS provide protection to the biofilm inhabitants by concentrating nutrients, preventing access of biocides, sequestering metals and toxins and preventing desiccation. Poultry processing biofilm have a high food and mineral contents that originate with product and process water. These constituents also provide protection to microorganisms held within biofilm. The biofilm community exhibits primitive homeostasis, a primitive circulatory system, genetic material exchange, and metabolic co-operation. Biofilms allow the organisms to act synergistically and provide an area for reproduction.

Biofilms have been around years, but are increasingly in the lime light because of better detection methods, the demand for longer shelf-life of the product/s and longer production runs. Biofilms may be beneficial or harmful, depending upon where they are found. In the production of some fermented foods, biofilms are an essential element for optimum production. As it is seen in the production of vinegar, acetic acid bacteria are allowed to grow on wood chips and the biofilm that is formed helps in conversion of substrate to acid more efficiently. The greatest concern is that biofilm contributes to the production of contaminated products-products with shortened shelf-life and may contain microorganisms of public health significance. Other problems that biofilm can create in poultry processing plant include impedance of heat transfer, fouling of probes, mechanical blockage in fluid handling system and plugging of filters and strainers resulting in higher running cost of processing operations.

Biofilm in processing plant can have a high level of organization, as they can exist in single or multiple species communities, form a single layer or 3-dimensional structure, or take the form of aggregates such as flocks or granules. A normal biofilm commonly function through collective behaviour and coordinated activity, which assists survival of constituents cells in stress environments. Environmental stress such as low nutrient availability trigger phenotypic changes of planktonic (free living) cells to the sessile (attached) form. Biofilms can exist on all types of surfaces in food processing plants ranging from plastic, glass, metal, wood and food products. They can also appear on conveyor system, grinders, slicers or any surface in continuous touch with production process. Among the major spoilage organisms which can form biofilm are *Pseudomonas fragi*, *P. fluorescens*, *Bacillus* sp. and *Enterococcus* sp. During production such organisms can be sloughed off and can contaminate the food and accelerate spoilage. Harmful bacteria such as, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, *E. coli* 0157: H 7 and *Campylobacter jejuni* can also form biofilms. These pathogens can also be entrapped inside biofilms formed by spoilage bacteria and can contaminate the product.

Biofilm formation: Biofilm formation consists of initial attachment, microcolony formation and EPS production, followed by maturation.
Attachment of microorganisms: The first step in biofilm formation involves the attachment of organisms to the surface. Before attachment of organisms to the surface, transport of nutrients, inorganic and organic material to the surface and adsorption of conditioning film containing nutrients (organic and inorganic) is essential. Adhesion of microorganisms to the substratum can be active or passive depending on cell motility. Passive attachment of microorganisms is driven by gravity, diffusion and fluid dynamics. This initial attachment is very weak and at this point of time in the life of biofilm, removal is very easy. The infant film consists of organic film and the weakly binded bacteria. In active adhesion, the bacterial cell surface properties facilitates initial attachment. Cell surface properties such as flagella, pili, adhesion protein, capsules, and surface charge influence attachment. Flagella allow bacteria to move to a specific attachment site. Synthesis of EPS and cell aggregates also affect attachment.

Attachment often occurs within 5 to 30 seconds and can be classified as reversible and irreversible. Reversible attachment involves electrostatic forces and hydrophobic interactions while irreversible attachment results from the anchoring of appendages and/or the production of extracellular polymers. Repulsive forces usually prevent direct bacterial contact with the substratum (often both substratum and bacterial cells are negatively charged). Bonding between bacterial appendages (pili, flagella, adhesion protein) and the substratum involve short range forces such as hydrogen bonds, hydrophobic and ionic covalent binding. Irreversible attachment takes 20 min to a maximum of 4 hr at 4 to 20 °C and removal of cells is difficult and requires application of strong shear force (scrubbing or scraping) or chemical breaking of the attachment forces through application of enzymes, detergents, surfactants, sanitizers and or heat. Adhesion is affected by chemical and physical properties of the cell and contact surfaces and composition of surrounding medium. Adherent properties of the cell are influenced by the cell envelope whose chemistry changes in response to environmental stimuli and quorum sensing. Irreversible attachment is a physiological stimuli under genetic regulation. Genes responsible for surface protein, attachment and EPS production are activated in response to external stimuli such as population density, stress or nutrient limitation.

Properties of food contact surfaces: Maximum attachment of bacterial cells depends upon high free surface energy or wettability of surface. Surfaces with high free surface energy, such as stainless and glass are more hydrophilic. These surfaces generally allow greater bacterial attachment and biofilm formation than hydrophobic surfaces such as Teflon, nylon, rubber and fluorinated polymers. Hydrophilic region of the contact surface provide more free surface energy as compared to hydrophobic surface and that is why most bacterial adhesions have been found at the hydrophilic region than hydrophobic region at the hydrophobic-hydrophilic interface of a stainless steel surface. It has been seen that cleaning stainless conditions the surface temporarily changes its properties. Cleaning with alkali or strong acid caused the surface to be hydrophilic, while cleaning with weak acid produced a hydrophobic effect. Once stainless steel is exposed to air or water, it is passivated by forming a chromium-oxide layer. Organic soil adheres to the oxide layer, producing a conditioned substratum to which bacteria adhere. In most cases, bacteria attach to hydrophilic than hydrophobic surfaces. Substrate type also influences the attachment pattern. Bacteria tend to attach to a glass (a hydrophilic surface) uniformly in a monolayer, while on hydrophobic surface such as nylon and tin, they tend to adhere in clamps. Contact time between the cell and the substratum is required for irreversible attachment and in some strains of Listeria monocytogenes which are found in food processing plants require a short contact time for attachment.
Adhesive properties of bacterial cell surface: Adhesion of bacterial cells is influenced by the physico-chemical properties of cell’s surface, which in turn are influenced by factors such as microbial growth rate, growth medium and culture conditions (time and temperature). Bacteria usually have a net negative surface charge and usually behaves as hydrophobic particles, but the degree of hydrophobicity can change with growth phase. Cell surface hydrophobicity is based on compounds associated with outer membrane including lipopolysaccharides, lipoproteins, lipoteichoic acid, and lipomannan. Orientation of these compounds on the outer membrane determines cell surface hydrophobicity. Most gram negative bacteria have long polysaccharides regions of their lipopolysaccharides exposed, resulting in a hydrophobic structure while gram positive bacteria have the lipid portion of lipoteichoic acid extending outward from the cell, resulting in a hydrophobic surface. EPS excretion also affects hydrophobicity before or after attachment. EPS excretion before attachment can lead to enhance or inhibit the attachment process depending upon the hydrophobicity of the cell surface after excretion. Emulsion secreted by Acetobacter calcaceticus, changes hydrophobic surfaces to hydrophilic resulting in inhibitory effect on hydrophobic cell attachment. Attachment of bacteria to a surface is also affected by nutrient availability, nutrient concentration, pH, temperature, electrolyte concentration and the flow of the material.

Microcolony formation: This will proceed after irreversible is given appropriate growth conditions. Microcolony formation results from simultaneous aggregation and growth of microorganisms and is accompanied by the production of EPS. Aggregation involve recruitment of planktonic cells from the surrounding medium as a result of cell- to-cell communication (quorum sensing). The production of EPS occurs through quorum sensing such as the case in P. aeruginosa where alg C gene is transcribed upon attachment, which results in down regulation of flagellum synthesis and up-regulation of alg T gene for the synthesis of alginate, the major compound of EPS. Then there is production of acrylhomoserine lactones and other quorum sensing molecules regulate the formation of biofilm by P. aeruginosa. In other microrganisms, adhesion and biofilm formation are under distinctly different genetic regulation. P. aeruginosa, E. coli and V. cholerae loose their flagella and increase their EPS production upon attachment to a surface. Although EPS is also produced in response to attachment and environmental stimuli such as osmotic pressure, pH, temperature, and starvation. P. fragi only adhered to stainless steel under starvation conditions and produce EPS only to anchor itself to the surface. EPS production does not always occur immediately after attachment and in most of the gram negative bacteria it is initiated 5 to 6 hr after attachment, however, attachment EPS can also be produced by planktonic cells resulting in enhanced attachment.

Maturation of biofilm: If conditions are suitable for sufficient growth and agglomeration, biofilm can develop in an organized structure. The mature biofilm consists of a single layer of cells in porous extracellular polymers or multilayered loosely packed microcolonies held together with EPS and interspersed with water channels. Biofilm increase in size over time but not all constituents in biofilm actively grow. Biofilm can vary in thickness from monocell layer to several centimeters thick depending upon biofilm production and growth conditions. Detachment from the surface can benefit the bacteria since they can move on to a new growth niche and establish a new biofilm. Factors affecting biofilm detachment include the biofilm thickness, fluid shear stress, nutrient availability and fluid velocity.

Stress response of cells in biofilm phase: In food processing environments, bacteria both in biofilm and suspended forms encounter stress such as dehydration, high heat, low temperature, and antimicrobial agents. Biofilm bacteria can be physically and morphologically different from their planktonic counterparts. Biofilm bacteria have been found 500 times more resistant to antimicrobial agents. Campylobacter jejuni, a thermophilic enteric pathogen associated with
poultry and biofilm is a good source of \textit{C. jejuni} in poultry house water systems since they can protect constituent microorganisms from environmental stress. Treatment with quaternary ammonium compounds, peracetic acid and mixture of peracetic acid and peroctonoic acid could not eliminate \textit{C. jejuni} embedded in biofilm after treatment at 50 and 200 ppm for 45 seconds. Biofilm bacteria often have a slow growth rate. This slow growth rate is not associated with nutrient limitation but with a general stress response initiated by biofilm growth. High cell density induces the production of rpo S protein which is usually expressed only in stationary state of laboratory growth cells of various organisms. \textit{E. coli} cells that lack rpo S protein are unable to form a normal biofilm.

\textbf{Advantages of biofilm growth mode:} Advantages of biofilm growth mode are i) Protection of microorganisms from antimicrobial agents ii) Increased availability of nutrients for growth iii) Increased binding of water molecules, reducing the possibility of dehydration iv) Proximity of progeny and other bacteria, facilitating plasmid transfer.

i) Protection from antimicrobial agents: Biofilm formation protect the biofilm cells from many antimicrobial agents, including antibiotics and biocides. Some of the factors such as age of biofilm, bacterial encapsulation and previous growth conditions (growth medium and temperature) affect the resistance of biofilm to chlorination. EPS is thought to act as a physical barrier to penetrate the biofilm. Antibiotics cannot penetrate effectively to reach biofilm cells that are packed in clumps and microcolonies.

ii) Availability of nutrients: Nutrient availability especially in drinking water system remain low but nature of EPS matrix allow it to serve as an ion exchange column concentrating nutrients from the surrounding fluid.

iii) Increased binding of water molecules: Some times when processing plants are not in operation and water availability is limited at that time, EPS helps in maintaining water availability for bacterial cells. EPS binds water molecules within biofilm and, thus, is highly hydrated. This reduces the effect of desiccation to which the planktonic cells are subjected. \textit{C. jejuni} is the best example as it is being sensitive to dryness which can survive being present in biofilm during dry season.

iv) Proximity to progeny and other bacteria: Biofilms consists of mixed species of microorganisms and exchange of metabolites among species can occur due to this proximity. Proximity of cells with other species is useful in removal of toxins. This was seen while studying the penetration of hydrogen peroxide in wild type \textit{Pseudomonas aeruginosa} strains. Hydrogen peroxide was able to penetrate and partially kill \textit{P. aeruginosa} strain in biofilm formed by \textit{P. aeruginosa}. This means that presence of one species in biofilm can protect other species by decomposing hydrogen peroxide. Plasmid transfer in biofilm can occur at a high rate due to the close proximity of cells. This is beneficial to the cells since the transferred DNA may carry useful capabilities that enhance survival. The close proximity of cells results in a possibility of inter species and intra species transfer of some biocide markers conferring resistance to the otherwise sensitive strains of contaminated bacteria within biofilms.

\textbf{Biofilm removal and control:} Removal of biofilms are difficult with use of normal cleaning in place (CIP) operation. Good prevention of biofilm formation is a function of implementing several measures. Among these nutrition, water limitation, equipment design and temperature control are important in biofilm control. But it is not so easy to reduce water availability, improve equipment design or reduce operating temperature and therefore, biofilm control efforts must often focus an effective cleaning of potential growth sites. Cleaning operations should effectively remove food debris and other soils that may contain microorganisms or promote microbial growth. Most cleaning operations include removal of loose soil with cold or warm
water followed by application of chemical agents, ringing and sanitation. Cleaning can be accompanied by using chemicals or combination of chemical or physical force (water turbulence or scrubbing). High temperature can reduce the need for physical force. Chemical cleaners suspend and dissolve food residues by decreasing surface tension, emulsifying fats, and peptizing proteins.

**Cleaning:** Most important part of sanitation program is cleaning as it prepares the surface for sanitizing. If the surface is not cleaned properly then it will provide an excellent environment on which biofilm can more easily form. Secondly, application of sanitizer to dirty surface is both ineffective and waste of money.

Most chemical cleaning agents used in the food processing industry are alkali compounds that act detergents for fats and proteins. They can be used in combination with chelators and anionic wetting agents. Many situations require the occasional use of acid cleaners to clean the surface soiled with precipitated minerals or having high food/mineral contents. Nonionizing wetting agents are also used in some formulations since they are good emulsifiers and control foaming. Chlorine compounds are added to alkali help peptize proteins. The cleaning process can remove 90% or more of microorganisms associated with the surface, but cannot be relied to kill them. Bacteria can deposit at other locations and given time, water and nutrients can form a biofilm and therefore, sanitization in addition to cleaning must be implemented. In poultry processing plant food contact surfaces are cleaned and sanitized daily as infrequent cleaning provides the opportunity for biofilm formation if moisture is present. An effective cleaning procedure must break up or dissolve the EPS matrix associated with the biofilm so that sanitizing agents can gain access to the viable cells.

**Sanitizing:** Sanitizer application is essential to inactivate microorganisms on the surface after cleaning. The major sanitizer used in the food industry are halogens, peroxygens, acids and quaternary ammonium compounds. Effectiveness of chemical sanitizer is influenced by the presence of soil, water hardness, temperature of application and ability to physically contact the surviving microorganisms. Chlorine is commonly used as a sanitizer due to its oxidizing and disinfecting power. Chlorine and anionic sanitizers were better able to remove *Listeria* and *Salmonella* EPS material from stainless steel than quaternary ammonia compounds and iodine, however, chlorine is usually inactivated by organic material leaving to its reduced efficacy. Chlorine dioxide and chloramines are also used as sanitizers in the food industry. Monochloramines was better able to penetrate bacterial biofilms than chlorine but required longer contact time. Quaternary ammonia compounds are also good surfactant sanitizers and are applied as foam, which provides longer contact times on surfaces such as pipes, walls and ceilings than does water application. They are effective against gram positive and gram negative bacteria, molds and yeasts. It is non corrosive, non irritating and its activity is not affected by organic load.

**Equipment design:** Use of well designed and properly installed processing equipment is also a good biofilm prevention measure. Poorly designed or installed system can be difficult to clean for a number of reasons. Ideally, equipments should be designed to prevent the accumulation of soil and allow for easy cleaning so that biofilm may not develop. Cleaning problems often occur at dead ends and where gaskets must be used, such as pumps and joints. Instituting best precisions based on principles of sanitary equipment and facility design is one of the most effective ways to prevent biofilm from taking hold on equipment surface. Greater understanding of the attachment process and life in the biofilm can produce other means of control. The product surfaces in poultry processing plant can be modified to resist material attachment. When bacteria
attach to a surface, gene expression changes significantly. If we can interfere with the signaling mechanisms between biofilm cells, we may be able to cause them to detach and flushed out of the plant.

**Summary:** The presence of biofilm is common in food processing plant including poultry processing plant. Biofilms harbor and protect pathogens and spoilage organisms. Moreover, microbial biofilm on surfaces result in equipment damage, product contamination, energy losses and other infections. It is believed that biofilm formation is part of survival strategies of microorganisms in adverse environments. Advantages of biofilm growth mode are protection of microorganisms from antimicrobial agents, increased availability of nutrients and plasmid transfer, and increased binding of water molecules, therefore reducing the possibility of dehydration. Food-borne pathogens and spoilage organisms can attach to and produce on many food contact and environmental surfaces. Pathogenic bacteria can be co-exist in a biofilm with other environmental microflora. Biofilm are difficult to remove from food processing surfaces and environments due to production of EPS matrix and the difficulties associated with cleaning complex processing equipment and processing environments. Therefore, biofilm control relies on the implementation of effective cleaning and sanitizing procedures and design of processing equipments and food processing environments that allow easy soil removal.
Recent Advances in Bio-and Phyto-Preservation of Meat

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There is growing quality consciousness among consumers for foods with high nutritional value, free from chemical preservatives and microbiological safety. Meat is an ideal medium for many bacteria to grow because it is high in moisture, rich in nitrogenous compounds (essential amino acids, proteins), a good source of minerals, vitamins and other growth factors. Furthermore, its pH is favorable for the growth of most microorganisms. In general, for controlling microbial growth in food, one needs to consider hydrogen ion concentration, water activity (aw), oxidation-reduction potential (Eh) and efficacy of antimicrobial ingredients. Combined with the effect of storage temperature, these are collectively termed as "hurdles" which can be applied for the improved antimicrobial activity in food. Some of antimicrobial agents such as desirable microbes and/or their metabolites, certain antimicrobial constituents of other foods and extracts or essential oils of commonly used spices can be used in combination with other hurdles in overall strategy to eliminate growth of microbes in foods.

Microorganisms (bacteria, virus, molds, yeasts) are ubiquitous in nature, some are having beneficial role while others are harmful or undesirable causing food to spoil and, thus, limiting shelf-life and a few of them are called pathogens which can cause food-borne illness. The pathogenic and spoilage microorganisms can be introduced into the food chain at any point of production or may be from contamination during processing and storage of foods. Therefore, efforts are needed to effectively control the growth of both pathogenic and spoilage microorganisms in the poultry meat for extended storage life with improved food safety.

The main effect of any food preservative is to restrict microbial activity, enzymatic, chemical and physical reactions that cause deterioration and spoilage of food. Food preservation works by lowering the amount of substances in the food that pathogen/microbes prefer to grow on. The best way to preserve food is to either lower the water content by thermal treatment or lower the pH of the food or both. In addition, other methods of food preservation include storage of food at lower (refrigeration or freezing) temperature, irradiation or use of chemical preservatives including organic acids and humectants to lower aw. Chemical preservatives, however, are not preferred now a days due to their residual effect and declining consumer preference. Therefore, more and more emphasis is being laid on bio and phyto-preservatives i.e. use of natural preservatives either alone or in combination with other methods. The natural preservatives can be categorized into i) desirable microbes and/or their metabolites (nisin, natamycin and fermentate (fungal product) ii) certain antimicrobial constituents of other foods (lysozyme, phosvitin, cystatin and lactoferrin and iii) plant derived products (herbs and spices).

Desirable microbes and/or their metabolites:

Some microorganism (bacteria or fungi) produce proteins or protein based compounds/metabolites which possess antimicrobial properties against other microorganisms. The most important example is the lactic acid bacteria which acts as antimicrobial agents by i) lowering the pH of the substrate at a level where most bacteria can not grow ii) excreting a variety of inhibitory substances other than lactic and acetic acid such as ethanol, hydrogen peroxide, diacetyl, free acetic acids, benzoate, antibiotics and bacteriocins iii) consuming the resources that pathogen needs to survive. Nisin, a peptide produced from Streptococcus lactis and is most likely produced in many fermented milk products, inhibits Gram-positive spores by
increasing their heat sensitivity. It can be purified and can be used for increasing shelf-life of many dairy and meat products. This has also been found to inhibit *Clostridium botulinum* growth in pasteurized cheese spreads. Lactic acid bacteria as live cultures can be used for the preservation of meat and other food products as they produce lactic acid which lowers the pH of food substrate at which most of the spoilage bacteria generally do not grow. Further, studies are needed for exploring use of nisin or lactic acid bacteria in food preservation specially in combination with essential oils of other phyto-products. Bifidobacteria have also found to be very good antimicrobial and it inhibits growth of pathogenic bacteria by producing acids and other antimicrobial compounds such as bacteriocins.

Natamycin (pimaricin), an antifungal agent is produced by *Streptomycyes natalensis* and this has been found effective against yeast and molds and can be used for preservation of various foods and the surface treatment of cheese Another food preservative from microbial origin is from fungus *Monascus purpureus*. The red mold species of this fungus can be cultivated on starch containing substrates such as rice and produces a product called fermentate. The main application of fermentate is its use as food additive in particular to meat as preservative and *Monascus* extract containing meat products were generally classified as better tasting than comparable products without *Monascus*. This relishing effect of *Monascus* could be by a flavour enhancing oligopeptides produced by a partial hydrolysis of rice proteins by *Monascus* enzymes. The antimicrobial effect of fermentate has been found against *Bacillus*, *Streptococcus* and *Pseudomonas*. Pigments from *Monascus purpureus* has also been found to have bacteriostatic effect against *Bacillus subtilis* and *Staphylococcus aureus* if used in low concentration. More research is required to explore the role of pigments from this fungus for food preservation especially replacing nitrite as food preservative.

**Antimicrobial constituents of other foods:**

Antimicrobial constituents present in some of the foods can be used as food preservatives such as lysozyme, cystatin and phosvitin from egg and lactoferrin from milk. Lysozyme, an enzyme derived from egg white has a bacteriocidal effect on a wide variety of spoilage bacteria such as *Lactobacilli*, *Leuconostoc* and *Pediococci* and has also been found to check the growth of *Clostridium tyrobutyricum*. This may have selected application in food preservation, especially when thermophilic spore formers are problems, and as a safe guard against food poisoning caused by *C. botulinum* and *L monocytogenes*. Lysozyme has been recognized as GRAS (generally recognized as safe) for using as antimicrobial agent in meat and poultry products. In natural cheese applications, it attacks *Clostridium tyrobutyricum*, which produces undesirable gas resulting in late blowing of cheese. It also prevents flavour degradation without affecting the cheese culture during ageing.

The dimeric form of lysozyme exhibits therapeutic, antiviral and anti-inflammatory properties, however, its limited antimicrobial efficacy against Gram-negative bacteria restricts its potential application in food industry. But the antimicrobial spectrum of lysozyme may be enhanced when it is used with other substances, such as chitosan that is known for its antimicrobial function. The enhanced antimicrobial activity of lysozyme with chitosan and synergistic effect of chitosan-lysozyme conjugation broaden the application of lysozyme in ensuring food safety and quality. In all lysozyme applications, ingredients panels must state “egg white lysozyme” to alert egg–allergic consumers of its presence.

Cystatin, a component from egg white, has been found to possess antimicrobial, antiviral and insecticidal effects, however, the greatest problem in utilizing cystatin as food preservative is its high cost. Phosvitin, a component from egg yolk lipids which has potent antioxidant property and can be used in food preservation after isolation and purification. Egg yolk phosvitin conjugates with galactomannan thereby producing a novel macromolecular antioxidant with significantly improved emulsifying activity and emulsion stability. Therefore, its
applications for the preservation of foods should be explored and its antimicrobial action against a variety of bacterial and fungal agents needs to be investigated.

Lactoferrin, a bioactive milk protein, which plays an important role in the immune system and protects the body from many infections. In January 2002, USDA approved activated lactoferrin for use in fresh beef, providing beef processors with a potentially powerful technology to protect consumers from pathogenic bacteria. ‘Activated Lactoferrin’ describes a unique combination of natural ingredients that mimic the optimum environment necessary for lactoferrin's maximum antimicrobial activity. Activation biases lactoferrin to its iron-free and immobilized forms, in effect returning lactoferrin to its most natural and functional state and use of this form of lactoferrin in preservation of foods needs to further investigations.

**Plant based preservatives:**

The use of spices and herbs as flavoring agents in foods is being practiced for centuries but it is only in recent years that modern science has started paying much attention to the exploitation of desirable properties of spices. However, despite the wide range of potential natural antimicrobials, relatively few are suitable for use in practice in particular as food preservatives. The aroma and anti-microbial properties of spices and herbs are related to their essential oil contents which are volatile oils obtained by distillation and enzymatic action. Spices in the form of powder, extracts or essential oils to check growth of many spoilage bacteria and fungi in foods have been well documented (Meena and Sethi, 1997; Subbulakshmi and Naik, 2002; Rajkumar and Berwal, 2003), though the antimicrobial activity of spices depends on type of test organism, nature and concentration of spice/herb, test medium and other factors for testing the activity. Most of the work on spices is going on to isolate their active principle in pure form and to use them for commercial use. Many companies are interested to investigate the beneficial properties of these spices especially in the marketable form just like vitamin supplements. For this, they need to know exactly what is the active ingredient present in a particular spice and how much of it is required for preservation of meat or other food ingredient. Recent research has been directed towards evaluation of the effectiveness of spices, period for which it can be effective and in finding out which bacteria are suppressed by which spices.

Results of some studies carried out in the past indicate that the growth of both Gram-positive and Gram-negative food-borne bacteria, yeast and mold can be inhibited by garlic, cinnamon, cloves and other spices. Antimicrobial effect of these spices/herbs can be seen in food products such as pickles, bread, rice and meat products, however, presence of fat, protein, salt and water contents of food influence microbial resistance. Therefore, higher levels of spices are necessary to inhibit microbial growth in foods than in culture media. Antimicrobial effects of spices such as garlic, cinnamon and clove against many bacteria such as Salmonella typhimurium, E. coli, Staph. aureus, Bacillus cereus, mycotoxigenic Aspergillus and Candida albicans have been described (Shelef, 1983).

**Garlic (Allium sativum):** Intact garlic bulb contains the precursor of allicin i.e. allin which hydrolyses to yield allicin by the enzyme allinase when the tissue of the bulb is disrupted and this allicin is the active ingredient which possess antimicrobial property. Some reports also indicate that if garlic is taken in small quantities daily then it can inhibit cancers and reduce cholesterol and plasma lipids thereby lowering the risk of heart diseases. Garlic has been used for treatment of respiratory problems such as asthma, bronchial congestion, fever, pneumonia, cough and ear, nose and mouth infections. It has also been used in the treatment of roundworms and hook worms. There is some evidence that fecal bacteria generate superoxide free radicals that produce mutagens and genotoxins and spices extracts of garlic, cinnamon volatile phenolic compounds such as eugenol, menthol and thymol exhibit bacteriostatic properties, thereby inhibiting the growth of...
high superoxide generating bacteria. Since garlic is a low acid vegetable with pH range of 5.3-6.3 and in this pH range it will support the growth and toxin production by the bacteria Clostridium botulinum especially when improper conditions are provided. These improper conditions include improper preparation and storage of fresh herb, moisture, room temperature, lack of oxygen and low acid condition as present in garlic. When this bacterium grows it produces extremely potent toxin. Similarly, when garlic is stored in oil at room temperature it produces botulinum toxin as the conditions (low acidity, no free oxygen in the oil and warm room temperature) for the production of this toxin are ideal.

Potentiality of using garlic as food preservatives has been evaluated in culture media as it inhibited bacterial pathogens such as Staphylococcus aureus, Salmonella typhi, E. coli and Listeria monocytogenes in culture broth, however, E. coli was found to be most sensitive and Listeria as most resistant (Kumar & Berwal, 1998). Aqueous garlic extract (AGE) has been recently evaluated for its effectiveness in preservation of poultry meat and in one study conducted on minced chicken meat it was found that incorporation of AGE at 4.0% (v/w) level in minced chicken meat (MCM) extended shelf life of MCM to one more day as compared to control (untreated) group during refrigerated (4 ±1°C) storage (Yadav et al., 2002). In another study AGE at 4.0% (v/w) level in combination with Lactobacillus acidophilus (LA bacteria) inhibited mesophilic and psychrophilic bacteria and both these in combination exerted synergistic effect and extended the shelf-life of minced chicken meat to 6 days as compared to 4 days shelf-life of control (untreated) group. The combination of AGE and LA culture was also found very effective in checking the growth of spoilage and pathogenic bacteria such as Aeromonas hydrophila in minced chicken meat during refrigerated storage. The combination of AGE and lactic acid at 0.08 % (v/w) level in model chicken meat system was found to delay the toxin production by E. coli during refrigerated storage. Aqueous garlic extract at 4.0 and 8.0% (v/w) was found to possess antimicrobial activity against Aeromonas hydrophila in minced chicken meat stored both at ambient (37°C) and refrigeration (4°C) temperatures and extended shelf-life of minced chicken meat (Yadav et al, 2004). Garlic have also been found very effective as antifungal agent and its antimicrobial activity against C. albicans have been found against cells in planktonic, adherent and sessile phases during its role in biofilm formation though fresh garlic have been found to have higher antifungal activity against C. albicans than garlic powder extract (Lemar et al, 2002).

Cinnamon (Cinnamomum zeylanicum): It is one of the large number of spices that has been known to preserve foods. Bark of cinnamon acts as an antimicrobial component and it contains 0.5 to 1.0 % volatile oils of which 75 % is cinnamic aldehyde (main active ingredient) and 8 % eugenol. Microbiologists have been testing the effectiveness of cinnamon and other spices in eliminating one of the most virulent bacterial causes of food poisoning E. coli O157. The researchers found that cinnamon added to apple juice that had been contaminated with E. coli was able to kill 99.5% of the bacteria within 3 days at room temperature. In an another study carried out on meat and sausages, it was found that cinnamon, clove and garlic all had a powerful ability to stop the growth of bacteria.

Recently, antimicrobial effect of cinnamon was also evaluated in model meat system involving chicken meat and it was found that incorporation of alcoholic extract of cinnamon at 0.4% (w/w) level alone or in combination with Lactobacillus acidophilus (LA culture) in minced chicken meat exerted potent antimicrobial effect in controlling the growth of total aerobic bacteria by 1 to 2 log scale (Yadav and Singh, 2004) during refrigeration (4 ±1°C) storage. However, antimicrobial effect of combination of cinnamon extract and LA culture was more as compared to either of them alone. These were also found to be good antioxidant as indicated by lower thiobarbituric acid (TBA) value in extract and LA culture treated group.
Cloves (Caryophyllum aromaticus): Cloves are actually the dried buds of the clove tree. Clove contains 14-21% essential oil and 95% of it is eugenol. The herb keeps the foods fresh as the main active ingredient of clove is eugenol, which has long been known to kill bacteria and viruses. Results of the recent study carried out in minced chicken meat indicated that incorporation of alcoholic clove extract at 0.15% w/w level alone or with LA culture exhibited potent antimicrobial effect during storage at refrigeration temperature. The antioxidative effect of either of these was evident from the fact that TBA values in clove extract or LA culture treated group remained lower as compared to untreated control group. The combination of both clove extract and LA culture doubled the shelf-life of minced chicken meat up to 8 days as compared to 4 days shelf-life of control group during storage at refrigeration temperature. Clove extract was also found to inhibit the growth of *Salmonella* Typhimurium (Singh et al., 2004) and other bacteria such as *Aeromonas hydrophila* and toxin production by *E. coli* in model meat system involving chicken meat and meat products during refrigerated storage. In another study carried out recently it was found that ethyl extract of cinnamon at 0.4 and 0.8% (w/w) had a very good bacteriostatic effect on *Aeromonas hydrophila* in minced chicken meat stored both at ambient (37°C) and refrigeration (4°C) temperatures (Yadav et al, 2004).

It can be summarized that a number of bio and phyto-compounds from natural sources have been found to possess antimicrobial and antioxidative properties and these can be used for the preservation of foods in combination with other food grade antimicrobials. Since the concept of bio or phyto-preservation of various foods has been gaining greater importance in recent years due to obvious reasons and therefore, more thrust should be laid on the isolation, characterization, standardization of application levels and methods, etc. of bio and phyto-preservatives to evaluate their efficacy in extending the shelf-life and improving microbial safety aspects of foods.

References:


Poultry meat has been accepted worldwide as a wholesome and nutritious food throughout the ages. In recent years, the consumption of poultry products has shown an increasing trend. However, poultry products are often involved in foodborne diseases, and the costs of such disease are considerable. Therefore, it is essential that the poultry industry ensure the production and supply of safe product to consumers in order to safeguard their health from pathogenic microorganisms that commonly contaminate the poultry and their products.

The need for pathogen control or for the introduction of eradication programmes is highlighted by the economic and health impact of poultry-borne human diseases. The economic impact of foodborne disease occurs in two areas: a) the costs of disease in human health terms, and b) the cost in trade or commercial terms. The public health impact of foodborne disease, on the other hand, is felt in the utilization of scarce medical and hospital resources by cases of preventable or avoidable disease. The economic and health impact of poultry-associated foodborne diseases is often obscure, as only the number of reported human cases is known but not the real number of cases or the number of cases specifically related to poultry. The number of campylobacteriosis cases has been shown by sentinel studies to be grossly underreported (more so than salmonellosis cases) in the UK (Palmer et al., 1996) and in the Netherlands (Notermans and Hoogenboom-Verdegaal, 1992). The zoonosis account being kept in Denmark provides a more precise determination of the relative importance of poultry. In 1996, 2-7% of registered human salmonellosis cases were related to poultry. Consequently, the relative economic impact of Salmonella in poultry may be estimated to be 2-7% of the total costs of human salmonellosis in that country. However, like most of the countries, no estimate of the total costs of human Salmonella infections in Denmark has so far been reported.

Epidemiological reports all over the world incriminate poultry meat as a source of outbreaks of human foodborne disease. Since poultry meat is usually not consumed raw, these outbreaks are caused by undercooking or cross-contamination of ready-to-eat products with microbial contaminants from the raw poultry or others introduced during preparation of the food (Anon, 1996). Therefore, there is need to identify the options on the use of specific production and processing techniques or decontamination methods to prevent further transmission of these pathogens from poultry and poultry products to consumers. In many cases of food poisoning like that caused by Campylobacter, the preparer of the food can be infected directly (hand-to-mouth) from the raw product. The aim of the poultry industry is to find ways to avoid contamination of live poultry and poultry products to consumers. In many cases of food poisoning like that caused by Campylobacter, the preparer of the food can be infected directly (hand-to-mouth) from the raw product. The aim of the poultry industry is to find ways to avoid contamination of these organisms. The aim should be to deliver live poultry free of pathogens to the processing plant. However, at present, the processing industry has to cope with contaminated flocks coming...
to slaughterhouses, where there are many operations that present opportunities for cross-
contamination; microbial contaminants are thus transmitted from contaminated to non-
contaminated carcasses or equipment. Consequently, additional treatment of products before or
after they leave the processing plant, and intensive consumer information and education about
the potential risk of the consumption of poultry products, should be part of the poultry industry
strategy for the future.

Poultry and poultry products must be handled properly during transport, in retail outlets,
and by the consumer within the household to preclude the problem of microbial multiplication
and the spread and cross-contamination of other foods. Microbial contamination of food may
result in spoilage and loss of product if the food is recalled or if a food borne disease has
occurred. Most recalls tend to be expensive; many cost millions of rupees, and some have
contributed to company bankruptcies. Even in the developed countries like USA, the reports
from the Centers for Disease Control (CDC), Atlanta, Georgia, have confirmed that the number
of outbreaks of foodborne disease has remained relatively constant over the last 5 years with
bacterial pathogens consistently accounting for approximately two-thirds of the outbreaks where
etiological agents could be identified. The most common bacterial pathogens transmissible to
humans by ingestion of raw or undercooked poultry include \textit{Escherichia coli, Campylobacter
jejuni, Salmonella, Staphylococcus aureus, Clostridium perfringens} and \textit{Listeria} (Malik and
Rawool, 2004). On some occasions, cooked or otherwise processed poultry may become
contaminated by \textit{Clostridium botulinum, Bacillus cereus, Yersinia spp., Aeromonas spp.} and
\textit{Shigella spp.} as well by bacteria found in raw and undercooked poultry. However, \textit{Salmonella, Campylobacter,} and to a lesser extent \textit{Listeria,} are considered to be the major foodborne
pathogens in the poultry industry, especially in the developed countries (ICGFI, 1999). Other
pathogenic microorganisms transmissible to humans by inhalation of aerosols or dust or by
contact with avian tissues include \textit{Chlamydia psittaci, Erysipelothrix rhusiopathiae} and
Newcastle virus. Major bacterial pathogens transmitted through poultry and poultry products as
well as their public health importance (Bachhil and Malik, 2005) is briefly presented below.

\textbf{Campylobacteriosis}

The disease is caused by members of genus - \textit{Campylobacter} (Group 2) consisting of 14
species, of which \textit{C. jejuni, C. fetus} (sub-species \textit{venerealis}) and \textit{C. coli} are the most important
human and veterinary pathogens. Campylobacters are slender, spirally curved rods with a polar
flagellum at one or both ends. The three species namely, \textit{C. jejuni, C. coli} and \textit{C. laridis} belong
to a thermophilic subgroup which fails to grow at 25°C but grows well at 42-45°C.

\textbf{Distribution of the disease:} Campylobacters are widely distributed in nature with most of their
species adapted to intestinal tract of warm-blooded animals and birds. The food and companion
animals, birds and wild animals act as reservoir hosts. Birds form the largest single reservoir for
\textit{C. jejuni} while pigs are the main host for \textit{C. coli}. The prevalence of campylobacters in faecal
samples usually varies 30-100%. Surveys of farm animals in a number of countries have
revealed an incidence of up to 70% in sheep, cattle and pigs, and up to 100% in chickens.

In USA, \textit{Campylobacter} is the leading foodborne pathogen. In most of the developed
countries, incidence of the \textit{Campylobacter} enteritis decreases with advancing age. On the
contrary, in developing countries where hygiene and sanitation is poor, the incidence of infection
in young children (<5 years of age) is as high as 40,000 per 1,00,000 or 0.4 episodes per child
per year. The ‘Travellers’ diarrhoea (TD) cases due to \textit{Campylobacter} is as low as 1-3% in
Mexico, 11-15% in Indian subcontinent as as high as 29% and 39% in travellers to Morocco and
Thailand, respectively.
In India, the status of campylobacteriosis remains largely unknown. However, the pathogen has been recovered from faecal samples of different animals and birds (chicken, quails etc.) as well as from beef, mutton and poultry meats in Bareilly and Varanasi (U.P.), and Hisar (Haryana).

**Sources and transmission:** (a) *Direct transmission* of the disease accounts for only small percentage of human cases, that too only in young children with acute diarrhoea. Person-to-person transmission is infrequent. Direct transmission from animals-to-man can occur especially in occupational exposed individuals, and from kittens or pups to man. (b) *Indirect transmission* is the prime route in humans through consumption of contaminated food, milk and water. The chicken meat constitutes the greatest risk among all the foods implicated, such as uncooked or poorly cooked meat products, unpasteurized dairy products and, uncooked foods subjected to possible cross-contamination by meat and poultry products. The pathogen is able to multiply only in foods kept at normal temperature, therefore, rarely may cause large outbreaks. The infective dose is small.

**The Disease:** Human beings, following an incubation period of 2-5 days, suffer with watery diarrhoea to dysentery, which is usually accompanied with fever and abdominal cramps that may be severe at times. Many patients also experience malaise, headache, nausea and vomiting. The illness lasts for 3-5 days and is followed by shedding of the organisms. *Campylobacter enteritis* has been associated with development of a reactive arthritis or arthropathy, which lasts for 3-7 days before resolving completely in most of the cases. A less common but potentially more devastating complication of *Campylobacter* infection is Guillain-Barre syndrome (GBS), a disorder of peripheral nerves characterized by ascending paralysis. Occasionally there is abortion, endocarditis, meningitis and septicemia. The diseases to which *C. fetus* has been linked are meningitis, pneumonia, arthritis, fatal septicemic infection in the newborn, and occasionally, sexually transmitted proctitis in adults.

A high proportion of healthy birds harbour the bacteria in their intestines. In diseased chickens, the egg production is markedly lowered and there may be enteritis, hepatitis, and loss in weight.

**Diagnosis:** 1. *Direct demonstration of pathogen* (with characteristic ‘S’ or comma shape) in fresh stool through dark field microscopy 2. *Culture and isolation* of the organism from the faeces, and rectal swab of patients by employing special media such as Skirrow’s medium. Camphylobacter Agar that are incubated at atmosphere of 5% O₂, 10% CO₂ and 85% N₂, preferably at temperature of 45°C. 3. *Serodiagnosis* can also be done using a flagellin antigen in the CFT. A rising antibody titre in a passive haemagglutination gives a positive diagnosis. An ELISA has also been described for the purpose. 4. *Molecular diagnostic tools* such as PCR has been developed for identification of genus as well as species-specific campylobacters from foods, semen and blood (Barua, 2003).

**Prevention and control:**

**In birds:** 1. *Good hygiene and management:* (i) Sanitation at farms, lairage and hatchery. It is the most accepted efficient method for prevention and control of *Campylobacter* infections, (ii) proper and hygienic disposal of and other body discharges, and (iii) adequate treatment of excreta before disposal. 2. *Vaccination:* live attenuated or subunit vaccines have met with limited success. An oral killed whole-cell vaccine has been shown to be safe and effective in animal models.

**In man:** 1. *Personal hygiene and sanitation:* (i) Safe and potable water for drinking, (ii) proper and compulsary heat treatment of poultry products. Milk contaminated with poultry and cattle faeces constitute the major sources of infection though, *Campylobacter* mastitis is also a source of contamination, (iii) adequate treatment of human excreta before its disposal. and (iv)
strict observance of personal hygiene helps much in avoiding the infection. 2. Health education: Persons, especially those engaged in poultry and animal husbandry, agriculture, food plants and food catering establishments must be educated about the disease and the protective measures. 3. Vaccination: An oral killed whole cell vaccine for animals is currently being tested in humans during phase I.

**Clostridial infections**

The causal agents of clostridial diseases belong to genus - *Clostridium* (Group 18, Bergey’s Manual, 9th ed.), and are Gram-positive, spore-forming rods, which can be differentiated from *Bacillus* based on anaerobic and catalase-negative attributes of the former. The three *Clostridium* species involved in food poisoning are *C. perfringens* types A and C, and *C. Botulinum*. Distribution of the disease: *Clostridium perfringens* type A infection, which accounts for a mild illness in humans has been reported as one of the most important causes of food poisoning world over, and ranks next to the leader - the *Salmonella*, in terms of incidence. Most documented outbreaks due to this pathogen have occurred in establishments where large quantities of food are served such as in restaurants, schools, cafeterias, hospitals, prisons and banquets. The number of persons affected in a single outbreak, therefore, is usually very high. The largest reported outbreak involved 13,300 persons. The isolation of the pathogen from food poisoning cases has been reported from many countries including England and Wales, West Germany, USA, Canada, Japan and India.

In India, *C. perfringens* has been isolated from poultry meat, fish, buffalo meat, beef, pig meat, infant foods, and also from diarrhoeal cases of food poisoning.

**Sources and transmission:** *C. perfringens* is ubiquitous in nature. The diseased or apparently healthy carriers among humans and animals carry the pathogen in their intestine, which serve as primary reservoir and source of the infection for susceptible hosts, foods and environment. The foods implicated in food poisoning are normally animal flesh such as chicken, beef, pork, turkey, fish paste, stews, meat pies and gravy which have been mishandled in some manner and, vegetables such as beans that have not been cooked thoroughly enough to destroy the spores.

The clostridial disease in poultry include enterotoxaemia of fowls due to type F (now known as one of the subtypes of type C) and haemorrhagic enteritis of chickens due to type D. *C. perfringens* type A in chickens has been implicated in necrotic enteritis in chickens.

**Diagnosis:** Although clostridia are common isolates, their clinical significance is not always immediately known and their diagnosis as cause of the disease depends on the microbial load, type of food implicated, the persistence of the isolate on resampling, and the condition of patient. The laboratory diagnosis includes: 1. Testing for morphological and cultural characteristics 2. Profiles of exoenzymes. carbohydrate fermentation.3. Detection of enterotoxin producing *C. perfringens* or enterotoxin in food and/or stool by PCR, ELISA, Reversed passive latex agglutination (RPLA) or gas chromatography. 4. Detection of anti-enterotoxin antibodies in blood of patient by ELISA. 5. Toxin production and pathogenicity testing in mice, guinea pig and rabbits. 6. Serotyping with antitoxin neutralization test.

**Prevention and control:** Thorough cooking of foods, quick subsequent cooling and adequate reheating before consumption can effectively prevent the clostridial food poisoning.

**Colibacillosis**

Based on disease syndromes and characteristics as well as effect on certain cell cultures and serological groupings, over 200 recognized serotypes of *E. coli* are divided into 6 virulence groups namely: (a) enteroaggregative (EAEC), (b) enterohaemorrhagic (EHEC), (c) enteroinvasive (EIEC), (d) enteropathogenic (EPEC), (e) enterotoxigenic (ETEC), and (f) diffuse-adherent (DAEC). The EHEC produces vero-cytotoxin (VT) also known as Shiga-like toxin/Shigella-like toxin (SLT) and, hence, these are abbreviated as VTEC and SLTEC, respectively. Of various groups, the EPEC and ETEC are important causes of acute enteritis whereas the EHEC has been implicated in a syndrome of diseases from mild diarrhoea to severe haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS).

Among bacterial infections, colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) is the first cause of morbidity and mortality in poultry, and is responsible for significant economic losses, especially in Europe, where poultry breeding is a major economic production, and takes a large place among exportations of food products. However, no reliable methods can be presently used to identify the causative strains of *E. coli*. Serotyping only allows the characterization of 16 to 60% of isolates from cases of colibacillosis. Thus, the characterization of APEC could be greatly improved if the diagnostic would be directly supported by the detection of virulence factors. Vaccines that are presently available for avian colibacillosis are not fully effective. This is probably due to the large variety of *E. coli* strains, which are able to cause colibacillosis infections in poultry. Thus, antigens corresponding to common virulence factors of APEC would have to be explored more widely in this purpose. The potential risk for human health that could be induced by APEC has never been evaluated even several data and observations argue in favor of such a risk. Therefore, there is need to investigate the characteristics of APEC on a large collection of avian *E. coli* strains by serotyping, molecular typing. Moreover, the search for virulence genes and pathogenicity islands, which are involved in virulence of *E. coli* for humans, and the survey of antibioresistance in APEC isolates would greatly improve the diagnostic methods and will allow further study of vaccine(s), based on virulence factors common to APEC isolates (Acha and Szyfres, 2003).

**Distribution of disease:** The pathogenic strains of *E. coli* are frequently associated with infantile diarrhoea, the greatest single cause of diarrhoea and mortality among babies. In some areas of the world, about 15-25% of children below five years die of diarrhoea. The rate of infection is higher in crowded tropical regions where sanitary facilities are poor, water supplies are contaminated, and adults carry pathogenic strains to which they have developed the immunity. *E. coli* strains also cause 50-80% of urinary tract infections (UTI). In a study involving veterinarians with different professional specialities, the prevalence of *E. coli* isolates that were resistant to several antibiotics turned out to be the highest in poultry and cattle practitioners and the lowest in swine practitioners. The first human case of infection with *E. coli* 0157:H7 was reported in 1982 and since then it has been increasing in incidence to an estimated 10,000-20,000 cases per year. The infectious dose of *E. coli* 0157:H7 is less than 100 cells. The SLTEC infection in man is present in most countries of the world, however, there is a wide variation in incidence among different countries and in many of them, its incidence appears to be increasing. The proportion of patients who develop HUS is still uncertain though it may be as high as 10% among patients suffering from *E. coli* 0157-associated gastroenteritis. The elderly and the children seem to be at higher risk.

In India, human infections due to *E. coli* causing enteric diseases have been reported. However, the true prevalence of this organism and the epidemiology has not been worked out. SLTEC strains have also been isolated from UTI, haemorrhagic enteritis and gastroenteritis cases. Moreover, in some patients with HUS syndrome, significant antibody titres against SLTEC have been detected. The ecology of SLTEC 0157 and other SLTEC is poorly understood. SLTEC have been isolated from cattle and foods of animal origin including poultry meat, however, their
prevalence in meat, milk and poultry is highly variable. The animals are usually asymptomatic and the excretion is intermittent. There appears to be very few reports on the isolation of E. coli 0157:H7.

**Sources and transmission:** E. coli is mainly transmitted through contaminated feed and water. Since E. coli is a normal inhabitant of intestinal tract, the environmental contamination is also thought to play a vital role in the spread of disease in a flock. The infected animals, birds or asymptomatic carriers can transmit the disease by contact. Most clinical diseases of E. coli are transmitted exclusively among humans mainly through (i) consumption of faecally contaminated animal food, food contaminated by cross-contamination and contaminated water, (ii) direct contact of infected man or carrier.

**The Disease:** The ETEC strains cause sudden profuse watery diarrhoea, particularly in children and adults, which is accompanied with abdominal pain, severe cramps and at times vomiting, leading to weakness and dehydration and rarely the fever. E. coli often invades sites other than intestine to cause neonatal meningitis, pneumonia, septicaemia, and wound infections. The EHEC strains are responsible for HUS and HC syndromes mediated through SLT. The HUS, first detected in 1985, is characterized by hemolytic anaemia, thrombocytopenia and acute renal failure. The HC syndrome, which was first seen in 1982 in diarrhoeic patients who consumed ground beef, leads to bloody diarrhoea with severe abdominal cramps besides nausea and vomiting, and at times fever.

In case of poultry, pathogenic serotypes of E. coli have been isolated from cases of septicaemia, salpingitis, pericarditis and ‘swollen head’ syndrome. Colibacillosis in adults, chicken and turkeys affects the lung, though it may also invade the circulatory system leading to septicaemia and death (Acha and Szyfres, 2003).

**Diagnosis:** The approach for diagnosis of colibacillosis is same in case of man and animals, and includes:

1. **Screening of samples for SLTEC:** It is done to demonstrate the presence of the pathogen in samples by means of (a) cytotoxicity assay in vero cells, and (b) tests like PCR and DNA hybridization aiming at verocytotoxic genes.
2. **Serology:** Immunospecific tests are still under experimental stage of development. The infected individuals produce antibodies against the lipopolysaccharide (LPS) of the pathogen. In the absence of positive isolation and toxin analysis results, the antibodies to the LPS can be detected by using haemagglutination tests, ELISA or immunoblotting.
3. **Isolation of the pathogen:** This gives the most conclusive diagnosis of infection. The positive samples in screening must be further analysed for the presence of SLTEC by cultural method employing indicative or selective agars such as sorbitol MacConky agar (SMAC) and a set of biochemical tests. In an outbreak setting, additional typing methods such as toxin gene analysis, plasmid analysis and pulsed field gel electrophoresis must be used.

**Prevention and control:** There is really no prevention other than avoiding the ingestion of the pathogen, particularly in case of E. coli 0157:H7. The preventive measures include,

In Man:
1. **Strict hygienic measures:** These are necessary (i) to prevent contamination of foods at all stages of processing, production and distribution, (ii) to prevent cross-contamination of foods, and (iii) to prevent nosocomial infection to newborn child through contaminated hands and instruments.
2. **Proper cooking of foods:** The thorough heat treatment, especially of meat and milk, is a must, and these should not consume in raw or semi-processed stages.
3. **Environmental sanitation:** The human excreta and waste should be properly disposed off.
4. **Health education:** Persons engaged in food production and processing such as farm managers, housewives and cook should be educated about the disease and preventive measures to be adopted.
In Birds: 1. **Good management**: It includes (i) feeding of fresh, clean, unspoiled and uncontaminated feeds to poultry, (ii) use of antibiotics in feed and water as a prophylactic to prevent outbreaks during.
2. **Environmental sanitation**: It refers to (i) proper cleaning and disinfection of poultry sheds, and (ii) proper disposal of farm sewage and dead birds.
3. **Immunization**: The birds may be vaccinated with commercially available vaccines against ETEC.

**Listeriosis**

The disease is caused by members of the genus *Listeria* (Group 19) which includes six species namely *Listeria monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. grayi*. Out of various listeriae, only *L. monocytogenes* is an opportunistic pathogen in human beings as well as various animal and bird species whereas *L. ivanovii* primarily affects only the bird and animal species, mainly as a cause of abortion in the ruminants, and only occasionally the man. The remaining species are non-pathogenic barring a rare human case report due to *L. seeligeri*.

Like other listeriae, *L. monocytogenes* is a small, Gram-positive, nonsporulating, facultatively anaerobic rod which measures 1-2μ x 0.5μ, grows in a range of 3-5°C (optimum 30-37°C) and show characteristic tumbling motility at or around 25°C. All the 13 recognized serovars of the pathogen are known to cause human listeriosis but serovars 1/2a, 1/2b and 4b are associated with most of the cases. The occurrence of listeric infections in man and animals in Indian subcontinent have been reported and reviewed (Malik et al., 2002).

**Distribution of disease:**

Human listeriosis is prevalent all over the world, causing several food borne outbreaks particularly in the developed countries. Economic losses attributable to it have been estimated to be $ 480 million in USA. The diseases accounts for 28% of the deaths caused by food borne organisms which ranks only second to the 36% caused by *Salmonella*, and the mortality among neonates and immunocompromised individuals is even higher (30-40%) making it a serious public health hazard.

In India, only 2 cases of human listeriosis have been reported, and the seroprevalence of the disease varies from 2.77 to 7.0%. However, the epidemiological data available to-date in the country is neither adequate nor reliable as it is based on the conventional diagnostic tests which show considerable cross-reactivity, and the disease largely remains undiagnosed and under reported. Of late, a study conducted at IVRI, Izatnagar revealed a seropositivity of 12.42% among 161 abattoir-associated persons in indirect plate as well as dot-ELISAs employing the haemolysin called listriolysin O (LLO) as antigen (Barbuddhe et al., 1999).

The cases of animal listeriosis have been reported in almost all species of domestic animals as well as from many species of poultry, fish, wild animals and rodents from worldover. Sheep and goats are the most commonly affected species. The prevalence of *L. monocytogenes* in raw broiler meat has been reported to range from 0-64%, which might be due to contamination from improper cleaning and disinfection of the processing line and the environment, and to lesser degree from the broilers. A wide variety of meats, particularly their surfaces have been found to be contaminated with *L. monocytogenes*, and the predominant serovar found in meats worldwide in serotype 1. The reported incidence of the pathogen varies greatly from 0-92% in case of fresh meats and 5-30% in ready-to-eat meat products.

In India, listeriosis was first described in sheep from Hyderabad in 1936-37, however, the pathogen was isolated for the first time in 1950 from an infected sheep in Madras and subsequently from goats, cattle, poultry, buffaloes, infertile cows and pigs. The outbreaks of
Listeriosis have also been reported among poultry, goats, buffaloes and pigs. In a series of recent works carried out at Indian Veterinary Research Institute (IVRI), Izatnagar (U.P.), pathogenic *L. monocytogenes* isolates were recovered from meats of 6.66% and 7.08% goats (in 2 separate studies), 7.40% sheep and 3.07% buffaloes; and milk of 1.56% goats and 6.25% buffaloes (Malik et al., 2002).

**Sources and transmission:** *L. monocytogenes* infects almost all domestic animals, many species of rodents, poultry (hens, ducks, geese, turkeys etc.) and wild animals. Chickens are thought to be the carriers and the prime reservoirs. The organism is ubiquitously present in the environment.

Foodborne transmission is the prominent means of infection. A wide variety of food stuffs including milk, soft cheese, ice cream, cook-chill foods, raw meats, ready-to-eat poultry, pates, unprocessed vegetables, salads, raw fish, fish products, sandwiches and fried rice have been found to be contaminated with *L. monocytogenes*. However, handling of newborn calves and infected material may also result in the infection. Nosocomial and person-to-person spread, though recognized, are uncommon. Direct contact with animals is also of little importance in transmission except in case of highly susceptible persons.

In case of animals, silage contaminated with *L. monocytogenes* is the prime source of infection. *Listeria* infected cows can excrete about 1x10^3 cells per litre in their milk. The pathogen is mainly transmitted through ingestion of food and water contaminated with faeces, saliva, nasal secretions, milk and aborted material from infected animals.

**The Disease:** Human listeriosis may present itself as meningitis or, more rarely, encephalitis. However, the infection can also cause septicaemia, abortion in pregnant woman, stillbirth or infection of the newborn. Minor skin infections particularly in farmers or veterinarians after handling bovine calvings or abortions have been recorded. The symptoms such as headache, vomiting, fever, malaise, pneumonia and conjunctivitis have also been seen. The meningitis is characterized by high temperature, stiffness of neck, often ataxia, tremors, seizures, and fluctuating consciousness. The onset is sudden and death may follow within 24-48 hrs.

Young birds are affected most. Outbreaks are infrequent and mortality may range from few cases to high. The septicaemic form is the most common, with degenerative lesions of the myocardium, pericarditis and focal hepatic necrosis. On rare occasions, the meningoencephalitic form, with marked torticolis is seen. With the generalised use of antibiotics in poultry feed, the cases of listeriosis in poultry have decreased to few (Acha and Szyfres, 2003).

**Diagnosis:** The disease can be diagnosed on the basis of (i) clinical symptoms and (ii) demonstration of the organisms in smear of CSF by Gram's staining, peroxide-anti-peroxide method or FAT, but its confirmation is done by (iii) isolation of the pathogen from clinical specimens (blood, CSF, faeces, mucus of newborn, vaginal secretions and foetus in abortion cases), food stuffs (Meats and cheese) and animal feed on media like PALCAM, PALCAMY and DRIA; along with (iv) Pathogenicity testing of *Listeria* isolates either by in vitro methods such as haemolysis on sheep blood agar, PI-PLC (Phosphatidylinositol-specific phospholipase C) assay and CAMP test as well as in vivo tests such as i/p inoculation of 3 weeks old mice and inoculation of 10-day old chicken embryo through CAM (chorioallantoic membrane) route, (v) detection of soluble antigen in CSF especially in meningitic cases of humans, but it is not reliable, (vi) examination of CSF for any rise in protein concentration (>0.4g l^-1^) and WBC count about 1.2x10^7 l^-1^ may be taken as suspected for listeriosis in encephalitic cases of animals (viii) serodiagnostic methods such as, serum agglutination, CFT, haemagglutination test, haemagglutination inhibition test, antibody precipitation test, growth inhibition test etc. have been reported. however, the detection of antibodies against LLO by plate- or dot-ELISA has been reported to be useful for diagnosis of both septicaemic and abortion forms of listeriosis. (ix) Recently, modified PI-PLC assay and a multiplex PCR based on virulence-associated genes
(plcA, prfA and hlyA) of Listeria spp. (Rawool, 2004; Shakuntala, 2004) have shown great promise as rapid and reliable in vitro alternatives to in vivo pathogenicity tests, which is the only reliable technique available at present for ascertaining the pathogenic potential of Listeria isolates. However, large number of the Listeria isolates need to be tested by these tests for the revalidation of observed results.

Prevention and control: Listeriosis can be prevented in man by taking due care during handling the abortion cases in humans as well as in animals, avoiding consumption of contaminated foodstuffs and avoiding cross-infections especially in hospitals among infants. For prevention and control of listeric infection in birds and animals following measures should be adopted.

1. Culling of infected bird and animals, 2. Care in use and preparation of silage as the pathogen grows luxuriantly at pH greater than 5, particularly when fermentation is ineffective and mould growth takes place. 3. Avoiding contamination of feed 4. Immunization: So far no vaccination is available, although a live, attenuated vaccine developed in Bulgaria, based upon serovars 1/2a and 4b of L. monocytogenes in some European countries is claimed to be effective for use in sheep.

Salmonellosis

The disease is caused by many species of genus Salmonella (Group 5, subgroup1, Family Enterobacteriaceae), which includes two species covering 2501 serovars identified so far. Serovars of other subspecies of S. enterica and those of S. bongori are designated only by their antigenic formulae. Salmonellae are Gram-negative rods that move by peritrichous flagella.

### Table 1: Serovars in each species and subspecies of salmonella

<table>
<thead>
<tr>
<th>Species</th>
<th>Subspecies</th>
<th>Year 1997</th>
<th>Year 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. enterica</td>
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<td>arizonae</td>
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<td>houtenae</td>
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<td>71</td>
</tr>
<tr>
<td></td>
<td>indica</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>S. bongori</td>
<td>-</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>2435</td>
<td>2501</td>
</tr>
</tbody>
</table>

Distribution of disease: With the exception of S. typhi and the paratyphoid serotypes particularly A and C, which are species specific for man, all other infections caused by salmonella may be considered zoonoses (Acha and Szyfres, 2003). Salmonellosis perhaps the most widespread zoonoses in the world involving various species of domestic as well as wild animals besides poultry, ducks, birds, amphibians, reptiles and rodents. S. Enteritidis phage type 4 (PT4) with its unique ability to infect contents of intact eggs has not only become a major problem in chicken eggs and meat in many areas of Europe and England but also a major cause of salmonellosis in humans in England and Wales. Salmonella is the single leading cause of gastroenteritis cases occurring in many countries, particularly North America and Europe as it is responsible for about 10% of total recovered diarrhoeal episodes. Salmonellosis caused by non-typhoidal species is not only more prevalent but has also shown an increasing trend world over with majority of cases being caused by S. Enteritidis and S. Typhimurium. S. Enteritidis, in particular, has emerged as an important cause of gastroenteritis, food-borne infection and a significant health problem in many countries like United States, England and Wales. In the
United States, it has been estimated that approximately 1,300,000 *Salmonella* infections and over 100 deaths due to salmonellosis occur annually at a cost of close to 1 billion dollars.

In India, human salmonellosis is endemic and one of the most widespread zoonosis (Table 1). However, it remains grossly underreported despite a marked increase in its cases during recent years.

**Sources and transmission:** Animal-to-animal, animal-to-man, man-to-man and environment to animal or man cycles continue uninterruptedly.

In human beings, eggs have been positively identified as the chief vehicle of transmission/infection; however, meat from broilers is also important. Man acquires *Salmonella* infection through various pathways including (i) In most cases, consumption of contaminated foods (mainly of animal origin) (ii) Faecal-oral route (very important in neonates). (iii) Direct transmission from animal-to-man which often involves pets or persons who are exposed occupationally. (iv) Person-to-person transmission which may be (a) vertical (infected mother to the offspring) or (b) horizontal (patient-to-hospital employee or vice-versa, patient-to-patient, and student-to-student in educational institutes etc.).

In birds, (i) Shedding of salmonellae in large numbers in excreta of clinical cases or symptomless carrier animals leads to widespread contamination of the environment (pastures, sheds, water and feeds) and, in turn, their oral transmission to healthy animals and man. Animals during their transportation and housing in lairages mostly become infected in this manner, (ii) Contamination of animal feeds, (iii) Utilization of waste products especially the poultry litter, (iv) In poultry, the salmonellae are transmitted transovarially.

**The Disease:** The incubation period varies from 6 to 73 hours and symptoms may last from 24 hours to 12 days. The infected persons usually have diarrhoea, abdominal pain, nausea and sometimes vomiting. Fever, although rare, lasts from 2 to 4 days. Complications leading to hospitalization include dehydration, sepsis, meningitis and osteomyelitis - Although overall mortality is very low (probably less than one percent), death rate is higher in infants and old people usually on account of septicaemia.

In case of poultry, the two serotypes, *S. pullorum* and *S. gallinarum*, are adopted to domestic fowl. They are not very pathogenic for man, although salmonellosis caused by them have been described in children. Many other serotypes are frequently isolated from domestic poultry; for that reason it is considered as one of the principle reservoirs of salmonellae. Pullorum disease caused by serotype *S. pullorum* and fowl typhoid caused by *S. gallinarum* produced serious economic losses if not adequately controlled. Both diseases are distributed worldwide and give rise to outbreaks with high morbidity and mortality. Pullorum disease appears during the first two weeks of life and causes high mortality. The agent is transmitted vertically as well as horizontally. Carrier birds lay infected eggs that contaminate incubators and hatcheries. Fowl typhoid occurs mainly in adult birds and is transmitted by the fecal matter of carrier fowl. On an affected poultry farm, recuperating birds and apparently healthy birds are reservoirs of infection.

Salmonellae unadapted to fowl also infect them frequently. Nearly, all the serotypes that attack man infect fowl as well. Some of these serotypes are isolated from healthy birds. The infection in adult birds is generally asymptomatic but during the first few weeks of life, its clinical picture is similar to pullorum disease (loss of appetite, nervous symptoms, and blockage of cloaca with diarrhoeal fecal matter). The highest mortality occurs during the first two weeks of life. Most losses occur between 6 and 10 days after hatching. Mortality practically seizes after a month. The clinical apparent form of the disease is rare after three weeks of life, but many birds survive as carriers. The most common agent in duck and geese is *S. Typhimurium*. It may be transmitted from infected ovary to the egg yolk as in pullorum disease, or by contamination of the shell when it passes to the cloaca.
Diagnosis: Human cases of salmonellosis can be suspected (1) on the basis of clinical symptoms and history of case (particularly in food poisoning episodes), and can be confirmed by (2) isolation and identification of the pathogen from food, clinical or morbid material, serological typing, and when necessary, phase typing and plasmid profiling. (3) Immunodiagnosis: A specific fimbrial antigen based latex slide agglutination test and flagellar antigen based competitive ELISA are being used for identification of S. Enteritidis (4) Simple PCR protocols with or without pre-enrichment of food samples have enabled detection of Salmonella within 24 hours (Deepa Surendaran, 2002).

Most cases of salmonellosis in poultry, particularly in carriers may not show any sign of the disease, and therefore, generally go unnoticed and undiagnosed. The serological diagnosis is important for identification and elimination of the individual cases of S. Pullorum and S. Gallinarum in fowl. As a screening test, the S. Pullorum antigen can also be used in detecting antibodies for the lipopolysaccharide of S. Enteritidis in chickens.

Prevention and control: In poultry: 1. Good hygiene and management: (i) Sanitation, plus formaldehyde treatment alone or in combination with heat, undertaken at farms, lairage and hatchery is the most accepted efficient method (ii) Carriers should be segregated from healthy ones. (iii) Animal/poultry excreta and waste should be properly disposed. (iv) The infected animals/carcasses detected at slaughter must be discarded. (v) The infected and carrier person should not be allowed in farm premises. 2. Pathogen-free animal feeds: Various animal feeds like meat, fish and bone meals should either be irradiated or heat treated for making them Salmonella-free. Pelleting, ionizing radiation, treatment of processed feed with formic acid will eliminate salmonellae. 3. Immunization: Vaccination by live or attenuated Salmonella vaccines should be used to protect poultry and other animals. Poultry are vaccinated by S. Gallinarum strain 9R (live). 4. Antibiotic treatment: The use of antibiotics with or without competitive exclusion of Salmonella can be adopted as an additional control measure. However, it has an inherent risk of selection of drug-resistant strains.

In humans, (i) strict observance of personal hygiene, (ii) proper sewage disposal, (iii) proper handling of foods to avoid cross-contamination, (iv) proper heat treatment of foods i.e. pasteurization or cooking of foods (particularly of animal origin), (v) supply of potable water. and (vi) periodical testing of foods and persons to detect reservoirs of infection.

Table 2. Important diseases caused by consumption of poultry and poultry products

<table>
<thead>
<tr>
<th>Causative agent and type of illness</th>
<th>Symptoms and Incubation period</th>
<th>Type of poultry product(s) involved</th>
<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni (Bacterial food borne Infection)</td>
<td>Watery, bloody diarrhea, fever, abdominal pain, headache (2 - 5 days)</td>
<td>Raw chicken, cooked meat contaminated with raw poultry meat</td>
<td>Properly handle and cook foods, avoid cross-contamination</td>
</tr>
<tr>
<td>Listeria monocytogenes (Bacterial Infection)</td>
<td>Healthy: flu-like symptoms Immuno-compromised: septicemia, meningitis, encephalitis, abortion (1 day - 3 weeks)</td>
<td>Raw poultry meat, refrigerated ready-to-eat foods.</td>
<td>Properly store and cook foods, avoid cross-contamination. rotate processed refrigerated foods.</td>
</tr>
<tr>
<td>Salmonella Enteritidis (Bacterial Infection)</td>
<td>Nausea, fever, vomiting, abdominal cramps, diarrhea (6 - 48 hrs)</td>
<td>Raw poultry, eggs, food handlers</td>
<td>Properly cook foods, avoid cross-contamination.</td>
</tr>
<tr>
<td>Escherichia coli (Bacterial Infection or Toxin-mediated Infection)</td>
<td>Hemolytic uremic syndrome (HUS), kidney failure, bloody diarrhea, thrombotic thrombocytopenic purpura</td>
<td>Raw poultry meat, undercooked hamburgers</td>
<td>Practice good food sanitation, hand washing, properly handle and cook foods</td>
</tr>
</tbody>
</table>

90
<table>
<thead>
<tr>
<th><strong>Microorganism</strong></th>
<th><strong>Prevalence (%)</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>0-100</td>
<td>Bryan and Doyle, 1995</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>63</td>
<td>Roberts, 1972</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>0.3</td>
<td>Anon., 1971</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>1.5</td>
<td>Doyle and Schoeni, 1987</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>0-100</td>
<td>Bryan and Doyle, 1995</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>88</td>
<td>Roberts, 1972</td>
</tr>
<tr>
<td><strong>-----do-----</strong></td>
<td>29</td>
<td>Isigidi et al., 1991</td>
</tr>
<tr>
<td><strong>-----do-----</strong></td>
<td>7</td>
<td>De Boer et al., 1991</td>
</tr>
</tbody>
</table>

**Table 3:** Techniques or methods to be implemented in the poultry production chain

<table>
<thead>
<tr>
<th>Area</th>
<th>Technology/method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchery</td>
<td>(i) Dipping of eggs (gentamicin sulphate, neomycin sulphate) (ii) Fumigation of eggs (formaldehyde) (iii) No re-use of transport trays, alternatively thorough cleaning and disinfection (iv) Cleaning and disinfection of containers</td>
</tr>
<tr>
<td>Genetics</td>
<td>(i) Production of resistant breeds</td>
</tr>
<tr>
<td>Husbandry/Management</td>
<td>(i) New litter systems (ii) Application of colonization resistant (CE-competitive exclusion) microflora (iii) Specific pathogen-free housing</td>
</tr>
<tr>
<td>Feed</td>
<td>(i) Pelleting, heating and heat extrusion techniques (ii) Addition of organic acids (iii) Addition of probiotics (e.g. oligosaccharides or microbes)</td>
</tr>
<tr>
<td>Processing</td>
<td>(i) Flock monitoring (ii) Effective transport crate washing and disinfection (iii) Clean-in-place systems (iv) Combined scalding, plucking (v) Cleaning and scalding in multistage scalders (vi) New evisceration techniques (vii) Rapid detection tests</td>
</tr>
<tr>
<td>End Product</td>
<td>i) Lactates/Lactic acid (ii) Inorganic phosphates (iii) Ionizing radiation (Source: Mulder et al., 1993)</td>
</tr>
</tbody>
</table>

**Table 4: Prevalence of potential human pathogenic bacteria in raw chicken in world**

<table>
<thead>
<tr>
<th><strong>Microorganism</strong></th>
<th><strong>Prevalence (%)</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>0-100</td>
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</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>63</td>
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<tr>
<td><em>Clostridium botulinum</em></td>
<td>0.3</td>
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<td><em>Escherichia coli</em> O157:H7</td>
<td>1.5</td>
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<td><em>Salmonella spp.</em></td>
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<tr>
<td><strong>-----do-----</strong></td>
<td>29</td>
<td>Isigidi et al., 1991</td>
</tr>
<tr>
<td><strong>-----do-----</strong></td>
<td>7</td>
<td>De Boer et al., 1991</td>
</tr>
</tbody>
</table>
Listeria monocytogenes 5 De Boer et al., 1991
-----do----- 60 Pini and Gilbert, 1988
Yersinia enterocolitica 8 De Boer et al., 1991

(Cited from: ICGFI, 1999)

Table 5: Prevalence of pathogens on poultry carcass sites in slaughterhouses in India

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Slaughterhouse</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Organised</td>
<td>Unorganised</td>
</tr>
<tr>
<td>S. aureus</td>
<td>9.13%</td>
<td>82.21%</td>
</tr>
<tr>
<td>B. cereus</td>
<td>Absent</td>
<td>47.49%</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>14.99%</td>
<td>39.23%</td>
</tr>
<tr>
<td>E. coli</td>
<td>17.46%</td>
<td>81.52%</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>Absent</td>
<td>10.55%</td>
</tr>
<tr>
<td>Campylobacter jejuni/coli</td>
<td>-</td>
<td>80 to 100%</td>
</tr>
</tbody>
</table>

Summarily, Chicken, like all fresh meats, is perishable and should be handled with care to maintain top quality. It is recommended to refrigerate raw chicken promptly; never leave it on countertop at room temperature. Packaged fresh chicken can be refrigerated in original wrappings in the coldest part of the refrigerator (40°F) or Freezer (0°F) it is stored. The attempts to decontaminate poultry have concentrated in three main aspects: 1) chemical methods (lactic acid, hydrogen peroxide, trisodium phosphate); 2) physical methods (-ionizing radiation, non-destructive heat treatments), and 3) novel methods (biopeptides and new preserving technologies which are a combination of physical and chemical treatments of end-products). The chain of poultry production and processing should be described in terms of the Hazard Analysis Critical Control Point (HACCP) concept. Corrective measures, including end-product decontamination when necessary, must be introduced at defined critical points. Unlike liquid foods such as pasteurized milk, which has a critical control point (CCP) to ensure the absence of pathogens, raw fresh and frozen poultry lack such a CCP. Currently, irradiation is the only available control measure and hence CCP that can ensure the absence of pathogens in such products need to be identified. It is up to the regulatory authorities and the consumer to decide whether to accept poultry in its present form or to introduce this effective decontamination measure to produce pathogen-free, raw fresh and frozen poultry. Directives or international and national regulations prohibiting the use of proven, safe, effective treatments to decontaminate poultry end-products need to be changed.

Measures to be taken by industry to avoid infection or contamination of live birds with potentially pathogenic microorganisms such as Salmonella and Campylobacter should be based on scientific data. To reduce the level of infection and contamination of live and processed poultry, new technologies and methods, as well as the introduction of monitoring and control programmes should be implemented. Information on the potential of genetic resistance of poultry breeds, the capability of microorganisms to colonize the gastrointestinal tract of poultry, the use of vaccines and antimicrobials, as well as the development of competitive exclusion microflora, are examples in this respect. Nowadays, the poultry industry in several countries, together with national governments, have introduced measures to be applied in breeder flocks to reduce the colonization and attachment of undesirable microorganisms in live animals. Continuation of these efforts through the entire production chain up to consumer-ready meat, eggs and egg products, may eventually help in reaching a pathogen-free status.

References: on request
India is a vast country with people of various religions living together. Their food habits vary according to their religion. They may be vegetarian or non-vegetarian. The number of non-vegetarians forms a sizable proportion. That is why the meat industry has become one of the important industries in our country.

One of the major or most common economic fraudulence in meat industry is the misrepresentation of costlier meat with cheaper one. It becomes more important because of some religious prejudices in countries like India. Hindus prefer to eat chicken, mutton but not the beef. Likewise Muslims do not take pork, the pig meat. In the meat industry, correct breed information in food labeling is required to assure meat quality.

In view of the above, there is a necessity to have a reliable test for confirmed detection of adulteration. Most of the present day tests suffer with cross-reactivity and are not very stringent. Thus development of accurate, authentic and precise method for detecting misrepresentation is a major challenge before the meat analysts. Several detection methods have been developed for identification of meat from different species (Patil 2003). Some of them with their merits and demerits have been discussed below:

1. **Anatomical, histological and organoleptic methods**

   These methods are reliable only in unprocessed raw meats. But the absence of characteristic structures to identify the species is one of the major limitations (Sherikar, 1987).

2. **Chemical methods**

   These methods rely on fat and carbohydrate components in meat but they are of little use due to the changes in the constituents during processing and storage. Moreover, they are time consuming, cumbersome and are unable to distinguish between marker and other components of similar activity (Hayder, 1979).

3. **Electrophoretic methods**

   Electrophoretic methods presume that composition of meat proteins is similar within the species and differ between them. However, composition of protein and type variation are seen in animals of different age, sex, stage of postmortem changes, stress, nutritritional status, temperature etc. All these factors were considered for developing species-specific electrophoretic profile for meat identification (Koh et al, 1998). SDS-PAGE (Scope and penney, 1971) and PAGE-IEF (Tinberg and Olsman, 1976) are the two are most commonly used methods for meat identification. Several workers used SDS-PAGE for identification of fresh beef, buffalo meat, mutton and chicken on the basis of number of bands, their relative position, intensity and difference in ratio of fronts (RF) values (Nath, 1986) and chevon on the

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*Ph.D.Scholar*
basis of cooked meat on thermostable muscle protein (TMP) (Radhakrishnan et al., 1988 and Bhilegaonkar et al., 1990). One limitation of SDS-PAGE is that the protein patterns with different species had quite uniform positions (Hoffman, 1985).

As meat proteins are amphoteric, IEF is one of the best methods to identify meat protein (Wilson and Walker, 1985). Fresh meat of cattle, buffalo, horse, pig and Kangaroo was identified by IEF method (Sinclair and Slattery, 1982). Adulterant buffalo meat in beef was detected in the ratio of 1:99, since the isoelectric focusing point of the phosphoglucomutase isoenzyme in beef is lower as compared to other meat (King and Kurth, 1982). Densitometric scanning has also been used for meat identification in raw and heated samples (70 °C) (Sherikar et al., 1988), however, boiling and autoclaving cause diffuse bands patterns. In some cases, this technique shows complications as numbers of bands are thrice as that on SDS-PAGE gel (Hoffmann, 1985).

4. Immunological methods

These techniques are based on the principle of antigen–antibody reactions. Commonly used immunological methods are ring test, double immuno-diffusion, immunoelectrophoresis, counter immuno-electrophoresis and ELISA.

Ring test is based on single immunodiffusion (Oudin, 1946). In this method antigen is placed over the antiserum in the capillary and an immuno-precipitate is formed at the interface of two layers. This technique was previously used in meat identification (Tizard et al. 1982).

Double immunodiffusion (DID) TEST (Ouchterlony, 1948) is based on the fact that antigen and antibody both diffuses towards each other in an inert medium. This method has some added advantages like resultant lines do not diffuse after formation and constituents of antigenic mixture can be compared with reference antigen. Many workers used this technique for meat identification (Pike and Sulkin, 1957; Ramadas, 1972; Hayden, 1979 and Radharishnan et al., 1988). Antisera against native and boiling resistant ethanol precipitated (BE) adrenal gland antigens and heated crude IgG antigens from cattle and sheep were raised in rabbit and used for DID and immunoelectrophoresis (IE) (Reddy and Mandokhot, 2002) which showed that sera raised against BE antigens was able to detect meat adulteration at a level up to 1% in cooked meat and 2 to 10% in fresh meat. In immunoelectrophoresis (IE), antigen diffuses radically and the antibodies diffuse laterally that result in precipitation arcs. IE can identify meat species by using species-specific antisera. Differentiation by IE was possible on the basis of number and location of the precipitation arcs formed (Misra, 1981). Differentiation of fresh and cooked meat may be possible (Reddy et al., 1990) by IE. A kit was also developed for test for rapid identification of meat (TRIM) using antisera and corresponding BE antigen disc (Sherikar et al., 1998) that can be maintained at room temperature and can be used at field level.

Counter immuno-electrophoresis (CIE) used alkaline gel, which helps in electro-osmosis, and can change the negative charge of weak proteins including IgG which than move towards cathode. During CIE, proteins from meat extract and gamma globulin from specific antiserum moves towards each other and produce precipitation band at the point of joining (Zade, 2002). This test was more suitable than IE (Reddy and Giridhar, 1995) in identification of cooked and fresh homologous pork antigen. This technique can differentiate pork, beef, poultry meat or kangaroo meat in thermally processed food at concentration <1.5% (Necidova et al., 2002).

Enzyme linked immunosorbant assay (ELISA) is one of the best immunological assay that uses antibodies or antigens coupled with high turnover number carrying assayed enzyme. Initially, it was used to detect 5% added poultry meat (Lajon et al., 1989) and also for species
identification in heat processed meat (Patterson and Jones, 1989 and Mifek and Glawischang, 1990) with detection limit of 1% for beef, pork or poultry meat. Double antibody sandwich ELISA is used for detection of beef, sheep, deer and horse meat in diluted cooked products with sensitivity of 0.13% (Andrews et al., 1992). ELISA with biotin-avidin amplification was found suitable for products cooked at 800°C for 2h but not for sterilized or intensive cooked samples (Demeulemester et al., 1992). Species identification can also be done by ELISA based on internal organs and fatty tissue (Hoffmann et al., 1994 and Pickering et al., 1995). MAb was also used for identification of fresh or cooked meat (castroet et al., 1990 and Shyang, 1996).

5. Molecular techniques

Molecular techniques involve DNA analysis since it is stable at high temperature and its structure is conserved within all tissues of an individual. Not only the genomic DNA but mitochondrial DNA has also been used for meat identification, especially cytochrome b and mt 12S rRNA gene in raw and processed meat products (Unseld et al., 1995 and Kocher et al., 1989).

In an study, 33.6 fg of genomic DNA from raw beef and 0.32 pg of genomic DNA from cooked beef was identified by PCR amplification of 1.709 satellite DNA which gave 218 bp DNA fragment (Zhang-guoli et al., 1999). In another approach, actin multigene family was used for meat speciation (Fairbrother et al., 1998) that gave reproducible pattern of heat-processed muscle held at 1200°C for 1h. Growth hormone gene was also used for species detection in blood and meat meal by specific PCR (Bottero et al., 2001). Multiplex PCR with a common forward primer and six reverse primers was used for identification of raw and cooked goat, chicken, cattle, sheep, pig and horsemeat that gave products of 157,k 227, 274, 331, 398 and 493 bp for each species respectively (Matsunaga et al., 1999a). A real time PCR was also used for meat identification (Casard, 2001). 5S rDNA gene (Rodriguez et al., 1991) was also used for meat identification. A single strand conformation polymorphism-PCR (SSCP-PCR) was used for meat identification of Hanwoo, Holstein, Angus, Charolais and Hereford cattle by amplifying MC1R, a protein associated with cattle coat colour (Chung et al., 2001). Breed specific SSCP markers were identified showing distinct differences between Hauwoo and Holstein or Angus breeds.

Mitochondrial cytochrom b gene was amplified to detect to model samples containing known amounts of beef and pork, with a detection limit of 0.05% (Piknova and Kuchta, 2002). Mitochondrial ATPase 6/8 gene was also used for identification of bovine, ovine, porcine, and poultry species with a detection limit of 0.03% for bovine and ovine and 1% for procine specific primers. Mitochondrial D loop is another region for which species-specific primers have been designed and 531 bp band was amplified in pork (Montial et al.)

PCR- RFLP is another convenient, rapid and sensitive molecular technique, which can be used for meat species identification (Verkaar et al., 2002). In this technique a gene of interest is amplified with specific primer and then digested with restriction enzyme producing different fragment characteristic of the enzyme used and thus enabling the products differentiation. Calpstatin (CAST) gene has been considered as candidate marker for beef quality. PCR-RFLP analysis revealed one heterozygotes associated with dark colour and higher cooking losses as compared to both homozygous genotypes (Kubiak et al., 2004). Fatness in pigs is of prime economic importance due to market incentives for production lean pork. PCR-RFLP of exon 6 and 18 of leptin receptor (LEPR) revealed significant genotypic differences in back fat thickness (Chen et al., 2004). Meat taste is mainly determined by the quality and quantity of triacylglycerols stored in adipose tissue. PCR-RFLP of leptin gene revealed one allele to be associated with fater carcasses and another with leaner carcasses (Lim et al., 2004).
Detection of animal species in blood and meat meals can be done by growth hormone gene specific PCR (Bottero et al., 2001). Presence of cattle and swine DNA in heat treated animal feeds, samples of mixed bovine/porcine blood meal, mixed bovine/porcine meat meals, pure bovine meat meal and ciccioli (residual pork fat) meal could be done accurately; 108 and 130 bp fragments of growth hormone gene were amplified for swine and cattle, respectively. They suggested that PCR could be useful tool in the development of a surveillance scheme.

Calvo et al. (2002) suggested specific PCR amplification of a repetitive DNA element to be powerful technique for the identification of beef in processed and unprocessed food, because of its simplicity, specificity and sensitivity. Effectiveness and specificity of this technique was confirmed by testing 45 cattle blood samples (from different breeds). This method was found to be useful in detection of contamination of pork up to 0.01% in raw beef.

PCR-RFLP of satellite I DNA, actin and melanocortnin gene was used for identification of sheep and goat with Apa I restriction enzyme (RE). Chicken, turkey and Hanwoo meat from Holstein and Augus meat respectively (Chikuni et al., 1994a; Verkka et al., 2002 and Chung et al., 2000).

PCR-RFLP of mitochondrial DNA was also used by several workers for meat identification. Cytochrome b gene is the commonly used target, which gives 359 bp amplicon and when cut with Alu I, Rsa I, Taq I and Hinf I helps to identifying raw and marinated, heat treated or fermented products of pig, cattle, wild boar, buffalo, sheep, goat, horse, chicken and turkey breed (Meyer et al., 1995). Even closely related deer species could also be distinguished by application of 1 or 2 RE (Wolf et al., 1999). Recently, species-specific mutation on mitochondrial DNA of cytochrome b and cytochrome oxidase II was used for bovine species identification (Verkka et al., 2002).

Mitochondrial 12S rRNA gene is helpful in differentiation of species that can be amplified by universal primers, followed by nucleotide sequence analysis. (Patil 2003) suggested the use of PCR-RFLP of mt 12S rRNA gene for qualitative differentiation of meat from commercial meat species like cattle, buffalo, sheep, goat, chicken, ducks, turkey, guinea fowl and quails.

In addition to cytochrome-b gene, 12S and 16S rRNA gene of mitochondria were also used for identification and differentiation of Helix pomatia and H. luconum, two popular edible snail in Europe. Species-specific band patterns were obtained for both and this method could be successfully used for fresh, cooked and canned cooked snails (Abdulmarwjood and Buelte, 2001 and Borgo et al., 1996).

RAPD-PCR also known as arbitrary primed PCR is another molecular approach for meat identification of various species. Meat samples from different origin and those subjected to different processing conditions can also be identified by RAPD-PCR. As little as 250 pg of DNA was used for the development of RAPD fingerprint (Calvo et al., 2001a). It was found that RAPD is rapid, reproducible and highly discriminatory method for monitoring traceability of meat. This method was suitable for raw, cooked meat and meat products (frozen, smoked, dried, salted and graved meat) (Martinez and Danielsdottir, 2000). RAPD pattern were species specific and did not require previous knowledge of DNA sequence of species under study.

RAPD-PCR analysis revealed several species specific patterns which could be utilized for meat identification from Zebu cattle, riverine buffalo, sheep, goat and pig species.

Forensically informative nucleotide sequencing (FINS) is one of the first molecular techniques for meat identification. It involves PCR amplification, cloning and sequencing of
conserved gene and comparison. Mt cytochrome-b and 12S rRNA is the most conserved gene, which are used by different workers as molecular marker. For identification of buffalo, emu and crocodile meat via a phylogenetic comparison computer programme (Forrest and Carneige, 1994). Mt 12S rRNA gene was studied in a forensic case to prove unambiguously that skin was not from tiger but from bovine (Prakash et al., 2000).

Amplified fragment length polymorphism (AFLP) was used to detect candidate markers that were absent in Japanese Black but present in Holstein (Sasazaki et al., 2004). This marker was useful for discriminatory between Japanese Black and F1 and would contribute to the elimination of falsified breed labeling of meat.

DNA hybridization is a reliable and sensitive but time-consuming method for identification of meat samples. Biotin labeled chromosomal DNA was used through blot hybridization for the detection of fresh and cooked meat of chicken, pig, goat sheep and beef meat (Chikuni et al., 1990). But it was found that the probe of ruminants reacted with other ruminant DNA. To avoid this problem specific genomic DNA probe were developed (Kirsten et al., 1991a). Dot blot DNA hybridization was used for detection of beef and pork in raw, pasteurized and sterilized meat product (Han et al., 1993). Complementary DNA (cDNA) probe was used for actin multigene family for evaluation of meat speciation (Karren et al., 1998). This method was useful to allow discrimination even between closely related species but not of the same breed within a species.

Species identification by oligonucleotide hybridization showed that repeated freezing and thawing of meat samples did not reduce the hybridization signals (Buntjer et al., 1999). But it was shown that this method is not superior to other methods of species identification of meat products since the strength of the signal is dependent upon tissue origin and sample processing.

In addition to qualitative methods, quantitative method has also been developed for pork in heat-treated meat products (Kirsten et al., 1991a). This method involves the immobilization of DNA on nylon membranes and hybridization with 32P labeled probe from genomic porcine DNA.

**Conclusion:** In the era of biotechnology, several DNA based reliable and precise detection systems have been developed for the detection of meat adulteration. There is a strong need to develop further simplified, field purpose, DNA based meat detection kits so that this malpractice can be checked. This is the high time to venture in this area. The need of the time is that the meat analysts and molecular biologists join hands together so that the set goals can be achieved at a much faster rate.

**Reference:** On request.
Poultry by-products accomplish the remaining material after the edible meat is taken out for consumption. By-products include the offal, feather, bone, blood, viscera, heads and feet. These are processed to obtain hygienic and nutritious products of economic importance. During recent years there has been a substantial development in processing and utilization of poultry by-products.

The amount of by-product left after selling the dressed bird or cuts is derived from the average dressing percentage. Percent dressing yield and yield of by-products of chicken and turkey is given below.

<table>
<thead>
<tr>
<th></th>
<th>Turkey</th>
<th>Broilers</th>
<th>Fowl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dressing Yield(%)</td>
<td>77</td>
<td>70</td>
<td>68</td>
</tr>
<tr>
<td>Offal(%)</td>
<td>12.5</td>
<td>17.5</td>
<td>12</td>
</tr>
<tr>
<td>Feathers(%)</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Blood(%)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Feathers/ Feather meal

Feathers represent about 7% of the total live weight. The feathers are made of a complex protein matrix and, as such, are a very rich source of protein (regular feather meal usually contains about 70-80% crude protein) that can be used for animal feed once the protein complex has been broken down. The feathers can also be used for bedding, ornaments, fertilizer, filler for chemical fertilizer and sporting equipment.

Feathers used in animal feed are usually hydrolyzed to break down the complex protein (keratin) structure, otherwise they would remain indigestible. Because feathers are mainly composed of protein, they represent a good source of dietary protein for animal feed. The feathers are washed to remove dirt and then dewatered. Those feathers will commonly pick up between 7-15% moisture during the scalding and defeathering operations. After some of this water is removed (usually by compression), the feathers are cooked for 1-2 hours in order to hydrolyze the complex protein structure. Heating is commonly done under pressure (2-3 Atm) that results in a temperature of >100°C, thereby increasing the hydrolysis rate. The feathers’ digestibility is related to the cooking temperature and time, where higher temperatures result in higher amino acid availability. The cooked feathers are then dried by air and ground, resulting in a product known as feather-meal. As with other feed materials, the feather-meal is passed through a metal detector to ensure that no metal objects are present. The common composition of a feather-meal is as follows:

- 75% crude protein (some contain up to 90%)
- 10% moisture (maximum)
- 3-4% fiber (maximum)
- <6% fat (maximum or minimum as specified)

The meal is rich in sulfur-containing amino acids, such as cysteine, and is also rich in arginine and threonine but is deficient in histidine, lysine, methionine and tryptophan. When the meal is fed to monogastric animals, such as poultry or swine, the limiting amino acids should be supplemented. The common feeding level is 0.5-1.5% of the diet. When fed to ruminant animals (beef cattle), feather-meal efficiency can be improved by urea supplementation.
Discarded feathers are currently used to produce feather meal through thermal processing resulting in a low nutritional value product. But recently developed biotechnological approaches using keratinase, the enzyme produced by bacterial strain Chryseobacterium sp. Kr 6 produce hydrolysed feather meal of high quality. In addition, keratin hydrolysates have potential use as organic fertilizers, production of edible films and rare amino acids.

Offal and bone

The classification of offal varies in different countries. The meat industry considers everything produced by or from the animal, except dressed meat, as a by-product or offal. In this category, there are the “edible” and “inedible” parts. The former includes a variety of meats such as livers, hearts and gizzards that are referred to as the giblets in the poultry industry. Blood which sometimes is used as an edible item, is also included in this category. The inedible portion includes parts such as the viscera (gut), heads and bones that are commonly sent for further processing into pet food, growing fur-bearing animals (e.g., mink), and feeding fish and hogs. Because of the danger of transmitting pathogenic organisms to other animals, the offal is usually cooked at a high temperature (>100°C, under pressure) to ensure the destruction of all pathogens. The treated offal material is usually high in protein and can be mixed with other ingredients (e.g., cereal, vitamins) to produce a balanced pet food diet. Meat-cum-bone meal, poultry by-product meal and hydrolysed feather meal are produced by rendering industry. The raw materials consist of by-products from the meat packing and processing industries and trimming from meat handling establishment like retail stores and restaurants. Rendering operations recycle the inedible tissues of poultry and other animals into nutritious feed ingredients for the livestock and poultry industry.

Pet Food

India has a large number of canine population (dogs, cats) which are kept as pet animals. The slaughterhouses produce large quantities of raw materials for pet food, which need to commercially exploited. However, there are only few companies, which have recently come forward for producing pet food. The international market is vast and demand of pet foods runs into billion dollars. Presently pet food manufacturing and marketing has gained momentum in India, particularly in metros. The offals and bones which were of little use earlier, have now been converted to pet food for feeding to dogs and cats in households. This is the most convenient and efficient utilization of processing wastes in a most profitable way. The material is extensively cooked and then mixed with other ingredients to produce a balanced diet for different pet food categories. Ingredients such as corn meal, soybean and vitamins are commonly used. In developed countries different types of pet food like canned cat food, canned dog food, dry dog food, etc. are available. The composition of a sample of dry dog food is given below.

**Ingredients:**  Ground corn, wheat shorts, poultry by-product meal, corn gluten, soybean meal.  poultry fat preserved with mixed tocopherols (to preserve flavor), rice, molasses, tripolyphosphate, dry whey, calcium carbonate, salt and vitamins.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (min)</td>
<td>22%</td>
</tr>
<tr>
<td>Fat (min)</td>
<td>10%</td>
</tr>
<tr>
<td>Crude fiber (max)</td>
<td>5%</td>
</tr>
<tr>
<td>Moisture</td>
<td>10%</td>
</tr>
</tbody>
</table>

The protein content of pet food can range from 12-50% depending on the way it is sold. Canned food usually has about 12-14%, semisolid food 21-25% and dry food 20-50% protein. It
should be mentioned that today, pet food companies invest substantial amounts of time and
money to explore the nutritional needs, as well as flavor and textural preferences (e.g., ground,
chunked) of different animal groups. Sophisticated processing equipment such as extruders are
often used to texturize and shape the product to make it more attractive and palatable in this
highly competitive pet food market.

A recent work carried out at CARI, Izatnagar on preparation of different types of pet food
biscuits and their evaluation in dogs suggested that pet food containing poultry processing
wastes, constituting about 25-30% of live wt. of poultry, was highly palatable and nutritious and
contained 5% moisture, 24% crude proteins, 4200 kcal ME/kg, 0.8% lysine, 0.5% methionine
with high (70%) pepsin digestibility and had shelf life of 6 months at mean ambient temperature
(26°C) in LDPE (300 G) pouches.

Blood

If blood can be collected hygienically, it can be utilized for human consumption. To be
cost effective this can only be achieved in a meat plant with a high throughput. Smaller plants
generally have blood spread on land, a practice not appreciated by environmentalists.

In animals, approximately 4-5% of the live weight of an animal is collectable blood,
which represents around 10% of the protein available in an animal. Dried blood is high in protein
(80-90%) and rich in lysine. The blood to be used for edible purpose is collected from cutting
line in a hygienic way. Normally an anticoagulant such as sodium citrate (0.2% w/v) is used to
prevent clotting. High speed centrifuse is done to separate solids. Thereafter plasma is frozen or
spray-dried at low temperature in order to maintain its solubility and binding properties. The red
cells can be used for black sausages or blood puddings or dried into blood meal.

For inedible purpose, it is essential that water does not come in contact with the blood for
two reasons: (1) water will cause haemolysis of the red blood cells and prevent the adequate
separation of plasma and red blood cells, and (2) should blood get into the plant effluent system
it will greatly increase the BODs by around ten times and the suspended solids by three times.

Before being allowed to enter the collecting tank, inedible blood should be strained to
exclude foreign matter. Drying of blood meal by removing the water is carried out in 3 steps. (i)
Direct batch drying carried out in batch dryers similar to rendering batch coolers. The raw blood
is dried to 2-10% moisture by simply boiling off the water.(ii) Batch coagulation followed by
batch drying. The raw blood is initially coagulated by injecting direct steam into an open tank
containing the raw blood. The coagulum, which is around 25% total solids (TS) is then separated
by draining or hand pressing and dried in a batch dryer. (iii) Continuous coagulation before
drying is the most common method of processing blood. Strained blood from a blood holding
tank is pumped into an intermediate pre-heating tank equipped with a low-speed agitator and the
blood is pre-heated to 60°C by steam. The blood then passes to the coagulator and as a result of
steam injection nozzles, positioned at several points in the coagulator, at the exit, the blood is at
an optimum temperature of 90°C. A decanter then separates the solids, which are dried, and the
liquid.

Bones

Bone may be classified as “edible” or ‘inedible’ depending on its source and its handling.
‘Edible’ bone must be handled speedily in a hygienic manner. Today there is an increasing
amount of ‘edible’ bone available because of the large amount of boneless meat being produced
at source. The end-products of edible bone processing are fat, bone meal and gelatin, with meat-
and-bone meal being produced when there is meat in the original raw material. Some, mainly chicken necks along with pig bones, are emulsified and used in pet food.

Owing to its high calcium and phosphorus content, bone meal is used as a constituent of poultry feeds and fertilizer. Calcined bone, obtained by roasting in air, is used in the manufacture of high-class pottery and china, in the refining of silver and in copper smelting. Bone charcoal is utilized in bleaching, sugar refining and case-hardening of compounds in the manufacture of steel. Special bone powders are employed for the removal of fluorine from drinking water.

Gelatin is produced from 'edible' bones subsequent to the extraction of fat and under carefully controlled pressure. It is used in making brawn, pies, ice cream and capsules for medicines, in photography, as a culture medium for bacteria and in the production of smokeless gunpowder. Some of the gelatin used for these purposes comes from veal and a smaller amount from beef. Nowadays pig skin supplies a large quantity of gelatin.

**Inedible rendering process**

While some meat plants have rendering departments for the treatment of condemned and other inedible material, it is better, from a public health standpoint as well as the efficiency of processing that the premises should be located away from food outlets and be large enough to handle material from a large area.

The best and most economical method of processing unfit meat and offal is by heat treatment in a jacketed cylinder, which gives complete sterilization and maximum return from the rendered material. A number of different methods are available for handling inedible material, all of which are concerned with the separation of the three main constituents, fat, water and fat-free substance, and the production of sterilized technical fat and meat-and-bone meal.

There are four categories of rendering systems, three of which are:
- Conventional batch dry rendering with mechanical and/or solvent defatting.
- Continuous dry rendering with screw press defatting
- Semi-continuous wet rendering with centrifugal defatting
- Continuous wet pressing and centrifugal defatting

The batch systems are more labour intensive but less expensive to install than continuous systems. In these three systems the raw material is cooked to sterilize the components (water, fat and meal) for separation, the fat being finally purified. There are major differences in the type and order of the various operations.

In the continuous dry rendering process (which resembles batch dry rendering except that the operation is continuous), the system operates at atmospheric pressure. After cooking, the material is pre-strained and discharged to a screw press for defatting. The length of the cooking process depends on the method of filling the cooker size.

In the fourth process, various models of this process have been developed but the main principle consists of mincing of the raw material, melting to liberate the fat. wet pressing and drying/sterilizing. Water, fat and fine solids are separated by centrifugation. A disadvantage of this process is that, similarly to continuous to dry rendering with screw press defatting, it does not fulfil existing sterilizing regulations in some countries, necessitating a further sterilization of the meal.

It is, thus, observed that the poultry by-products are also valuable materials in terms of nutritious composition and low cost for processing and use as feather meal, meat-cum-bone meal, blood meal, pet food. etc. which have great potential for further explottati
Harmful substances in poultry products include a wide range of chemical contaminants such as residues of drugs, hormones; pesticides, heavy metals etc. The chemicals get entry into livestock and poultry through oral (feed, water)/nasal route and excreted through faeces/urine/droppings. These substances are widely prevalent in biosphere including soil, water, air, food commodities, etc. The accumulation of residues in body is a function of level of occurrence and duration of exposure to the contaminant.

A residue is defined in EC directive as a ‘residue of substances having a pharmacological action, of their metabolites and of other substances transmitted to animal products and which are likely to be harmful to human health’. Almost all chemicals administered/fed knowingly or unknowingly to poultry result in some trace residue remaining in the carcass. The residues of chemical contaminants are deposited primarily in liver, kidney, muscle and adipose fat tissues and bring about changes at molecular leve in human beingsl. Now-a-days it has been observed that mostly new-born babies, children and women experience abnormal syndromes due to the effect of chemical residues arising from environment.

Residues can occur for a variety of reasons. Apart from feed, water and air treatment of poultry with veterinary drugs, use of feed additives also form a base for contamination of poultry by chemicals. In the preservation and processing of food, additives are employed to prevent the onset of spoilage, to promote binding properties and to enhance flavour and nutritive value. These additives include antioxidants, emulsifiers, humectants, firming reagents, sequestrants, colouring agents, stabilizers, sweetners, tenderizers, etc. At both production and processing stages, residues or contaminants may enter the food chain from intentional or accidental exposure to these chemicals.

Currently, in India, the production of eggs and poultry meat stands at about 44 billion and 1.4 million tons respectively, of which approximately 5% eggs and 20% chickens are marketed as processed products. India ranks fourth in egg and fifth in poultry meat production in the world. The domestic growth rates of egg and poultry meat have been 5 to 7% and 10 to 15% per annum respectively over the last decade. Poultry meat constitutes about 25% of total meat production in the country. The export volume in both the products have concurrently elevated to the tune of 6587 tons in table eggs, 8924 tons in liquid eggs and 119 tons in frozen chicken. The export of dried egg products, however, has recorded a steep increase from 1775 tons in 2001 to 3400 tons in 2002.

Undoubtedly, India has enormous potential to strengthen economy through expansion of domestic market and promotion of the export of raw or processed value added poultry products. The importing countries’ have imposed strict and stringent standards for residue of bio- and chemical-contaminants. This calls for regular monitoring and appropriate control measures to produce exportable poultry products conforming to International standards. In the prevailing WTO regime, need for adherence to sanitary and phyto-sanitary (SPS) measures through
adoption of GMP or more preferably Hazard Analysis and Critical Control Point (HACCP) system of quality assurance system to ensure food safety is assuming greater significance in recent years in accelerating export trade of poultry products. Export/import trade regulations governing occurrence of residues within permissible level in poultry products have been enforced by various international bodies like FAO/WHO/USDA/EC.

No chemical in safe under all conditions of use. Hence, it is important that all are fully evaluated for safety, as the parent compound and or as its metabolites, and that the result of these evaluations determine acceptability. Maximum residual limit (MRL) is the level of residues in food product which should occur at maximum and beyond this level, it is rejected for acceptance as an edible item. As international markets become increasingly harmonized, standardization of MRL is being attempted. Since some countries/union of states have their own standards for trade purposes, harmonization of standards will result in a common platform on global level suiting to all countries.

Occurrence of residue of chemicals should be studied and monitored in poultry products i.e. egg, meat etc. on a massive and wider scale on regular basis. In our country reports in this regard is scanty and not systematic. There is a need to organize the information and formulate the strategy to monitor the occurrence of residues so that these occur at below MRL in finished products. Some of the categories of chemicals known to occur as residues in poultry products are given below.

<table>
<thead>
<tr>
<th>Veterinary drugs</th>
<th>Tetracyclines, streptomycin sulfonamides etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth promoters/Hormones</td>
<td>GH, TRH, oestradiol, clenbuterol etc.</td>
</tr>
<tr>
<td>Feed additives</td>
<td>Coccidiostats, anthelmectics</td>
</tr>
<tr>
<td>Food Preservatives or chemicals used during product development</td>
<td>Humectants, emulsifiers etc.</td>
</tr>
<tr>
<td>Pesticides (insecticides, herbicides, fungicides)</td>
<td>BHC, DDT, PCBs etc.</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Cd, Co, Cu, As, Hg etc.</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>Aflatoxins; Ochratoxins, etc.</td>
</tr>
</tbody>
</table>

Drugs/antimicrobials

The chemicals under this category are antibiotics which inhibit the growth of or to kill microbes. These drugs are taken therapeutically to control diseases or infections. Fraction of the chemicals remain as residues in poultry and get transferred to consumers through consumption of contaminated products. Tissues like liver, muscle, kidney, fat and egg products are potential points of deposition. Public concern about the use of drugs in intensively reared livestock/poultry has forced researchers/ planners for re-examination of this practices. In some European countries use of certain antibiotics have been strictly limited.

Besides consumer point of view, antibiotics residues also interfere with further food processing where fermentation process is to be carried out. They cause allergic reactions in highly sensitized consumers. A small number of antimicrobials have carcinogenic properties. Compounds like zinc bacitracin, spiramycin, tylosin and virginiamycin have been banned in Great Britain.
The occurrence of residue of drugs in poultry meat and egg needs to be assessed countrywide. Efforts have been initiated by APEDA to evolve a data-base and formulate a monitoring plan at production & processing stage to contain the residues in poultry products so that large scale export of commodity is achieved. The residue of drugs can be detected by High Performance Liquid Chromatograph.

Pesticides

Pest control chemicals are toxic to living organisms to fulfil their role. Depending on the pest being controlled, they may be termed insecticides, fungicides, etc. Among insecticides, the chlorinated hydrocarbons are extremely durable, persistent and bio-accumulating compounds. BHC and DDT are two organochlorines which were generally used and have been banned for their persistency in the environment and food chain. Unacceptably high tissue concentrations of BHC, DDT, endrin have also occurred in broilers fed grains treated with these chemicals. These chemicals have high affinity for lipids and thus deposited in the adipose tissues, yolk, etc. On the other hand, organophosphates (malathion, cumaphos, dichlorophos, diazinon, etc.) are more toxic but excreted rapidly and do not persist to the same extent as organochlorines in environment because they can be hydrolysed chemically and enzymatically.

The MRL values specified of different pesticides for meat and meat products are 0.2ppm, 1.0, 0.2, 0.1, 1.0, 0.05, 0.05, 0.2ppm for aldrin/dieldrin, DDT, α-HCH, β-HCH, γ-HCH (lindane), endrin, chlordane, dichlorovous and heptachlor respectively.

The work done at CARI revealed that BHC level in spent hen muscle tissue ranged from 0.05 – 0.1ppm whereas in liver and adipose fat it was in the range of 0.1 – 0.25ppm and 0.2 – 0.5ppm, respectively. The level of DDT was recorded to be 0.08 – 0.2ppm in muscle, 0.15 – 0.25ppm in liver and 0.2 – 0.3ppm in adipose tissues.

There are other more durable organic environmental contaminants such as polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs), their residues in poultry products also pose threat to human health. The residue of pesticidal chemicals can be analyzed by Gas Chromatograph and High Performance Liquid Chromatograph.

Heavy Metals

Intake of heavy metals in feed/food/water cause accumulation of their residue in tissues. Occasionally, toxic concentrations occur in animal and poultry tissues and their products. Heavy metals are the metals having higher atomic weight and having a specific gravity of 5 or above. Some of them are lead, arsenic, mercury, cadmium, copper, cobalt, etc. Sources of contamination are mining areas, soil, water, paints, smoke, industrial & vehicular emissions, etc.

Lead contamination occurs through feed, smoke, paints, etc. and residues are detected in liver, bones, kidney. Acute cases are rare but chronic cases are often encountered. Arsenic is second most important metal to cause poisoning in animals. Poultry may be exposed to organic or inorganic arsenic compounds through contaminated feed/water or medicines containing this metal. Generally it takes 40 days for complete withdrawal from the animals.

Mercury preparations containing inorganic salts or organic mercurial compounds have been widely used in crop production, vety. medicines etc. Antifungal drugs used during storage
of grains also contain mercury compounds. Absorbed inorganic mercury is stored in the liver and kidney but organic preparations are more widely distributed.

Cadmium has received much attention because of its reported toxicity to humans. This metal accumulates in body tissues and said to cause kidney failure. The greatest concentrations occur in liver and kidney. The origin of the residues may be contamination of grain/feed with sewage sludge or organocadmium fungicides. Concentrations as high as 200 μg/g (dry wt.) in kidney have been observed in farm animals. Feeds supplemented with copper tend to accumulate residues in liver and kidney. Eggs also found to contain residue of copper.

The residues of heavy metals and other chemicals contaminants in market poultry products have been little studied in our country. A study was conducted at IVRI on the prevalence of residues of lead in samples of poultry feed, egg and poultry tissues was studied in organized and unorganized (rural) sectors in and around Bareilly district of Uttar Pradesh. It was found that poultry feed contained lead at the level of 0.45-0.48 μg/g in two organized farms. Lead concentration in egg albumen and yolk was found to be 0.37, 0.44 and 0.42, 0.47μg/g in organized and backyard poultry, respectively. In a recent study of similar kind in Chennai, egg and poultry meat samples from local market had levels of lead, cadmium, mercury, except copper, higher than permissible limit (TANUVAS Report, 2003).

A study at CARI found out the occurrence of residue of lead in layer feed (0.326 ppm), whole egg (0.183 ppm), egg albumen (0.302 ppm), egg yolk (0.349 ppm) and kidney (0.316 ppm) in market samples. The overall concentration of residual Cd was found significantly higher in poultry liver (0.09 ppm) and kidney (0.108 ppm) followed by egg yolk. Layer feed had lower level of Cd than others. The level of copper in liver (2.041 ppm) was observed at the top followed by kidney (2.01 ppm), layer feed (1.042 ppm), muscle (0.932 ppm), egg yolk (0.735 ppm), whole egg (0.534 ppm) and egg albumen (0.378 ppm).

Detection and quantification of metallic residues in tissues can be done by Atomic Absorption Spectrophotometer.

Mycotoxins

These are metabolite products of toxigenic moulds growing in food and food stuffs, and their residues in poultry meat, eggs pose cause for public health. However, the risk to human health from direct consumption of contaminated grain is much higher than arising from animal products.

Aflatoxins are produced by Aspergillus flavus and Aspergillus parasiticus. (AFB, AFB2, AFG1, AFG2). AFB1 in most toxic & potent among them. Much of the ingested toxin is excreted within 24 hours and excretion is almost complete within 96 hours after ingestion. Liver, kidneys & adipose tissue retain detectable quantities of toxin for longer periods. At CARI, the residual level of aflatoxin B1 in muscle, liver and adipose tissue samples collected from the local market was determined and was found to be on an average of 0.05ppm and it occurred in 20% of the samples.

Another mycotoxin, Ochratoxin A is produced by Penicillium spp. & some Aspergillus strains. This toxin is also most common and very toxic to poultry. Kidney is the major target
organ but liver damage has been reported. The presence of these toxins can be detected by HPLC, UV-VIS spectrophotometry and immunoassay kits.

**Control of occurrence of pesticides**

The occurrence of contamination can be checked at sources viz. soil, water, feed, medicines etc. In case of feed ingredients (grains)/compounded feeds the contamination occurs during crop production and storage of grains. Regular monitoring of residues of chemicals and minimizing/judicious use of contaminated sources would greatly reduce the occurrence in poultry products. When the products are found to be contaminated with chemicals few cooking methods are of some help. The research work conducted at IVRI and CARI revealed that dry heat treatment in form of broiling was most effective in reducing the BHC level (70-80%). The other mode of cooking such as microwave and pressure cooking was also found effective to some extent. Similar observation was also made with DDT, where broiling and pressure cooking resulted in minimizing the residues by 60% and 50% respectively. Metallic residues are poorly destroyed by cooking.
Necessity and Drawbacks of IPR Issues for Poultry Products

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While addressing the Second Agricultural Science Congress in 1995 at Hyderabad, Dr. M.S. Swaminathan, former Director General of ICAR, emphasized about the augmenting role of Intellectual Property Rights in Indian economy through assertive role of agricultural sciences. India, the world's third largest producer of agricultural products after the United States and China, produces about 150 million tons of fruit and vegetables per year and is the largest producer of eggs at 45 billion/annum. But nearly, 40 percent of our agricultural produce is reportedly wasted before reaching the market. On the other hand, India happens to be one of the potential major markets for food exports and joint ventures as evinced by the outcome of 20th annual food products exhibition,” Aahar 2005” in New Delhi, the platform for international companies seeking markets for poultry, dairy, horticultural products, processed food, packaging and refrigeration and for joint business ventures. American food exporters are virtually targeting India's middle class society, which is mostly concentrated in the metros, with their consumption behaviors comparable to westerners. Growing health consciousness among the educated middle class has further added to the specialty food imports leading to the incessant launch of fast food and western style restaurants. Ultimately, the vibrant domestic food processing industry is compelled to create substantial markets for an increasingly broad range of food items. Shift from elite to "middle class" does not compromise with the demand for correct quantity and quality and diversity in variety of food supported by proper grading, sorting, packaging and refrigeration to enhance the shelf life of the product. Perpetual increase in the income of our society also leads to more protein consumption in the form of poultry, eggs and milk etc. In order to produce quality and cost efficient food products, it is imperative to address issues related to high tariffs, higher transaction costs, trade facilitation, licensing system and protection of intellectual property rights (IPR). According to Indian Poultry Industry Informatics (2000), lack of cold chain facilities particularly for poultry meat products, fragmented marketing channels, less attended food laws, complex tax structure, increasing use of SPS [sanitary and phytosanitary] measures work against the incentives to the exporters. To attract the FDIs or the investments needed to stimulate the economy through livestock production, proper IPR protections must be adopted and enforced accordingly. As per Confederation of Indian Industry (CII), the Indian food processing industry a sunrise sector is ought to continue with its strong product base and great export potential especially for Indian poultry and fishery.

Intellectual Property Rights (IPR) in India

The Indian concept for IP system was developed about 150 years ago based on British law (1856). After independence, the Indian Patents & Designs Act came into force in 1911. Later on, India acceded to the Paris Convention and Patent Cooperation Treaty (PCT) w.e.f December 7, 1998, and began operating as a PCT Receiving Office, Designated Office and Elected Office for the purposes of international applications filed under the PCT. Bill to amend the Patents Act (1970) was passed by both Houses of Parliament in March 1999, to contain the "black box" medicines and drugs. Exclusive Marketing Rights (Articles 70.8 and 70.9) of TRIPS came into existence from January 1, 1995. Protection of intellectual property rights in India continues to be
strengthened further. The year 1999 witnessed the consideration and passage of major legislation with regard to protection of intellectual property rights in harmony with international practices. The Indian intellectual property legal community is extensive, well established and sophisticated. The Controller General, Patents, Designs and Trade Marks maintains a register of practitioners (agents and attorneys) that are qualified and permitted to represent applicants before him/her. The improvement of the IPR system likely to be the infrastructure of the knowledge-based economy varies from country to country. The laws dealing with protection of IPR are practically insufficient, and marketing of counterfeit and pirated products is now an international issue. The issue of IPR protection is not limited to one country, and economy of Asia Pacific Economic Cooperation (APEC) member countries should work together to improve and prevail, despite the differences in systems. Therefore, in recognition of the common goal of protection and the enforcement of the control of IPR, a system that enables the members to coordinate their activities is very much needed. In the year 2002, Japan proposed for establishment of the "APEC IPR Service Center Network" at the first APEC Senior Officials' Meeting (SOM1 in Mexico City). It was agreed to back this proposal through after a study report is available for protecting and enforcing control of intellectual property rights.

**Legal Framework**

India has a well-established statutory, administrative and judicial framework to safeguard intellectual property rights related to patents, trademarks, copyright or industrial designs. The Ministry of Commerce and Industry (MCl) maintains an extensive and comprehensive inter-ministerial and private sector consultative network. Our IP policy is aimed at economic development through trade and is treated as an important component of technological innovations. Ministry of Science and Technology, Ministry of Human Resource Development and the Ministry of Agriculture are also close associates of MCI in implementing and regulating our IP policies.

National Intellectual Property Organization (NIPO) is another appreciable initiative for putting India on the map of intellectual superpowers. It is aimed to be an umbrella organization for Intellectual Property (IP) laws and related matters in India to promote and develop IP and to interact with other organizations in India and abroad, which are active in this field. Scientists, authors, academicians, technocrats and senior civil servants and eminent legal experts support the NIPO.

The Office of the Controller General of Patents, Designs and Trade Marks is a subordinate Office under the Department of Industrial Policy and Promotion in the Ministry of Commerce and Industry. This Office has statutory responsibility for administration of patents, trademarks and industrial designs and serves as a main source of policy advice to the Government of India on industrial property matters.

The Copyright Office, in the Ministry of Human Resources Development, provides policy advice to Government with respect to copyright and neighboring rights. India happens to be the member of following international conventions and treaties regarding intellectual property:

- Berne Union
- Convention for the Protection of Producers of Phonograms
The awareness of intellectual property rights (IPR) among domestic firms has greatly increased with the nation's rapid economic and scientific development. However, we still need to make long-term and unremitting efforts to further improve our IPR system. Five intellectual property Chairs in Universities have been established in various regions of the country. Government has communicated the main technical and management-training institutions in the country to encourage their participation IP training programmes. Professional training in IP has also been provided through foreign Universities and at the WIPO Academy in Geneva. India continues to play a leadership role in the South Asia Association for Regional Cooperation (SAARC) and has hosted conferences and seminars on IP in that context. In addition, India has presented the views of the region and developing countries in general, with respect to the need to encourage transfer of technology, to protect biodiversity and to extend protection of geographical indications of goods in WIPO and at the WTO, most recently at the Doha Ministerial.

Patenting of poultry products

As far as poultry products are concerned these are processed and sold in all kinds of locations and different level of processors. In this profession, organized as well as unorganized sector contribute very significantly. Hawkers to big fast food outlets emphasize upon their quality and cost benefit to the consumers. Product should be available in the market suit to the requirement of customers and their purchasing capabilities. Though, the growing demand for processed poultry meat or egg products has further spurred the wider involvement of entrepreneurs in this direction but some how, the consumer, generally, remains unaware about the standard of food he is getting or he is authorized to get. In fact, we have to develop our own standards for every kind of food and make it mandatory for the processors to follow and market their products accordingly. In wake of this ardent requirement, Bureau of Indian Standards and Patent Office (Govt. of India) are quite vigilant and endeavoring their level best to formulate standards and patent the processing technologies, respectively. Patenting of processing technology passes through following stages-

- Development and standardization of original technology/ innovation,
- Filing of application for patent,
- Examination of document with repeated modifications as per satisfaction of Patent Office
- Grant of acceptance of patent,
- Gazette notification of patent for wider publicity and challenges if any,
- Sealing of patented technology (Optional).

Division of Post Harvest Technology at this institute pioneered in patenting five technologies listed hereunder-

**Indian Patent 180509**- Method of preparation of mustard oil based chicken gizzard Pickle. By A.K. sachdev, Ram Gopal and S.S. Verma

**Indian Patent 184430**- Method of preparation of mixed chicken meat loaf.

   By A.K. sachdev, Ram Gopal and S.S. Verma


Indian Patent 188420- Method of preparation of cooked chicken roll. By A.K. Sachdev, Ram Gopal and Rajvir Singh

After patenting the product processing specifications, the important aspect of transfer of technology comes into effect. Certain requirements are to be addressed to provide pace to this activity.

**Impediments in transfer of technology**-

The acquired patent in poultry products technology is confronted with following drawbacks-

- The patent is granted for a particular recipe and the technique, which is not already reported. Any minor deviation in the recipe or processing technique can take it out of the legal framework.
- The Heads of the institutes, where technology has been developed, need be vested with power of selling the patented technology. Hierarchal obligations could generally delay the transfer of technology to the needy entrepreneurs.
- Since the patented technology for edible food products suffers threat of copying, it could be sold to many of the interested entrepreneurs that too at a very meager cost because, by transferring the know-how we are helping unorganized poultry meat processing sector to generate employment opportunities. Heavy costs could only be obtained from the big establishments, which are generally interested in getting the idea instead of the technology after paying its cost to the concerned institute.

**Suggestions for propagating IPR**

If we view Chinese system, it has built a complete patent system over the past 20 years, and the system meets international standards with the country’s accession to World Trade Organization. Further it is reported that the awareness of intellectual property rights (IPR) among domestic firms has increased with the nation's rapid economic and scientific development. Their lacuna to this aspect is that the users can either buy from the patent pool or have nothing to buy. This is one of the symptoms of IPR abuse. In order to popularize the concept of IPR in India particularly for poultry products, certain suggestions are made as under:-

1. Time taken for grant of patent from the date of its filing has to be reduced drastically to few months instead of 3-4 years.
2. The patent should be for the product instead of a specific technique to prepare that product.
3. The process of selling patents needs be extremely simple and target oriented.
4. Patented technologies for poultry products should preferably be transferred to economically depressed class of people so that concept of “Work to every hand for food to every mouth” including unemployed youth, uneducated housewives and unskilled workers could come true.
5. Cost of patent should be very meager so the more people could be encouraged to avail this facility.

India has to grow through persistency, hard work and educational awareness to overcome the threat of socioeconomic depression and paucity of resources. IPR could be a link between researchers/technologists and the users giving due weightage to all the concerned.
Pragmatic approaches for increased and efficient poultry production, processing, quality control and marketing have become more important for sustaining growth in poultry production activities, particularly in the competitive environment of trade liberalization brought out by WTO regime. Ensuring safety of poultry products has received greater attention in the recent years because of the need for meeting the requirements for export trade and also due to the implications of liberalized trade regulations. In order to meet the quality requirements it is necessary to understand different quality aspects of poultry products, their production, processing and utilization and regulatory requirements both of National and International.

Changing consumer priorities:

Consumer demands that the product is appealing to the eye, tasty, tender, wholesome and safe for consumption, nutritious, at an affordable price and of late ethnical practices are involved in production. The following have been the consumer concerns in different decades:

- 1970- Price
- 1980- Price, Fresh food, Quality, Product range·
- 1990- Price, Fresh food, Quality/safety, Product range, Service, Welfare
- 2000- Price, Fresh food, Quality/safety, Product range, Service, Welfare + Ethics, Ethnic/traditional, Recycling
- 2010- Above of 2000 + Environmental ethics, Organics, Time, Pack size, Reliability, Zero risk

Food Safety Issues

Food safety is concerned with preventing animal products, not fit for human consumption, reaching consumers. As food can become contaminated at several steps in the production, processing and utilization controlling food borne pathogens is a constant challenge. Food safety concerns pertain to pesticide and veterinary drug residues, antibiotic resistance, chemical residues, adulterants, food borne-pathogens, emerging pathogens, reemerging old pathogens, risks from irradiation of foods and genetically modified foods. International organizations concerned with food safety are FAO, WHO, OIE, IPPC and Codex. A number of National standards and regulations such as of USA Code of Federal Regulations (CFR 9: Animals and Animal Products), Australian, European and Canadian play an important role in International food trade and need to understood. Food safety is ensured through quality control programmes which are in plant as well as regulated by approved agencies. Several approaches are employed in ensuring food safety. Some of these include TQM, HACCP, GMP, GAP, ISO etc. Production aspects have major implications to food safety particularly with regard to pesticide and veterinary drug residues and introduction of pathogens and food spoilage organisms. Observing proper withdrawal times for drugs and chemicals and practice of hygiene in production, products processing and distribution would ensure to a large extent production and consumption of wholesome poultry products.
Risk analysis: A number of newer approaches have emerged in the understanding and assessment of risk to ensure food safety. A number of risk analysis terms related to food safety have been used in food standards: hazard, risk, risk assessment, hazard identification, hazard characterization, exposure assessment, risk characterization, risk management, risk communication, dose-response assessment, scenario set. HACCP is a risk management tool that provides a more structured approach to the control of processing or manufacturing products than that achievable by traditional inspection and quality control. Rather than by testing the end product for failure it functions to prevent failure by systematically controlling the process. It anticipates potential problems or failures and does not depend only on final inspection. HACCP can also yield potential cost savings in product wastage, re-processing, or recall should problems occur. The Codex Alimentarius Commission has promulgated the concept of HACCP by adopting Guidelines for the application of the hazard analysis critical control point system during its 20th Session.

Precautionary principle: The principle states that potential environmental risks should be dealt with even in the absence of scientific certainty. It has long been advocated by environmentalists, who see it as a more effective way of managing hazards than traditional scientific risk assessments, which call for numbers and hard proof as prerequisites for action (Nature, 2000, 407:551).

Traceability: In the identification of risk in food trade traceability has become important but associated with difficulties particularly in developing countries due to a large number of small holders. Animal products, which are processed and packaged, could be identified to the country and processing plant but not to the raw material stage as keeping records and sample identity are not feasible. Newer approaches to identify the source of risk are a challenge.

Indian situation of animal products safety

Indian situation is unique in that it is production by masses rather than mass production observed in western countries. While production, processing and utilization is of small scale but monitoring quality and safety aspects and following international trade requirements has been a problem of multi dimension.

A number of constraints are observed in assuring food safety in Indian situation:
1. Small scale production with multimillion units distributed over a range of agro-climatic and economic zones resulting sampling problems.
2. Inadequate means for adopting quality control aspects by primary producers.
3. Unreasonable economics of quality control programmes. In ability to demonstrate investments on quality aspects would fetch beneficial returns.
4. Lack of modernization in production and processing.
5. Inadequate and poor quality infrastructure for monitoring food safety aspects particularly microbes and residues.
6. Processing and consumption pattern
7. Low per capita consumption and lack of awareness
8. Lack of risk demonstration
9. Diverse feed resources and climatic condition
10. Indiscriminate use of chemicals and pesticides
11. Unorganized production and processing
12. Constraints of potable water supply affecting hygiene
13. Lack of appropriate sampling plans
14. Natural calamities and consequences
15. Lack of cold chain facilities

Chemical and Pesticide residues

The per capita use of chemicals and pesticides in Indian agriculture is quite lower compared to other developed countries and hence the chance of finding residues in meat and eggs above the permitted levels may not exist. However in the absence of an acceptable monitoring plan to demonstrate the status of contaminants a full advantage may not be realized.

Indian Regulations in Livestock and Poultry Sector:

Slaughter of animals is a state subject regulated under State Animal Preservation Acts and rules made there under. Slaughter houses including poultry processing are controlled by local bodies. Other acts, orders and rules are:

The prevention of cruelty to animals act, 1960.
The prevention of food adulteration act, 1954.
Air (prevention and control of pollution) act, 1981.
Environment (protection) act, 1986.

Export (quality control and inspection) act, 1963.
Export (Quality control and Inspection) Rules, 1964.

WTO, OIE and Codex

Harmonization of food standards is generally viewed as a prerequisite to the protection of consumer health as well as allowing the fullest possible facilitation of international trade. Agreement on the application of sanitary and phytosanitary measures (SPS Agreement) and Technical Barriers to Trade (TBT) Agreement of WTO recognizes international standards, guidelines and recommendations, including the Codex Alimentarius as reference points for facilitating international trade and resolving trade disputes in international law. The Codex Alimentarius Commission (CAC) shall be responsible for making proposals to, and consulted by FAO and WHO on all matters pertaining to the implementation of the joint activities significantly in a number of practical ways. The WHO is a joint sponsor of the CAC with FAO. To adopt Codex standards, countries require an adequate food law as well as a technical and administrative infrastructure with the capacity to implement it and ensure compliance. WTO under SPS Agreement recognizes for food safety, the standards, guidelines and recommendations established under the auspices of the International Office of Epizootics (OIE); for plant health, the international
standards, guidelines and recommendations developed under the auspices of the International Plant Protection Convention (IPPC) and for matters not covered by the above organizations, appropriate standards, guidelines and recommendations promulgated by other relevant international organizations open for Membership to all members, as identified by the Committee.

Codex standards

One of the principal purposes of the Codex Commission is the preparation of food standards and their publication in the Codex Alimentarius. Codex develops commodity standards which are product specific and general standards which have across the board application to all foods and are not product specific. A number of codex standards have dealt with safety aspects of animal products. Some important standards with a brief mention of scope and content are as follows:

Hygiene standards

Effective hygiene control is considered vital to avoid the adverse human health and economic consequences of food borne illness, food borne injury, and food spoilage. Codex developed a general standard ‘Recommended international code of practice general principles of food hygiene’. These general principles should be used in conjunction with each specific code of hygienic practice, where appropriate and the guidelines on microbiological criteria.

Code of Hygienic practice for meat: Meat has traditionally been viewed as a vehicle for a significant proportion of human food borne diseases. A contemporary risk-based approach to meat hygiene requires that hygiene measures should be applied at those points in the food chain where they will be of greatest value in reducing food borne risks to consumers. The scope of this code covers hygiene provisions for meat from the time of live animal production up to the point of retail sale. Meat hygiene is by nature a complex activity and this code refers to standards, texts, and other recommendations developed elsewhere in the Codex system where linkages are appropriate.

Code of hygienic practice for processed meat and poultry products

The code applies to processed meat and poultry products and contains the minimum requirements of hygiene in the production, handling, packaging, storing and transportation of processed meat products to assure a healthful and wholesome supply of such products. HACCP system has been applied to the code and a number of other codes have been considered in preparing the code.

Contaminants and toxins in foods

The standard ‘Codex general standard for contaminants and toxins in foods’ contains the main principles and procedures which are used and recommended by the Codex Alimentarius in dealing with contaminants and toxins in food and feeds, and lists the maximum levels of contaminants and natural toxicants in foods and feeds which are recommended by the CAC to be applied to commodities moving in international trade.
Reduction of source directed food contamination with chemicals

Codex has formulated 'Code of practice for source directed measures to reduce contamination of food with chemicals' with the main objective to increase awareness of sources of chemical contamination of food and feed, and of source directed measures to prevent such contamination. This means that measures recommended in the document may lie outside the direct responsibility of the food control authorities and Codex. Essentially, these approaches consist of a) measures to eliminate or control the source of contamination, b) processing to reduce contaminant levels and c) measures to identify and separate contaminated food from food fit for human consumption.

Good Laboratory Practice in pesticide residue analysis

Codex developed 'Guidelines on good laboratory practice in pesticide residue analysis' and the components of the code covering the analyst, basic resources (the laboratory and equipment and supplies) and the analysis comprising avoidance of contamination, reception and storage of samples, standard operating procedures (SOPs), validation of methods, maintenance of overall analytical performance, confirmatory tests, the concept of lower practical levels for the determination of residues of pesticides and expression of results.

Control of the use of veterinary drugs

The Code 'Recommended international code of practice for control of the use of veterinary drugs' set out guidelines on the prescription, application, distribution and control of drugs used for treating animals, preserving animal health or improving animal production. Good practice in the use of veterinary drugs (GPVD), as defined by the CCRVDF, is the official recommended or authorized usage including withdrawal periods, approved by national authorities. To avoid the presence of unacceptable residues in meat or other by-products of animal origin, it is essential that the livestock owner adheres to the withdrawal period laid down for each product and dose regime or to a suitably lengthy withdrawal period, prescribed by a veterinarian, where none is specified. If animals are sold before the end of the withdrawal period, the buyer must be informed. Regular feedback or information to veterinarians and manufacturers on suspected adverse reactions should be encouraged.

Good Animal feeding

Codex has recommended 'Code of practice on good animal feeding' with the objective to help ensure the safety of food for human consumption through adherence to good animal feeding practice at the farm level and good manufacturing practices (GMPs) during the procurement, handling, storage, processing and distribution of animal feed and feed ingredients for food producing animals. All feed and feed ingredients should meet minimum safety standards. Codex Maximum Residue limits and Extraneous Maximum Residue Levels set for feed should be applied. MRLs for food set by Codex may be useful in determining minimum safety standards for feed. The presence of undesirable substances should be identified, controlled and minimized.

Food additives

'General principles for the use of food additives' were adopted by the Ninth Session of Codex Alimentarius Commission as an advisory text. All food additives, should have been or
should be subjected to appropriate technological testing and evaluation. This evaluation should take into account among other things any cumulative, synergistic or potentiating effects of their use. Only those food additives should be endorsed which so far as can be judged on the evidence presently available, present no hazard to the health of the consumer at the levels of use proposed. Use of food additives is justified only where they serve one or more of the following purposes: to preserve the nutritional quality of the food, to provide necessary ingredients or constituents for foods manufactured for groups of consumers having special dietary needs, to enhance the keeping quality or stability of a food or to improve its organoleptic properties (but does not deceive the consumer) and to provide aids in the manufacture, processing, preparation, treatment, packing, transport or storage of food not to disguise the effects of the use of faulty raw materials or undesirable practices or techniques during the course of any of these activities.

Microbiological criteria for foods

Codex recommended "Principles for the establishment and application of microbiological criteria for foods" to give guidance on the establishment and application of microbiological criteria for foods at any point in the food chain from primary production to final consumption. A microbiological criterion for food defines the acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms including parasites, and or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot. A microbiological criterion should be established and applied only where there is a definite need and where its application is practical. The criterion should be technically attainable by applying Good Manufacturing Practices. To fulfill the purposes of a microbiological criterion, consideration should be given to: the evidence of actual or potential hazards to health; the microbiological status of the raw material(s); the effect of processing on the microbiological status of the food; the likelihood and consequences of microbiological contamination and/or growth during subsequent handling, storage and use; the category(s) of consumers concerned; the cost benefit ratio associated with the application of the criterion; and the intended use of the food.

The microorganisms included in a criterion should be widely accepted as relevant—as pathogens, as indicator organisms or as spoilage organisms—to the particular food and technology. Organisms whose significance in the specified food is doubtful should not be included in a criterion. Where pathogens can be detected directly and reliably consideration should be given to testing for them in preference to testing for indicator organisms. Whenever possible, only methods for which the reliability (accuracy, reproducibility, inter-and intra laboratory variation) has been statistically established in comparative or collaborative laboratories should be used. Limits used in criteria should be based on microbiological data gathered at various production establishments operating under Good Hygiene Practices (GHPs) and applying the HACCP system. A well designed sampling plan defines the probability of detecting microorganisms in a lot, but it should be borne in mind that no sampling plan can ensure the absence of a particular organism. The test report shall give the information needed for complete identification of the sample, the sampling plan, the test method, the results and if, appropriate, their interpretation.

Microbiological risk assessment

Risk assessment of microbiological hazards in food are described in the code "Principles and guidelines for the conduct of microbiological risk assessment". Microbiological Risk
Analysis is a process consisting of three components—risk assessment, risk management, and risk communication, which has the overall objective of ensuring public health protection. There should be a functional separation between risk assessment and risk management for an unbiased process. Risk assessment should be conducted according to a structured approach that includes hazard identification, hazard characterization, exposure assessment, and risk characterization. Microbial pathogen levels can be dynamic and while they may be kept low, for example, by proper time/temperature controls during food processing, they can substantially increase with abuse conditions. Surveillance programs can provide an ongoing opportunity to reassess the public health risks associated with pathogens in foods as new and relevant data become available and a microbiological risk assessment may need to be revised.

**Ethics for International trade in foods**

The 'Code of Ethics for International Trade in Food' was developed in the light of the consideration that many countries—particularly developing countries do not yet have adequate food control infrastructures to protect consumers against possible health hazards in food and against fraud. Specific requirements of the code include: Food standards, Food Hygiene, Labeling, Food additives, Pesticide residues, Microbiological contaminants, Other contaminants, Irradiated food, Foods for infants, children, and other vulnerable groups, and Nutritional aspects concerning in particular vulnerable groups and regions where malnutrition exists.

**Food import control systems**

The code 'Guidelines for food import control systems' provides a framework for the development and operation of an import control system to protect consumers and facilitate fair practices in food trade while ensuring unjustified technical barriers to trade are not introduced. Appropriate level of protection (ALOP) has been defined as the level of protection deemed appropriate by the country establishing a sanitary measure to protect human life within its territory (This concept may otherwise be referred to as the ‘acceptable level of risk’). Food import control systems should have the following main characteristics:

(a) requirements for imported food that are consistent with requirements for domestic foods  
(b) clearly defined responsibilities for the competent authority  
(c) clearly defined and transparent legislation and operating procedures  
(d) precedence to the protection of consumers  
(e) provision of the importing country for recognition of the food control system applied by an exporting country’s competent authority  
(f) uniform nationwide implementation  
(g) implementation that ensures the levels of protection achieved are consistent with those for domestic food.

**Equivalence of sanitary measures**

In order to facilitate trade with mutual benefit it is desirable to consider the effectiveness of sanitary measures of the exporting country in achieving the appropriate level of sanitary protection of the importing country, consistent with the principle of equivalence as provided for in the WTO, SPS agreement (Article 2.3). Importing countries should avoid the application of unnecessary measures when they have already been carried out by the exporting country. For the purpose of determining equivalence Codex developed 'Guidelines on the judgement of
Equivalence is the state wherein sanitary measures applied in an exporting country, though different from the measures applied in an importing country, achieve, as determined by the exporting country, the importing country’s appropriate level of sanitary protection. Judgement of equivalence by the importing country should be based on a transparent analytical process that is objective and consistent, and includes consultation with all interested parties to the extent practicable and reasonable.

**Conclusion:** There is need for establishing appropriate regulatory body with adequate infrastructure facilities for assuring quality of poultry products for domestic and export trade along with large scale investments for developing processing sector.

**List of some codex standards**

- Codex Standard for Luncheon Meat (CODEX STAN 89-1981)
- Code of practice on good animal feeding (CAC/RCP 54-2000)
- Principles for the establishment and application of microbiological criteria for foods (CAC/GL 21-1997)
- Principles and guidelines for the conduct of microbiological risk assessment (CAC/GL- 30(1999))
- General principles for the use of food additives 1 (CAC/ MISC I-1972)
- Code of practice for source directed measures to reduce contamination of food with chemicals (CAC/RCP 49-2001)
- Recommended international code of practice for control of the use of veterinary drugs (CAC/RCP 38-1993)
- Guidelines on good laboratory practice in pesticide residue analysis (CAC/GL 401)
- Codex general standard for contaminants and toxins in food (Codex Stan 193-1995 (Rev.1-1997))
- Recommended International code of practice
- General principles of food hygiene (CAC/RCP 1-1969, Rev. 4-20031)
- Draft Code of Hygienic Practice for Meat (at Step 8)

**BIS STANDARDS FOR CHICKEN PRODUCTS**

<table>
<thead>
<tr>
<th>No.</th>
<th>Standard Code</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>2.</td>
<td>IS 4674: 1975</td>
<td>Dressed chicken (first revision)</td>
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<tr>
<td>3.</td>
<td>*IS 4723: 1978</td>
<td>Egg powder (first revision)</td>
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<td>4.</td>
<td>IS 5558: 1970</td>
<td>Chicken essence</td>
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<td></td>
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<td>----------------------------------------------------------------------</td>
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<td>7.</td>
<td>IS 5960 (PT 3): 1970</td>
<td>Methods of test for meat and meat products: Part 3 Determination of total fat content</td>
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<td>21</td>
<td>IS 6558: 1972</td>
<td>Code of practice for cold storage of shell eggs</td>
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<td>22</td>
<td>IS 6559: 1972</td>
<td>Code of practice for anti-mortem and post-mortem inspection for poultry</td>
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<tr>
<td>23</td>
<td>IS 7049: 1973</td>
<td>Code for handling, processing, quality evaluation and storage of poultry</td>
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<td>25</td>
<td>IS 9800: 1993</td>
<td>Day-old chicks (Layer/broilers)- Basic requirement (first revision)</td>
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<td>26</td>
<td>IS 9810: 1981</td>
<td>Method for evaluation of quality of fresh chicken eggs</td>
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<td>27</td>
<td>IS 10382: 1982</td>
<td>Edible egg albumen- powder</td>
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<td>28</td>
<td>IS 10697: 1983</td>
<td>Chicken, canned in brine</td>
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<tr>
<td>29</td>
<td>IS 12541: 1988</td>
<td>Chicken curry, canned</td>
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<td>30</td>
<td>IS 12543: 1988</td>
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<td>31</td>
<td>IS 12561: 1988</td>
<td>Pickled quail eggs</td>
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<td>32</td>
<td>IS 13400: 1992</td>
<td>Chicken sausages</td>
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<td>33</td>
<td>IS 13401: 1992</td>
<td>Determination of Thiobarbituric acid value in meat</td>
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### Table 1: EU microbiological meat standard (minced meat)

<table>
<thead>
<tr>
<th>Microbial types</th>
<th>n</th>
<th>c</th>
<th>m</th>
<th>M</th>
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</thead>
<tbody>
<tr>
<td>Aerobic mesophilic counts</td>
<td>5</td>
<td>2</td>
<td>500000/g</td>
<td>500000/g</td>
</tr>
<tr>
<td><em>Salmonella</em> absent in 10g</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5</td>
<td>2</td>
<td>50/g</td>
<td>500/g</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>5</td>
<td>2</td>
<td>100/g</td>
<td>1000/g</td>
</tr>
</tbody>
</table>

(Todd (2003))

### Table 2: EU microbiological standard for egg products

<table>
<thead>
<tr>
<th>Microbial types</th>
<th>Number</th>
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<tbody>
<tr>
<td>Aerobic mesophilic counts</td>
<td>500000/g</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Absent in 25g</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>100/g</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Absent in 1g</td>
</tr>
</tbody>
</table>

(Malik (2003))

### Table 3: BIS specification for microbial limits in egg powder

<table>
<thead>
<tr>
<th>Microbial types</th>
<th>Number (Maximum allowable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacterial counts</td>
<td>75000/g</td>
</tr>
<tr>
<td>Coliform counts</td>
<td>100/g</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Absent in 25g</td>
</tr>
<tr>
<td>Yeas and mould counts</td>
<td>50/g</td>
</tr>
</tbody>
</table>

(Source: IS: 4723-1979.)

### Table 4: Codex microbial standards for poultry meat

<table>
<thead>
<tr>
<th>Microbial types</th>
<th>Class A Satisfactory</th>
<th>Class B Acceptable</th>
<th>Class C Unsatisfactory</th>
<th>Class D Unacceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic plate counts</td>
<td>&lt;10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;5&lt;/sup&gt;-&lt;10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>&gt;10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>Not detected in 25g</td>
<td>N/A</td>
<td>N/A</td>
<td>Present in 25g</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>&lt;20</td>
<td>20-&lt;100</td>
<td>&gt;1000</td>
<td>N/A</td>
</tr>
<tr>
<td><em>E. coli</em> O157</td>
<td>Not detected in 25g</td>
<td>N/A</td>
<td>N/A</td>
<td>Present in 25g</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>Not detected in 25g</td>
<td>N/A</td>
<td>N/A</td>
<td>Present in 25g</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Not detected in 25g</td>
<td>N/A</td>
<td>N/A</td>
<td>Present in 25g</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>Not detected in 25g</td>
<td>N/A</td>
<td>N/A</td>
<td>Present in 25g</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>&lt;20</td>
<td>20-&lt;100</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;-&lt;10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>&gt;10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>&lt;20</td>
<td>20-&lt;100</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;-&lt;10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&gt;10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td>&lt;20</td>
<td>20-&lt;100</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;-&lt;10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&gt;10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>&lt;10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;-&lt;10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;-&lt;10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>&gt;10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

N/A = Not applicable

(Source: Codex Alimentarius Commission (Website: http://www.codexalimentarius.net/index_en.sim).
Table 5: Maximum residue limits (MRLs) of pesticides (ppm) in poultry meat

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>PFA rules</th>
<th>Codex standard</th>
<th>U.S. standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meat</td>
<td>Egg</td>
<td>Meat</td>
</tr>
<tr>
<td>Aldrin/dieldrin</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>Chlordane</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Benomyl</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>Chlorfenvinfos</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.1</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>0.02</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>DDT &amp; its metabolites</td>
<td>7.0</td>
<td>0.5</td>
<td>5.0</td>
</tr>
<tr>
<td>2,4-Dichlorophenoxyacetic acid</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Decamethrin/Ethyldecamethrin</td>
<td>-</td>
<td>-</td>
<td>0.03</td>
</tr>
<tr>
<td>Ethion</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Edifenphos</td>
<td>0.02</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>0.15</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lindane</td>
<td>2.0</td>
<td>0.10</td>
<td>0.7</td>
</tr>
<tr>
<td>Monocrotofos</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Phenthoate</td>
<td>0.05</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Propoxur</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>

- Data not available

Source: MOHFW (2004); FAO (2005); EPA (2000)
Table 6: Codex maximum residue limits (MRLs) and acceptable daily intake (ADI) levels of some important antimicrobial drugs in food of animal origin

<table>
<thead>
<tr>
<th>Antimicrobial Drugs</th>
<th>Acceptable daily intake (μg/kg)</th>
<th>Target tissue (ppm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>-</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Benzyl procaine</td>
<td>0-30</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Benzyl penicillin</td>
<td>-</td>
<td>0.300</td>
<td>0.300</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>-</td>
<td>0.030</td>
<td>0.030</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>-</td>
<td>0.400</td>
<td>0.400</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>-</td>
<td>0.100</td>
<td>0.200</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0-20</td>
<td>0.500</td>
<td>0.100</td>
</tr>
<tr>
<td>Sulfadimidine</td>
<td>0-50</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Sulfonamides (in combination)</td>
<td>0-50</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0-50</td>
<td>0.600</td>
<td>0.600</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0-30</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0-30</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>(OTC+CTC+TC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>-</td>
<td>0.050</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Data not available; Source: CAC (2005)

Table 7. Codex maximum residue limits (MRLs) in eggs

- Oxytetracycline, Tetracycline, 200 μg/kg
- Chlorotetracycline (Singly or combination)
- Amprolium 1000 μg/kg
- Chloramphenicol 0.1 μg/kg
- Flubendazole 400 μg/kg
Poultry Marketing in India – Problems, Prospects and The WTO

Sandeep Saran
Central Avian Research Institute, Izatnagar-243 122

Poultry is one of the fastest growing segments of agricultural sector in India with an average growth rate of 8 to 15 % per annum. As a result, India is now the world's 4th largest egg producer and the 5th major producer of broilers. The estimated egg and meat production in India has steadily gone up to 44 billion eggs and 1.6 billion broilers in 2004. India’s contribution to world’s egg and chicken production was nearly 4% and 2%, respectively (Bootwala, 2005). With a turnover of over Rs. 300 billion, the Industry provided direct-indirect employment to over five million people and has great potential to create gainful employment with every increase in egg/meat consumption. Poultry sector accounted for about 1% of the India’s Gross Domestic Product (GDP) and 10% of the livestock GDP (Mehta et.al. 2002). Consistent with the increase in production and productivity, the per capita availability also increased to 44 eggs and 1.76 kg poultry meat per annum, which is far below the recommended levels of 180 eggs and 11 kg of poultry meat, suggested by Nutritional Advisory Committee, Government of India. Above this, highly skewed distribution/consumption pattern of egg and meat further deteriorates the situation in rural areas. In fact, only 25% population residing in urban areas consumes 65% eggs and 70% of poultry meat and in a typical Indian village the egg consumption is not more than 10 eggs per capita per year. Bridging the nutritional gap to attain the recommended nutritional level is expected to provide nutritional security to all Indians besides creating over 10 million jobs (All India Poultry Business Directory, 2003-04). The selected indicators of development of the Indian poultry sector are presented in Table 1.

Table 1: Selected indicators of poultry development

<table>
<thead>
<tr>
<th>Indicators</th>
<th>1970-71</th>
<th>2003-04</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg production</td>
<td>0.27 MMT (5.3 billion)</td>
<td>2.2 MMT (44 billion)</td>
</tr>
<tr>
<td>Commercial broiler production</td>
<td>0.12 million MT</td>
<td>1.6 million MT</td>
</tr>
<tr>
<td>Human Population</td>
<td>548.2 million</td>
<td>1020 million</td>
</tr>
<tr>
<td>Per capita egg availability</td>
<td>10 / annum</td>
<td>44 / annum</td>
</tr>
<tr>
<td>Per capita meat availability</td>
<td>0.22 kg</td>
<td>1.76 kg</td>
</tr>
<tr>
<td>Turnover poultry sector</td>
<td>N.A.*</td>
<td>Rs. 300 billion</td>
</tr>
<tr>
<td>Total poultry population</td>
<td>138.5 million</td>
<td>457.4 million</td>
</tr>
<tr>
<td>Poultry feed production (by organized sector)</td>
<td>N.A.*</td>
<td>13.2 million MT</td>
</tr>
</tbody>
</table>

*Data not available
Despite the spectacular growth of the poultry sector in India, the market inefficiencies, as are inherent in any developing economy, lead to the marketing problems especially for perishable articles like poultry eggs and meat. The Indian poultry marketing system is plagued by erratic demand – supply patterns in respect of major poultry inputs, consequent unstable and volatile prices of poultry products, large scale operation of middle men, and too few hands controlling large poultry markets, etc. During 2001-2002, excessive supply of chicks and eggs forced poultry farmers to cull their birds prematurely. Even some of the hatcheries in southern parts of the country had to take a collective decision to stop setting of eggs (chick production holiday) for hatching for at least a week so as to balance supply and demand of chicks. Similarly on inputs front, Government is often faced with the challenges of safeguarding mutually conflicting interests of maize producers on one hand and the poultry industry as a major consumer of maize on the other. Obviously, deficiencies in the marketing system of egg and poultry meat are responsible for such situations. Growth in marketing infrastructure has not been able to keep pace with the fast expanding poultry industry, which has to suffer from frequent depressions in its growth path leading to auto adjustments in demand and supply of poultry products.

The Vicious Circle:

It has been observed that poultry industry suffers market related setback every 8-10 years, which triggers a sort of business cycle in the poultry sector. The following flow diagram illustrates the sequence of events leading to one another forming the vicious circle in poultry sector. A lucrative profit in poultry business, at one time, prompts and attracts new entrants leading to excessive demand for chicks and poultry feed on one hand and over production of eggs and meat on the other resulting in lowering of their prices. Thus, seemingly high profit margins in poultry business soon disappear due to high input cost (on account of increased feed and chicks cost) and low market prices. The situation forces small farmers and new players to down their shutters and thus auto adjustments in demand for feed and chicks take place. With large number of small players rendered out of business, the excessive supply situation in respect of eggs and meat is also automatically corrected leading to reappearance of profit margins. In this cycle, inability of markets to absorb over production of chicks and eggs leads to depression in prices of these products. If market infrastructure also keeps growing with the expanding poultry production, the poultry produce could be channelized to rural markets. However, actually this does not happen and poultry farmers are forced to incur losses on one hand and poultry products remain out of reach of rural people. Hence, marketing infrastructure needs to be strengthened so that with expanding poultry production, newer demand areas could also be tapped.
**Bottlenecks in Poultry Marketing:**

Marketing is simply the performance of all business activities in the flow of poultry products and services from the point of initial production until they reach the hands of consumer. The marketing machinery continues to make adjustments to changes in poultry production pattern and trends in food consumption. However, these adjustments in marketing machinery are limited by certain bottlenecks which are specific to the poultry industry in addition to the general shortcomings with which the whole agricultural marketing system suffers. Some of the important ones are discussed hereunder.

It has been estimated that that real prices of eggs (adjusted for consumer price index) in various important wholesale egg markets have gone down by over 29% during the last two decades. Highly perishable nature of poultry products and lack of suitable marketing agents such as cold chains are the most important factors due to which mostly small and medium farmers suffer. Eggs can't be stored for long due to their limited shelf life. Non-availability of proper storage and transport facilities hamper the product movement to far-flung areas. The product movement is restricted to certain well-defined transport routes and remotely connected areas remain out of bound for poultry products. India has certain well-defined poultry belts where bulk of production takes place (A.P., Tamil Nadu, Kerala and Karnataka in South and Haryana, Punjab, Delhi and West U.P. in north) as most of the poultry units are concentrated in these areas. However, due to limited processing and value addition facilities, any surplus production has a depressing effect on poultry prices. Lack of infrastructural facilities such as egg grading, washing, packaging, dry go-down and automatic dressing plants etc. at both production centres and key ports are other limiting factors reducing competitiveness of Indian products in international markets.
Further, non adherence to international quality standards (HACCP and GMP norms), unhygienic and unscientific methods of product handling and excessive use of pharmaceuticals without bothering for their toxic residual effects in poultry products deteriorates their prospects to find a place in export markets. By and large the Made in India label is considered to be sub-standard produce. Besides, some of the flaws in poultry marketing system are specific to rural markets such as excessive dependence of poultry farmers on middlemen who siphon off most of the profits by manipulating prices to their advantage, improper methods of sale (sale under cover, sale by counting of birds and not by weight) and improper methods of weighing etc. Consequently, prices which producers receive for their products have little relationship with supply/demand position since small producers are at the receiving end as price takers. Non-availability of quality chicks, feed, medicine and technical support in respect of health and hygiene of birds in rural areas due to non-existent input markets result in high mortality, reduced growth, poor feed conversion efficiency and increased production cost. Being in higher demand due to preference for ‘desi’ products and limited supply of commercial eggs and meat in villages, prices of poultry products usually rule higher in rural areas as compared to urban areas. Moreover, rural markets are unable to absorb even meager quantities of poultry products due to limited purchasing power of consumers and availability of other cheaper substitutes.

Traditionally, eggs and meat have not featured prominently in Indian diets. A significant proportion of vegetarian population considers eggs to be of animal origin. Another problem which applies to a cross section of population is the belief that consumption of eggs during hot weather could be harmful. Such factors depress the demand for poultry products during summers. This is reflected in a marked seasonality in egg chick placing.

<table>
<thead>
<tr>
<th>Months</th>
<th>Eggs-All India</th>
<th>Broilers-Delhi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>109.23</td>
<td>101.12</td>
</tr>
<tr>
<td>Feb</td>
<td>96.58</td>
<td>96.88</td>
</tr>
<tr>
<td>Mar</td>
<td>92.37</td>
<td>98.02</td>
</tr>
<tr>
<td>Apr</td>
<td>82.60</td>
<td>93.88</td>
</tr>
<tr>
<td>May</td>
<td>92.00</td>
<td>91.54</td>
</tr>
<tr>
<td>Jun</td>
<td>102.96</td>
<td>98.44</td>
</tr>
<tr>
<td>Jul</td>
<td>104.15</td>
<td>100.48</td>
</tr>
<tr>
<td>Aug</td>
<td>96.76</td>
<td>104.79</td>
</tr>
<tr>
<td>Sep</td>
<td>98.87</td>
<td>104.84</td>
</tr>
<tr>
<td>Oct</td>
<td>100.90</td>
<td>102.25</td>
</tr>
<tr>
<td>Nov</td>
<td>111.68</td>
<td>104.49</td>
</tr>
<tr>
<td>Dec</td>
<td>111.90</td>
<td>103.25</td>
</tr>
</tbody>
</table>


1 HACCP: Hazard Analysis Critical Control Point – an internationally recognized quality auditing method; GMP: Good Manufacturing Practices.
The seasonal behaviour of eggs and chicken demand is reflected in the seasonal indices (Table 2) worked out on the basis of monthly wholesale price data pertaining to major markets in the country. Egg as well as chicken prices were observed to attain a peak during winters whereas the prices dipped to their lowest levels during summers. The fluctuations in the prices of poultry products could also be attributed to festivals falling in specific months. In the Western region of the country, religious observances significantly reduced poultry consumption for about 3 months in a year. On the other hand, in West Bengal, an increase in poultry consumption was associated with the Durga Puja festival, and no significant seasonal down swings in consumption were reported. With limited frozen storage facilities or inter-regional movement of live birds, the seasonal swings in demand contributed to volatility in market prices of poultry meat in some regions. Preference of Indian consumers for fresh poultry products rather than frozen and preserved ones, is another factor posing a serious problem before producers and marketing agents. Maintaining live birds at retail outlets is difficult and involves huge cost in terms of feed and loss of body weight.

Prospects of Wet Markets:

Almost 95% poultry meat in India is marketed in the form of live birds (Jain, 2005) and the rest as chilled or frozen value added products. The cost of handling live birds, their transportation, losses due to shrinkage and mortality severely limit interregional movements. Therefore, Indian poultry markets (especially broilers) have a regional character and scope rather than national, which also limits the opportunities for low-cost producers to market their products in higher cost regions (Sachdev, 2001). This prevents them from reaping higher profits and also exposes them to vagaries of seasonality in demand and prices. Absence of cold chains and supportive infrastructure (electricity, cold storages, refrigerated vans etc.) along with consumers' preference for freshly slaughtered birds (Rosario, 1997) are the biggest hurdles in expansion of processing sector in India. Dominance of wet markets restricts the imports of frozen poultry products. Small domestic processing sector, which is likely to expand in near future, serves the demand for frozen poultry products by hotel industry and high-end urban customers. Thus, the live-bird market is expected to still dominate in India for the next few years (Shyam Sunder et al., 1999) till household consumers moved to opt frozen/chilled products.

It is important for poultry players to focus on wet market (retailing fresh products rather frozen or preserved ones) as well as processed products' market. Currently, less than 5% of total poultry output is processed which leads to substantial price differentials between processed and fresh meat (the production cost of fresh meat is Rs.40/kg while that of processed one is Rs 52/kg)(Anonymous, 2001). Due to price differentials and slow pace of development of modern retailing formats in smaller towns and rural areas, the fresh market is likely to dominate the marketing scene. Moreover, cost conscious consumers are also skeptical of the quality of processed meat and unaware of its weight benefits (1.3 kg fresh bird is equivalent to 1 kg processed meat). The wet markets which account for 95% of poultry sales are largely ignored by organized players because their costs are not competitive. However, backward integration and contract poultry farming can help organized players to bring down their cost and be competitive.
in market. Therefore, investments in modern abattoirs and distribution systems on one hand and aggressive campaigns for marketing processed products on the other are urgently needed.

**Post WTO Scene:**

The area that was supposed to benefit developing countries most as a result of the implementation of the Agreement on Agriculture was greater market access and larger amounts of exports to the developed countries, as a result of their being more competitive when subsidies in the OECD (Organization for Economic Co-operation and Development) countries are reduced and trade barriers are lowered. Unfortunately, after five years of implementation, the expected market access opportunities have not materialized. The FAO has reported that, 'On the whole, few studies reported improvements in agricultural exports in the post-UR period – the typical finding was that there was little change in the volume exported or in diversification of products and destinations'.

Ironically, there seems to have been more exports from the developed countries into the markets of developing countries. The FAO reports that for developing countries, 'Food imports were reported to be rising rapidly in most case studies. According to UNCTAD statistics, the share of developing countries in world agricultural exports remains low: from 31.7% in 1970-72, it fell to 25.4% in 1990-92 before increasing to 30.7% by 1996-97. This is a figure that is smaller than 25 years earlier. In contrast, between 1980 and 1996, the annual growth of exports by OECD countries of primary agricultural commodities and processed agricultural products was respectively, 2.5% and 6.5%.

**Tariff Rates of Poultry Products and Threats of Dumping:**

In 1999-2000, the range of tariff rates was 15% (of meat and edible offal) to 40% (of live poultry and food preparations of poultry products). During the same financial year, tariff rate of maize for use for poultry feed was nil, which was increased to 70% in the budget proposals of 2000/1. However, the tariff rate has been declined to 15%, with the adoption of Tariff-Quota Regime. All other products of the poultry sector attracted tariff rate of 35% during last two years because the Quantitative Restrictions (QRs) on these items were removed. The import policy of 2000-01 and 2001-02, has already removed QRs on all poultry products. Most of these products were restricted items before 1999-2000. After the removal of QRs of these items, tariff rates are the most important instrument in India’s import policy. In the budget proposals for 2000-01, the government had announced 35% tariff rate for items of the poultry sector (and items of other sectors) for which QR is removed (Mehta, 2002).

Following the removal of quantitative restrictions in the post WTO regime, the poultry farmers are apprehensive about possible tariff cuts which may trigger surge in import of chicken legs (drum sticks) from countries where breast fillets are preferred over drum sticks. As per 2002 figures, the price of imported eggs was Rs.2.84/each, that of chicken Rs.66.69/kg, chicken legs Rs. 34.86/kg and boneless meat Rs. 102.69/kg in India (the effective rates of import duty on

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2 Except for four tariff lines, whose tariff rate is 100 per cent see Table II.3 for details.
3 Poultry Times of India, Sept.2002. Vol. 9, pp.11
eggs being 40%, that on egg products 62.8%, chicken meat 19.6% and processed chicken products 57.2%). However, the domestic market prices for eggs, chicken, chicken legs and boneless meat are Rs.2.00 each, Rs.65/kg, Rs.90/kg and Rs.140/kg, respectively. Thus, differentials in market prices of domestic products and landing prices of imported products may trigger large-scale imports harming the interests of domestic producers and flooding the markets with imported stuffs. The local small-scale producers may be forced to pack up and leave the field. The domestic industry is price competitive only in eggs. However, some studies have shown that Indian whole chicken and chicken products do not show much competitive advantage over other suppliers. The price in India of whole chicken is around 30-40% higher than the import price of Brazilian chicken. In addition, it should be noticed that there is not much significant difference in prices of different cuts of chicken in India, while the prices in other countries vary significantly for different cuts, like breast meat, thigh meat and leg quarters. There are several reasons why local poultry products are relatively expensive compared to imported products.

First, there is a big difference in the size of poultry farms operated here and abroad. In India, there are about 1 million poultry farmers of whom 95% have 500 to 5000 birds. One with 50,000 birds and above is considered a big farmer in India. But in the United States, an average poultry farmer maintains a flock of 0.4-0.5 billion birds.

Second, a farmer in India has to buy maize feed (for poultry) at around $130 per tonne, while his counterpart in US pays only $80 per tonne. Since the feed cost accounts for nearly 75% of the cost of production of eggs and chickens, the relatively higher price of maize in India leads to higher costs of production.

Third, US and European poultry processors are said to earn their profits by selling their breast portion of chicken, which is conveniently promoted as lean/white meat at a premium price of around $3 per pound (or Rs.250 per kg) in their own markets. The leg portion (the leg quarter), on the other hand, is treated as dark meat and is targeted for dumping in Asian markets at a throwaway price of 20-25 cents per pound (i.e. around Rs.35 per kg). In the Indian market, the thigh and leg quarter is considered a delicacy and is preferred over the breast portion. Therefore, when the local markets are dumped by imported leg quarters at throwaway prices, local producers are definitely going to be hurt.

Fourth, foreign governments, especially the US and EU, support poultry exports with subsidies such as the Restitution Money Scheme of the European Union, and the Export Enhancement Scheme of US. The amount of subsidy works out to be more than 25% of the domestic price in EU, and 40% in the US. The result is an unlevel playing field in which the ball inevitably bounces towards the Indian goal. Therefore, suitable policy interventions are absolutely essential to safeguard the interests of indigenous poultry industry.

1 http://commerce.nic.in/wto_sub/Agri/sub_g37.htm
SPS as a Trade Barrier:

FAO's investigation has revealed that more developing countries are experiencing trade obstacles due to SPS measures. A major problem is that there is a lack of mutual recognition of inspections and standards. Several major importing countries are asking for 'sameness' in the process, rather than 'equivalence'. Moreover, so far, only a handful of equivalence agreements have been agreed upon, all amongst developed countries.

The variation and stringency of measures for imported food products can be gleaned from the example of imported poultry meat. The different standards raises the issue of how arbitrary standard setting can be. Of the 135 countries which are WTO members, 15 are currently allowed to export fresh, chilled or frozen poultry meat to the EC, five may export to the US, one to Canada and none are allowed to export to Australia. FAO rightly comments that indeed, 'Trade harassment' is 'considered a common problem'. The huge obstacle for developing countries is the lack of financial or technical resources to implement these stringent requirements or even to take a significant role in the standard-setting process.

Institutional Support to Poultry Industry:

The Indian poultry industry is not very old. There were a host of problems in 1970s and early '80s as is common with any new industry during its initial growth phases. Rising primary input costs such as medicines, feed, electricity, taxes etc. coupled with domination of middlemen led to the crisis of 1981-82 when egg prices fell drastically and over 20,000 marginal poultry farmers lost their only source of livelihood. At that point, despite high input cost, output prices were not even sufficient to recover the production cost. Then in 1982, with the efforts of a group of farmers led by late Dr.B.V. Rao, the National Egg Coordination Committee (NECC) was formed which is an institution performing its designated functions right from its birth in order to ensure remunerative prices of poultry products and to minimize the role of middle men in poultry marketing. The major activities of NECC are (a) declaration of egg prices (b) market intervention through NAFED and Agro Corpex India Limited (NECC prevents undue fall in market prices of eggs due to over production or lack of demand through procurement from market and storing them in cold storages) (c) advertising, sales promotion, publicity and consumer education, (d) extension activities, (e) market research, (f) market identification and development, (f) export promotion through grant of subsidies etc. Due to these multifarious activities of NECC, it is often termed as the voice and soul of Indian egg industry.

Apart from NECC, other agencies involved in promotion of poultry marketing in India and abroad are Agriculture and Processed Food Products Export Development Authority (APEDA) – to provide links between Indian producers and global markets and National Agricultural Cooperative Marketing Federation of India Limited (NAFED) – a national apex body of cooperative marketing in the country.

High cost of feed ingredients, domestic shortages coupled with limited integration of various economic entities involved in poultry production lead to reduction in the competitiveness of Indian producers in export markets. Brand creation is also an uphill and expensive task for poultry producers. In order to overcome these problems, the poultry industry needs supportive
measures from government in the form of policy, fiscal and infrastructural support. Some of these are as under.

**Policy Support:**
- Promotion of higher production of important feed ingredients such as maize and soybean and monitoring of their exports/imports so that domestic shortages do not occur.
- Emphasis on quality production of maize, soybean and other agriculture based inputs by taking suitable measures to limit the use of pesticides and other plant protection chemicals.
- Support price policy for eggs and chicken through intervention by NAFED and other such public sector agencies.
- 100% export oriented units may be allowed to channelize their surpluses in the domestic markets freely.
- Creation of a nodal agency on the lines of NDDB to oversee development of poultry industry in India

**Fiscal Support:**
- Exemption of processed and branded chicken meat preparations from duties and levies at least for certain time period till the brands are established.
- Extending freight subsidy for exports till destination
- Rationalization of customs duty on raw material for animal health products.
- Rationalization in customs duty on capital equipments for processing, packaging, washing, grading and stamping etc.

**Infrastructural Support:**
- Godowns and warehouses for feed ingredients.
- Cold chain for distribution of poultry produce in domestic markets at affordable prices.
- Facilities for storage and transport at airports and sea ports and speedy handling of cargo etc.
- Establishment of modern poultry disease diagnostic, feed analytical and meat testing laboratories.
- Provision of R&D backup support in all areas of poultry production, processing and marketing.
- Establishment of modern training centres to cater to the manpower needs of poultry industry.

As per the US department of commerce and foreign commercial service figures, the estimated size of Indian food processing industry is Rs.339,500 crores of which packages food alone accounts for about Rs.106.700 crores. India's share in the global market is less than 1%. Moreover, there exists a wide gap between the current per capita availability and recommended levels of eggs and meat consumption for Indian population. Thus, poultry and processed products have tremendous potential in Indian and export markets. It has been estimated that a unit increase in per capita egg consumption would create about 25000 new jobs. Thus, poultry has enormous employment potential as well. All round efforts of all the concerned quarters to overcome the deficiencies of marketing and production systems will definitely lead India to secure top position in production and exports in the world.

**References:** On Request
The adulteration of costly meat with a cheaper one is a common malpractice in processed meat market. The identification of species of origin of meat presents considerable problems for food analysts. Correct species identification is important for the consumer for other reasons also such as medical requirements of individuals who have specific food allergies or religious taboos, apart from possible economic losses. Identification of species of origin of fresh meat has been achieved using various methods. Anatomical, histological and organoleptic methods are reliable only in unprocessed raw meats. Further, these methods are cumbersome, time consuming and not so highly specific and sensitive, hence, often fail to detect the differences between closely related species such as sheep and goat, cattle and buffalo etc.

Advent of DNA based techniques, popularly known as molecular techniques has provided several powerful tools for species differentiation. Since DNA has stability at high temperature and has conserved structure within all tissues of an individual, these techniques seem to be more optimum for meat differentiation. Some of these molecular techniques which have been used for identification of species in meat and meat products include polymerase chain reaction (PCR); restriction fragment length polymorphism (RFLP); random amplified polymorphic DNA (RAPD) fingerprinting; DNA hybridization and DNA sequencing. In an animal cell, DNA is present in nucleus as well as mitochondria. Both types of DNA were used for meat differentiation. The mitochondrial DNA is one of the highly conserved sequences in different species of animals. Recently, mt 12S rRNA gene was sequenced and analysed in a forensic case to prove unambiguously that, skin sample, which was claimed to be of tiger, was from bovine. The universal primers for mt 12S rRNA gene can amplify corresponding regions from a wide variety of organisms, including birds and insects. Mitochondrial 12S rRNA gene was exploited earlier to differentiate snail species. Similarly, PCR amplification of mt Cytochrome b gene and mt 16S rRNA gene have also been used for meat species identification. The high homogeneity of mitochondrial 12S rRNA gene can be exploited for developing a simple, quick and easy method for meat differentiation. On the other hand, presence or absence of specific size satellite DNA bands in a species can also be used as a method of differentiation among different meat species.

Protocol for PCR-RFLP of 12S rRNA gene for meat species identification

A. Isolation of DNA

Following simple protocol may be used for isolation of genomic DNA from raw / cooked or processed meat sample.

- Mince the fresh / cooked / processed meat sample and take about 100 mg of it and mix with 1.0 ml phosphate buffer saline (PBS), pH 8.0 and triturated thoroughly in sterile pestle and mortar.
- Transfer the triturate to 1.5 ml centrifuge tubes and centrifuge at 3000 rpm for 5 min.
Transfer 600 μl of supernatant to another micro centrifuge tube and add 30 μl of 10% SDS (sodium dodecyl sulphate) and incubated at 37°C for 30 min.

Add Proteinase K (0.1 mg/ml), vortexed and kept at 50°C for 1 hour.
Add 630μl of phenol: chloroform: isoamylalcohol (25:24:1) and mixed thoroughly.
Centrifuge the mixture at 10,000 rpm for 10 min and transfer the upper most aqueous phase to another micro centrifuge tube.
Add 0.2 volumes (120μl) of ammonium acetate (1M) solution and 700μl absolute alcohol. Mix and centrifuge at 12000 rpm for 10 min. Discard the aqueous phase, leaving a pellet at the bottom.
Wash the pellet with 1 ml of 70% alcohol and centrifuge at 10000 rpm for 5 min.
Dry the pellet at room temperature and dissolve it in 100μl of IX TE buffer. Keep it in water bath at 60°C for 2 hr to inhibit Dnase activity and to dissolve pellet properly in the TE buffer.
After 2 hr of incubation, keep the dissolved DNA at -20°C for further use.
Evaluation of quality and quantity of DNA

Quality of DNA

Run the dissolved DNA sample, after adding loading dye in 0.8 % agarose gel to check the quality of genomic DNA. Following protocol may be adopted for horizontal gel electrophoresis in 8 x 8 cm gel casting tray and mini horizontal gel electrophoresis assembly of Bangalore Geneti.

Prepare the gel casting tray by sealing its ends with adhesive tape. Place the comb and keep the comb.
Prepare the 25 ml of 0.8 % agarose (w/v) by dissolving 0.2 g agarose in 25 ml of 1 X TBE and heating the solution over gentle heat until the agarose is completely melted and dissolved.
After cooling to 60°C, add ethidium bromide (10 mg/ml @ 5 μl per 100 ml of agarose solution) and mixed gently.
Pour the gel solution into the leveled casting tray gently by avoiding formation of bubble, especially close to the wells.
Allow agarose to set and then remove the comb gently gently. Remove the adhesive tape and place the gel in the electrophoresis tank containing 1.0X TBE buffer. The gel should completely submerged in the buffer.
Load 5 μl of dissolved DNA sample after adding 2 μl of 6 X gel loading dye (Xylene Cyanol and Bromophenol Blue).
Run the gel at 5 V/cm (40-50 V) for 3 hrs.
See the gel over an UV transilluminator.
Presence of a single intact band, free of smearing and RNA contamination reflect the good quality of genomic DNA.
Quantify the amount of DNA extracted by comparing the intensity of the band with the intensity of bands of lambda DNA of known amount.
Figure 1 depicts the quality of genomic DNA extracted from raw and processed meat (Boiled, steam cooked and autoclaved). No or very little smearing is observed in raw and boiled meat.
However slight to moderate smearing is seen in genomic DNA extracted from steam cooked and autoclaved meat.

**B. Amplification of 12 S rRNA gene**

Dilute the genomic DNA solution to the final concentration of 25-30 ng / ul.

Following primers for mitochondrial 12S rRNA gene may be used. Synthesize these sequence by any commercial firms.

**Sequence of primers for mitochondrial 12 S rRNA gene**

Forward primer: 5’CAA ACT GGG ATT AGA TAC CCA CTA T3 ‘
Reverse primer: 5’GAG GGT GAC GGG GGG TGT GT 3’

The PCR reaction may be set up in 25 μl reaction volume. Based on initial trial, the reaction mixture was optimized as follows, 2.5 μl of 10 X Assay buffer (100 mM Tris- HCl, pH 9.0, 15 mM MgCl₂, 500mM KCl and 0.1% gelatin), 200 μM of dNTP mix, 10 pm of forward and reverse primer, 1 U Taq DNA polymerase, 50 ng of purified DNA and autoclaved milli Q water to make up the volume. The PCR tube containing the reaction mixture was flash spun on a microcentrifuge to get the reactants at the bottom. The reactions were performed in a Thermocycler.

The cycling conditions contained an initial denaturation at 94 °C for 5 min followed by 30 cycles of 45 sec denaturation at 94 °C, 45 sec annealing at 60 °C and 1 min elongation at 72°C. After the reaction, tubes with PCR products were held at 4 °C until further analysis/ confirmation by agarose gel electrophoresis or stored at −20 °C for further use.

Figure 2 show the amplification of 12S rRNA gene from raw, cooked, autoclaved and processed meat. Note that cooking, autoclaving or processing though resulted in smearing of genomic DNA (Fig 1), but do not affect the amplification of 12S rRNA gene, except the slightly lower intensity of band in autoclaved processing.

**C. Identification of restriction enzymes capable of differentiating different meat species through PCR-RFLP**

Using the known sequences of 12S rRNA gene from different meat species from database (EMBL), a panel of restriction enzymes, which are supposed to give unique size of restriction fragments for each type of meat species or a particular meat species under question.

Table 1 shows such selective restriction enzymes, which are capable of generating chicken and pig specific restriction fragments.

**Table 1. Selected restriction enzymes for PCR-RFLP studies for meat differentiation.**

<table>
<thead>
<tr>
<th>Name of restriction enzymes</th>
<th>Size of restriction fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BseG I</td>
<td>Cattle Buffalo Sheep Goat Chicken Pig</td>
</tr>
<tr>
<td></td>
<td>NA NA NA NA NA 323 + 120</td>
</tr>
<tr>
<td>Dpn I</td>
<td>NA NA NA NA 341 + 99 NA</td>
</tr>
<tr>
<td>Eco 136 II</td>
<td>NA NA NA NA 248 + 202 NA</td>
</tr>
<tr>
<td>Eco 24 I</td>
<td>NA NA NA NA 246 + 204 NA</td>
</tr>
<tr>
<td>Mbo I</td>
<td>NA NA NA NA 339 + 101 246 + 197</td>
</tr>
<tr>
<td>Sac I</td>
<td>NA NA NA NA 246 + 204 NA</td>
</tr>
</tbody>
</table>

NA = No restriction site
D. Restriction fragment length polymorphism (RFLP)

PCR amplified product of mitochondrial 12S rRNA gene may be subjected to restriction enzyme digestion with suitable selected enzymes for that species.

The procedure was performed as follows

- Enzyme buffer (EB) mix was prepared by mixing 2 μl of restriction enzyme with 8 μl of respective buffer (10X).
- Reaction mix was prepared by mixing 10 μl PCR product with 2 μl of enzyme buffer mix.
- Volume was made up to 20 μl with autoclaved Milli Q water and reaction mixture was incubated at 37 °C overnight.
- The enzyme activity was stopped by freezing at -20 °C or by adding 2 μl of 6 X loading dye.
- Digested product was subjected to electrophoresis in 2 % agarose gel along with 100 bp ladder (Bangalore Genei).
- Agarose gel after electrophoresis was observed in gel documentation system for products of desired molecular weight.
Fig. 3 shows the restriction enzyme profile of chicken and pig with Mbo I restriction enzyme. Kindly note that the species-specific restriction fragments resolved on 2% agarose.

Fig. 1
DNA extracted from raw/processed meat samples

Fig. 2
Amplification of 12S sRNA gene raw/processed meat samples

R: Raw meat; A: Processed meat (cooked at 72°C for 30 min); B: Processed meat (steam cooked at 90°C for 30 min); C: Processed meat (autoclaved at 120°C/15 Ib/30 min); P: pork, ch: chicken. M: Marker, lDNA double digested with HindIII and EcoRI

Fig. 3
PCR-RFLP of 12S rRNA gene of in raw meat from different species with Mbo I restriction enzyme. M: 100 bp ladder; B: Beef; Bu:Buffalo meat; M: Mutton; C: Chevon; P: Pork; Ch: Chick