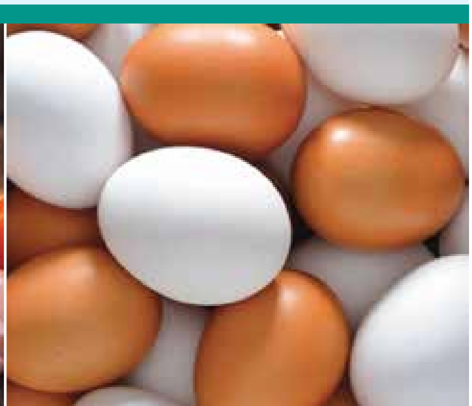
A Review on

Quality and Safety of Animal Source Foods



Quality Control Laboratory

for livestock inputs and its food products

Department of Livestock Services, Bangladesh

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Published by

Quality Control Laboratory for Livestock Inputs and its Food Products

Department of Livestock Services, Bangladesh, Dhaka

December, 2019

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Acknowledgement

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Printed by

STEP Communications
5th Floor, House-26, Road-02, Banani, Dhaka-1213

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December, 2019

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Now or Never

Human civilization is enjoying the highest peak of its success in medicine when early mortality of human is the lowest ever in the human history. This achievement was ensured by the invention of antibiotics in 1928. But in short time, the field of medicine received new challenge like antibiotics resistance leading to loss of drug of choice for treating infections. In fact, we are approaching towards an area of “No antibiotics” to treat infections. A single cut might lead to death due to absence of effective antibiotics. Therefore, it is urgent to react “NOW or NEVER”.

Abbreviations

AMR - Antimicrobial Resistance
BBS - Bangladesh Bureau of Statistics
BLRI - Bangladesh Livestock Research Institute
BDT - Bangladeshi Taka
BRAC - Bangladesh Rural Advancement Committee
BMPCUL - Bangladesh Milk Producers Cooperative Union Limited
BSTI - Bangladesh Standards and Testing Institute
BQ - Black Quarter
CFU - Colony Forming Unit
CAC - Codex Alimenterius Commission
DLS - Department of Livestock Services
DOC - Day Old Chick
DNA - Deoxyribo Nucleic Acid
DANMAP - The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
ETP - Effluent Treatment Plant
EU - European Union
EUCAST - European Committee on Antimicrobial Susceptibility Testing
FAO - Food and Agriculture Organization
FMD - Foot and Mouth Disease
GDP - Gross Domestic Products
HS - Hemorrhagic Septicemia
LC-MS - Liquid Chromatography-Mass Spectrometer
MRLs - Maximum Residue Limit
NGO - Non Government Organization
OIE - Office International des Epizooties
PCR - Polymerase Chain Reaction
PRAN - Programme for Rural Advancement Nationally
UHT - Ultra High Temperature
USDA - United States Department of Agriculture
USA - United States of America
US FDA - United States Food and Drug Administration
RNA - Ribonucleic Acid
WGS - Whole Genome Sequencing
WHO - World Health Organization
WPSA - World's Poultry Science Association

Executive summary

Livestock is one of the most important sub-sector in agriculture since many thousands of years in this region. Contribution of livestock in Agricultural GDP is 14%, and 1.66% in overall GDP in Bangladesh. Livestock production has been increasing in a rapid pace in Bangladesh since 2000. Currently, Bangladesh has achieved a remarkable growth in animal production which accounts 99.23 lakh metric ton milk, 75.14 lakh metric ton meat and 1711 crore eggs. Despite of high production livestock industry is still vulnerable due to existence of weak value chain. Currently, Bangladesh is moving towards exportation of the livestock products which would require a strong value chain system.

Intensified livestock industry has been growing exponentially in Bangladesh with the application of formulated feed, use of antimicrobials and other advance technologies. Hundreds of feed mills have been operating in Bangladesh with limited practice of feed quality control system. These feed mills used to collect feed ingredients from the local and international sources. The major feeds and fodder resources produced locally and used in feed formulation in those industries to enhance animal production. Overall, the local ingredients include residues of crops, rice, maize, wheat, minor cereals, pulses, sesame, rape and mustard, ground nut, coconut, cotton, sugarcane, potato, mango, pineapple, banana, jackfruits, vegetables and green grass. Besides the addition of vitamins, minerals and enzymes, antimicrobials used to add as growth promoter which is strictly prohibited in many countries of the world including Bangladesh. It is believed that addition of antimicrobials in feed as growth promoter leads to development of antimicrobial resistance which is a serious public health concern. Contamination of feeds by Salmonella spp., E. coli, Campylobacter spp. is closely associated with public health issues. Moreover, feed might contain toxins, and other contaminants like pesticides, heavy metals which might pass to the human body through food chain.

Animal originated foods are common menu in the daily food in every counties of the world and all of the people of the world are enjoying animal derived food since human life started in the world. Excluding raw milk, meat and eggs, the products produced from these raw food (meat, milk and eggs) are diverse and differed from culture, race and even regions. Bangladesh is one of the ancient civilizations in the Indian sub-continent with many thousands of year's old tradition and vibrant food culture which is home to wide array of animal derived food products. As for example, boiled milk, yoghurt, butter, cheese, sweet-meat, khir, cooked meat, dry meat, and other meat products, egg and egg products. These items are routinely used in preparation of different food and food products. Our food culture is "cook and eat", i.e. in most cases properly cooked foods are eaten here in Bangladesh. However, some dairy products are consumed without cooking like yoghurt, butter, cheese even though these are produced from processed milk.

Meat, milk, egg and their products used to contain normal microbiota including beneficial microbes, spoilage microbes and pathogens. Additionally, these products are prone to post contamination with potential food-borne pathogens like Salmonella spp. and E. coli. Beside the food-borne pathogens, milk and dairy products, meat and meat products and, egg and egg products may contain antimicrobial resistance bacteria and antimicrobial resistance genes which is a direct threat to public health globally including Bangladesh where antimicrobials are used in food animal farming without or limited regulations and control. Indiscriminate use of antimicrobials, chemicals, pesticides in the primary production might lead to residue findings in the feed and food products. Perishable products like milk, meat, egg and their products always prone to spoilage by spoilage microbes unless constant control and hygienic measures are in practice.

Preface

Food safety issue is a major concern in Bangladesh. Animal originated food products are the main source of protein. These mainly come from the intensified production where the use of antimicrobials, chemicals and other inputs are common practices. The quality of inputs of animal production is pre-requisite of receiving good foods for human consumption. Lack of regulation on the application of antimicrobials and chemicals lead to development antimicrobial resistance bacteria and residue findings in the products. Quality of these products (meat, milk and egg) also deteriorate due to activity of spoilage microbiota. Moreover, occurrence of pathogens like Salmonella spp., E. coli, Campylobacter spp. etc are threat to consumers. Development of resistance to antimicrobials in bacteria found in animal originated food products partly goes to the use of antimicrobials at the primary production level. Residues of antimicrobials, chemicals, heavy metals, pesticides in animal originated food products was found to be threat to public health.

This study includes reviews of residues in food and food products, contaminants and toxins in feed, quality of meat, milk, egg and their products to get better understanding of food quality of safety to intervene on these issues in near future.

This review work would not have been possible without commitment and support of the "Establishment of Quality Control Laboratory for Livestock Inputs and its Products" project. The Project Director wish to thank Gazi Consultancy for this review work on the food safety issues of animal origin and the quality issues of livestock production inputs.

Lasty, Gazi Consultancy express its deepest gratitude to the Department of Livestock Services (DLS), Ministry of Fisheries and Livestock, Dhaka, Bangladesh for the selection and support for this work.

CHAPTER 1

Current status of food animal production business in Bangladesh

Summary

Livestock, next to crop is the most important subsector since in Bangladesh. Contribution of livestock in Agricultural GDP is 14% and in overall production has been growing faster. In the recent past, Bangladesh achieved remarkable growth in this sector. The annual milk and meat production is 99.23 and 75.14 lac metric ton, while the number of egg production is 1711 crore. Even though, Bangladesh has been experiencing high productivity in the livestock sector but the industry is still vulnerable due to weak value chain, poor standard in food processing industry and lack of sufficient arrangement to conduct food quality control and ensure food safety. In this article status of livestock industry in Bangladesh, quality of different animal originated food and food products, antimicrobial use in primary animal production, antimicrobial resistance and food safety, feed technology, biological and Non-biological contaminants in animal source food products, environmental contaminants and toxin in animal feed were reviewed. Overall, the goal of this study was to evaluate the potential practices and threats associated with livestock inputs and animal originated food products.

Key words: Food quality, livestock inputs, food quality, food safety

Introduction

Livestock is an integrated part of Agriculture in Bangladesh. The significance of livestock is further increased in the era of "ever green revolution". Livestock plays a significant role in the national economy and in the socio-economic development of Bangladesh. The contribution of livestock sector to overall GDP in the year 2016-17 was 1.66 (2016-17) and its share to Agricultural GDP is 14.08% (provisionally estimated). Despite the fact that the contribution of livestock to overall GDP is low, livestock serves an essential role as a source of protein, employment generation, export earning, and provision of livestock security. This sector plays an important role in the sustenance of landless people, marginal farmers, livelihood options of the rural poor families and potentially important for poverty reduction in Bangladesh and in the south Asian region. There are about 420 million cattle and buffaloes and 360 million goats and sheep in the region (BBS, 2016). Average densities of livestock in this region is highest in the world, i.e. 70-137 animals/sq.km. against the world average of 29 animal/sq. km. The share of the South Asian countries of the world's meat production is only 5.4% despite rearing a large herd of animals while the share of Bangladesh is insignificant (Gurung et al., 2017). It is estimated that Bangladesh is home to livestock and poultry population of about 53.66 and 307.47 million respectively. According to species, cows 23.44 million, buffaloes 1.45 million, goats 25.61 million, sheep 3.16 million, chicken 259.42 million, and ducks 48.05 (Bangladesh Economic Review 2013).

Existing livestock situation in Bangladesh

Bangladesh is home to 160 million people and a densely populated country in the world promised with multifarious and enormous potential. Food animal production has been increasing rapidly to meet the demand of the local market. This industry has been generating employment opportunities and millions of people are living on food animal production. Food animal production industry include dairy and meat animals, and poultry production. The entire value chain include on-farm production, marketing of live animal, marketing of animal products like milk, meat and egg, processing of meat, milk and eggs and their sale to the market. Additionally, the entire process is supported by livestock input business like feed, pharmaceuticals, vaccines and nutritional product business. The entire value chain is supporting the economy and employment.

Status of Dairy Industry in Bangladesh

Farm size

Except few dairy farms under Department of Livestock Services, Ministry of Fisheries and Livestock most of the dairy farm in the country are belonging to the private sector. These can be categorized into 5 different groups based on local standard.

1) **Dairying for home consumption/ Family Dairy:** Most of the farms belong to this category. In this types of farm, one or two cows are kept to meet primarily the household demand for milk products, and the surplus are sold in the local market or through home delivery system.
2) **Dairying for dual purposes (draught and milk):** Bangladesh is a rural country where every farm house holds are depending mainly on draft power for cultivations. Therefore, they usually keep 2 and 3 cows including both bulls and dairy cows and often they have to use their dairy cows for ploughing. Milk production comes from those cows is usually consumed and the surplus amount is sold in the market and through home delivery system.
3) **Small-scale dairy farming:** The medium and small size farms are operated with business goal and generally these farms are housed with 2 to 5 cows. These farms are used to form and operated by receiving financial and technical support from the government, NGOs and co-operatives. Milk produced from these farms is usually sold in the market or to the middleman known as Farias/Gosh.
4) **Medium sized commercial dairy farming:** The medium sized commercial Dairy farm usually housed with 6 to 20 cows. This types of farms generally receive support from the co-operative or some time from the Bank. The products of these farms are sold in the market.
5) **Private large commercial dairy farms:** In recent times, large commercial dairy farms have been increasing in Bangladesh and most of them are financed by the Banks. The modern commercial dairy farms are used to house more than 20 cows. In contrast to the private sector, there are only eight government dairy farms throughout the country.

Around 6 million cattle in Bangladesh are dairy cattle among the total cattle population. The indigenous types include 85 to 90% and 10-15% are crossbred. The Indigenous group of cattle can be classified as a) non-descript deshi – a dwarf size zebu, **b)** Red Chittagong cattle, and **c)** Pabna cattle, etc. The crossbred cattle are mainly derived from crossing local with different exotic breeds, primarily Holstein-Friesian, and some crosses with Sindhi, Sahiwal, and Jersey etc. at different level of genetic combinations.

The government dairy farms are basically being used for breeding purposes to supply heifers to the small-scale farmers or for breeding bull production. In recent times, the Department of Livestock Services (DLS) and Bangladesh Livestock Research Institute (BLRI) started joint buffalo improvement program in public sector. A buffalo development farm has been established at Bagerhat district in the coastal region.

Buffaloes are highly useful for draught purposes and therefore buffaloes are managed in many households for dual purposes. Almost half of the population of buffaloes are reared in the coastal region of the southern part under extensive farming in 'Bathan' (Bathan is a farming system where a large number of cattle/ buffaloes are reared on herds in some isolated areas usually seen in coastal and riverine estuary areas). Buffalo development program has also been initiated by private sector. Lal Teer livestock limited is the pioneer in buffalo development programme in the private sector. Their main goal is to create dairy and meat buffalo in Bangladesh. Buffalos in Bangladesh is mainly indigenous origin and most of them are riverine type and some are swamp type. There are also some crossbred with Murrah, Nili-Ravi, Suri, and also Jafrabadi in South Eastern and northern border districts.

Black Bengal goat is the only major breed in Bangladesh which comprises 90% of the total populations of goats. Additionally Jamunapari breed and their cross with local variety are also seen.

Status of poultry Industry in Bangladesh

Bangladesh has a long history of poultry rearing under traditional and Backyard system. At the beginning, commercial poultry farming started on small scale involving the rural women, unemployed youths, semi-urban and urban poultry rearer. Poultry sector in Bangladesh is a fast growing developed sector and DLS has been facilitating the entire process of development since 1990s. Since then a significant annual average growth rate in the commercial poultry sector has been achieved. Poultry Industry Association in Bangladesh claim average growth rate in this sector is about 15-20%.

The catchy features regarding the poultry sector investment, activities, consumption of poultry product, poultry feed, hatchery operations, and quality issue etc. have been outlined as per proceeding of 9th WPSA conference, Dhaka 2015 as follows:

1. Poultry industry is generating huge employment opportunities next to the Garments Industry. In recent times, 2 million people employed in this industry half of which are female.
2. The annual per capita chicken consumption in Bangladesh is nearly 3.63 kg, in contrast to the global health standard of consumption of 18-20 kg.
3. FAO recommends annual per capita consumption of 104 eggs. In Bangladesh this number is counted only 40-50 eggs.
4. WHO and FAO's joint survey on health standards recommended 43.80 kg of annual per capita protein intake but Bangladesh achieved 15.23 kg.
5. Use of antibiotics in poultry feed is controlled by regulation, and compliance is in practice. Antibiotics are used for treating poultry diseases as per advice of the veterinarians.
6. The demand of poultry feed has been estimated to be 2.7 million metric ton annually. Around 2.57 million metric tons comes from the commercial feed mills and 0.13 lac metric tons comes from the local manufacturers.
7. Out of total annual demand in Bangladesh, around 95% of poultry feed is produced locally under advanced technology. More than 99% of poultry feed production was found to be devoid of tannery waste.

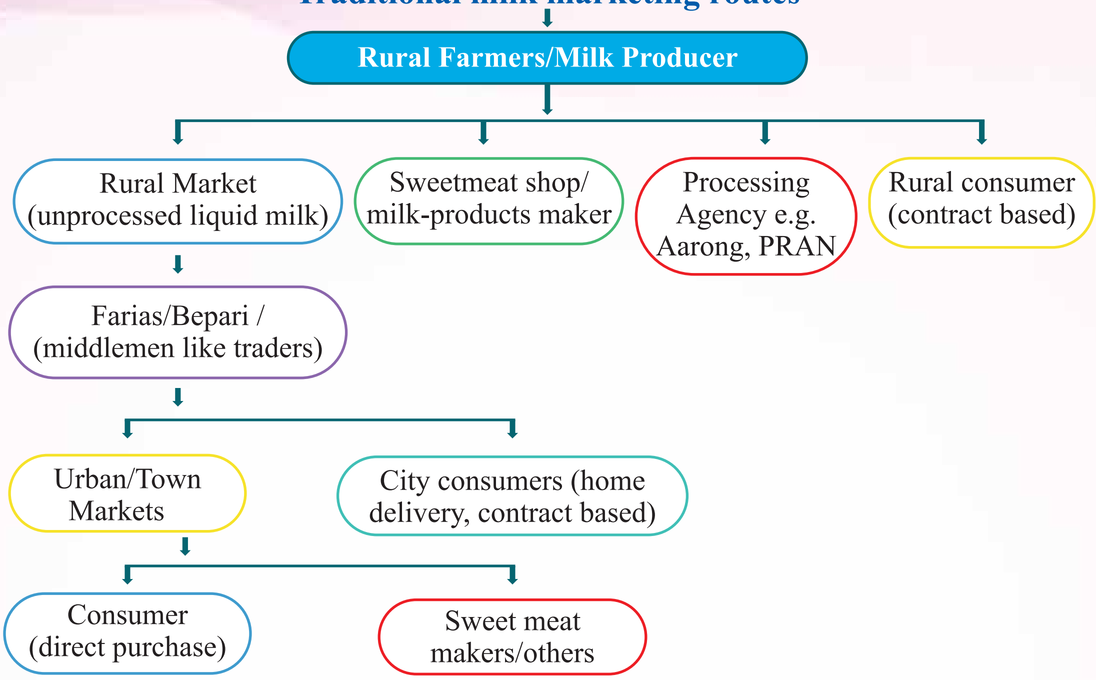
8. Currently the investment in poultry industry has been increased 17 times in contrast to 80's decade which accounted for 25,000 core BDT.
9. The implementation of effective policy and building infrastructure of the government and removal of the conflicting policies will facilitate poultry industry to grow to 50,000 crore by 2020.
10. It is estimated that currently the daily demand of chicken is 1,700 Metric Ton. Around 2 core eggs (estimated) are produced daily.
11. Weekly demand of day-old chick is about 1 core 10 lacs.
12. The number of grandparent stock farm under operation is 6.
13. Number of listed breeder farms is 140.
14. Production of parent stock is 60-70 thousand per week.
15. Production of day- old broiler chicks is million per week.

Animal product harvesting and marketing system

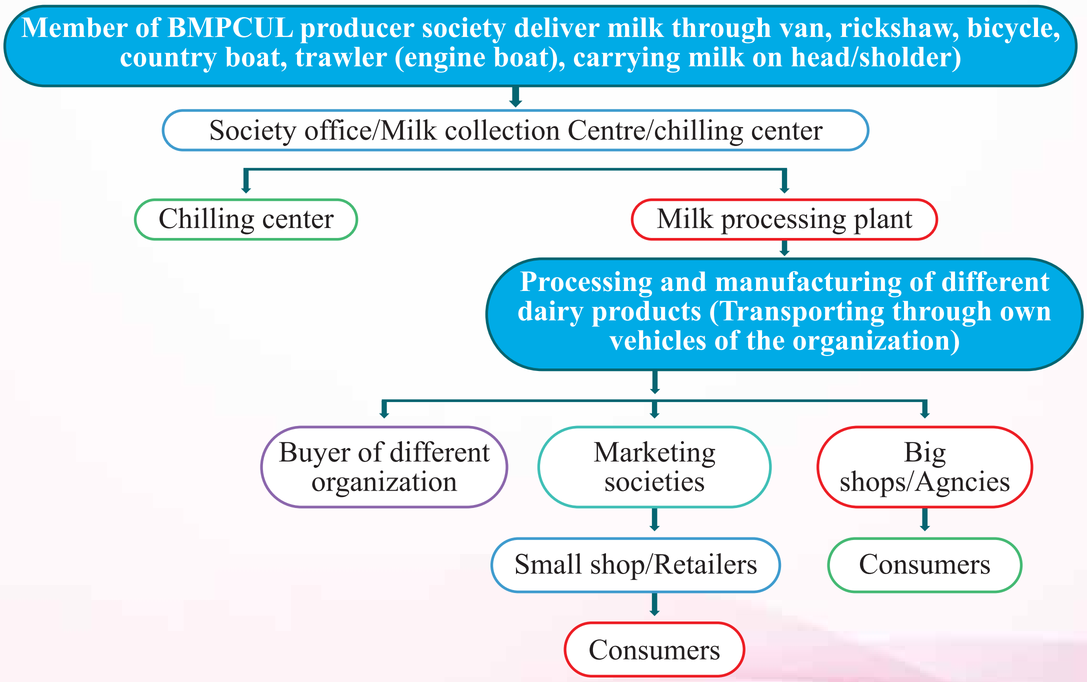
Livestock product harvesting system in Bangladesh is poorly defined. The harvesting process is poorly practiced with limited recourses, poor handling, poor hygiene etc. For example, both in backyard and commercial dairy farm, milk is drawn through hand milking system instead of machine milking. Hand milking is not always done following teat dipping in disinfectant or disinfection of milker's hand. In general, freshly drawn milk is expected to be sterile. But in practice, contamination of milk starts from teat cannels of mastitic animal, milker's hand, coat of the cow, environment of the milk barn, milk bucket etc. Soon after drawing of milk, it should be stored in chilling temperature (0-4°C) and maintained under chilling conditions until further processing. After drawing of milk, farmers carry this milk through van to the milk collection center of different milk proceeding industries 10-15 km away from the farm or farmer's home. During transportation of milk from the farm to the milk collection or chilling center, banana leaves are used for milk preservation purposes. There are many milk chilling centers located milk pocket zones which are owned by different dairy companies like milk vita, PRAN Dairy, BRAC Dairy, Bikrampur Dairy Rangpur dairy, Akij Food and Beverage etc. From the milk chilling centers, milk is being carried to the dairy factory by insulated road milk tanker. Once the road milk tanker arrived in the processing plant, raw milk is received following platform tests and stored in chilled condition until further processing. In general, all types of livestock products are being marketed in Bangladesh. The main products include meat, milk, eggs and their products.

Marketing system of milk prevailing in the country is both informal and commercialized. But the informal marketing system is more dominant over commercial marketing system. In case of informal marketing system, raw whole milk is directly sold by the dairy owner or through middle men like Farias or Ghosh. There are processed products of milk mainly in the form of pasteurized milk, UHT milk, yoghurts, powdered milk, butter, cheese, ice-cream, butter-oil (ghee) etc. Good number of sweetmeat makers are also processing milk to prepare delicious items known as "misti" or "rosgolla" a form of cheese in sugar syrup. The informal milk marketing routes are as follows:

Traditional milk marketing routes



Milk marketing routes of milk producer's cooperative union limited (BMPCUL):



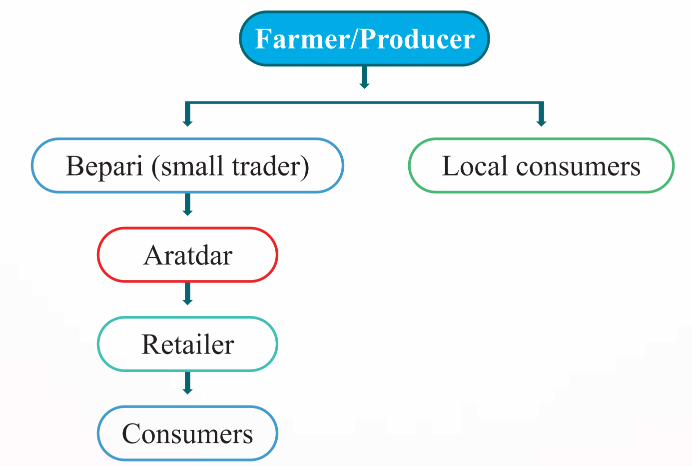
Meat marketing

Bangladesh is home to traditional butcher shop located in the rural villages and even in the big cities with similar characteristics. In those shop, animals are slaughtered on the open floor, skin is removed on the same floor; meats are cut on wooden board frequently without proper disinfecting and the overall hygienic condition of the premises are substandard. This kind butcher shop is the major source of meat trading in Bangladesh. On the other hand, the market of processed meat is extremely small which can be seen in the super-markets located in Dhaka and other cities. Public meat processing plants are few with substandard hygiene practice. In contrast, private sector has been operating some meat processing plants namely Bengal meat, BRAC, Aftab, CP Bangladesh, Kazi Farms, Golden harvest etc. Bengal meat has been marketing ready to cool meat, ready to eat meat products in big cities through many different customized outlets.

Egg and poultry marketing

In Bangladesh, eggs are sold mainly in numbers rather than weight. Egg grading system are not well established in Bangladesh. Generally eggs are sold in the open market, small shops, and super markets. Even though egg grading system are not standardized, recognized and ensured by any authority, some companies are marketing graded egg in packages. Native poultry birds (chicken, ducks, pigeon, geese etc) are sold in the traditional rural market by the backyard farmers. The birds are sold either directly to the consumers to the small farias who collect and supply those to the "Arat" located in the towns or cities. The traditional poultry and egg marketing system generally have the following routes.

Traditional poultry and egg marketing channels:



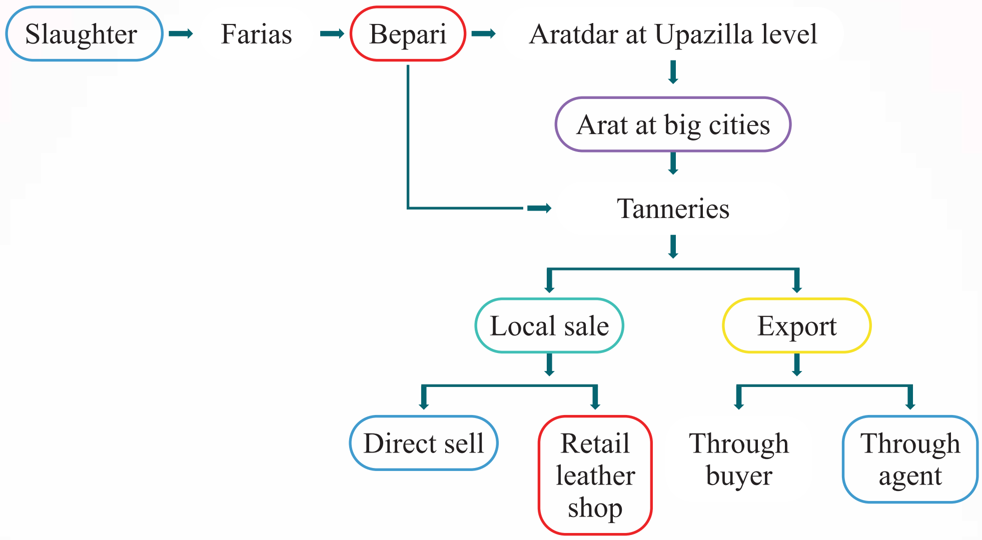
Pricing of livestock products

Bangladesh is a place where fair trade in terms of price, quality and quantity is merely under good practice. Therefore, price of animal originated products varies considerably from place to place. Instability in livestock product pricing system is one of the major constraints in livestock development. The price depends on many factors such as market demand particularly during religious festivals, social events, feed and other input's price, production flow and supply etc. The farmers sometimes bound to sell milk at lower price due to excess supply of milk during the flush season – mainly from January to June, also due to transportation problem during "hartal "(strike/political agitations).

Hides and skin marketing channel

Hides and skin marketing system is not well characterized but leather industry is growing fast. Hides and skins are marketed by a chain of middleman. Collection and marketing routes of hides and skins is as follows:

Marketing channel of hides and skins in Bangladesh is as follows:



Livestock product processing in Bangladesh

Informal livestock product value chain is prevailing in Bangladesh. Livestock product processing capacity is still in primary stage in Bangladesh. Bangladesh has ample of opportunity in processing industry of livestock and poultry resources through which we can supply good quality and safe food products through hygienic practices to the consumers. This sector has the potential to employ many thousands of youths including educated one as well. Few companies are operating in the market and there is room for the new companies to join and help the growth of this sub-sector up to the potential level. The livestock products include milk, meat, eggs and hides and skin. A brief account of the processing of these products is given below:

Milk processing

Raw milk is being processed to produce pasteurized milk, UHT milk, yoghurt, butter, butter-oil, and cheese. All of these products are being sold in the big cities of Bangladesh. There are many public and private processing company are involved in raw milk processing and marketing of dairy products. Milk Vita and Savar Dairy are the public enterprise while Aarong is the brand name of BRAC (NGO). Also PRAN, AFTAB, Farm Fresh and Tulip are the private companies involved in processing and marketing of dairy products.

Bangladesh milk producers' corporative union Ltd. (BMPCUL), widely known today by its popular brand name Milk Vita. This organization started milk processing in a corporative effort in 1965. Currently, it is the biggest dairy processing and marketing corporative organization in Bangladesh. Milk Vita shares 50% of the total pasteurized-milk market share (52%) in Bangladesh. Members of the cooperative deliver milk to collection points and pooled milk are transported to the chilling plant before processing. From the chilling plant, milk transported to the processing plants located several places in Bangladesh including the processing plants in Mirpur 7, Dhaka. Cooperative members receive technical training, animal breeding supports, veterinary and extension services and other different supports to enhance milk production.

BRAC Dairy and Food project

Bangladesh rural advance committee (BRAC) is an international NGO launched milk processing company with brand name "Aarong" to support its village organization in dairy production through providing microfinance loans for livestock and market development and also provide multifarious market service including transportation, pasteurization, processing, branding and distributions.

Programme for Rural Advancement Nationally (PRAN)

PRAN is one of the giant agro-processing and commercial company in Bangladesh. This company introduced ultra-high temperature (UHT) treated milk production for the first time in Bangladesh on 2002 which was aimed to run a School Nutrition Program. PRAN started its journey of milk processing based on a partnership with land-O-Lakes, Tetra Pak and United States Department of Agriculture (USDA). The technical expertise gained from this partnership was shared with other commercial companies to expand UHT milk processing in Bangladesh. Currently, PRAN is the third largest milk processing company in Bangladesh whose market share is about 10%.

Meat

Meat processing industry in Bangladesh is relatively small. However, broiler, mutton and beef are being processed for marketing as "ready to cook" or ready to eat form. There is no meat processing plant operated by the Government but in recent year private sector intervention in marketing of processed meat has been started. Bengal Meat is a private entity which came with process meat in the market of big cities. It is operating its business through 40 outlets in Dhaka city area. The main products include smoked roasted beef, beef pepperoni, beef bacon, beef sausage, sandwich meat etc.

Many other private companies like BRAC (NGO), CP Bangladesh, Golden Harvest and I.G. Foods are operating poultry processing plants and marketing poultry meat products. These products include, chicken nuggets, chicken meat ball, chicken fries, quick fry chicken fillet, sandwich meat, smoked chicken, chicken sausage, chicken shashlik etc. In every live-bird-market chicken are being dressed by using de-feathering machine.

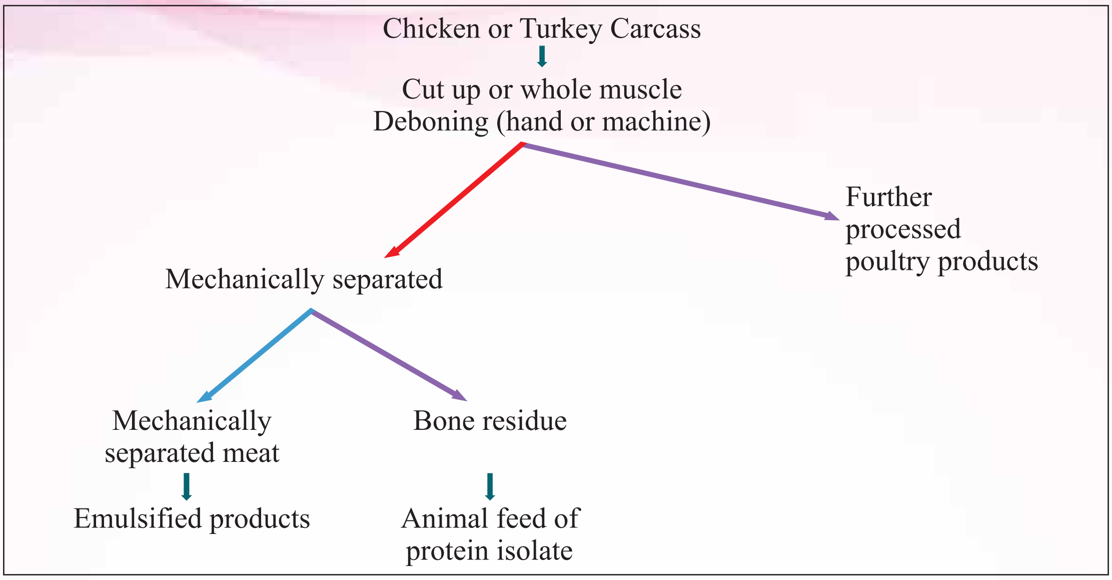


Figure: Mechanical separation of poultry meat.

Egg

In the developed world, there are many processed egg products are found in the market which is merely present in the country like Bangladesh. There does not exist any egg processing plant in Bangladesh but grading of egg has been started very recently.

Food safety issues of animal originated food products

Bangladesh is a country where chemicals and antimicrobials are used without any sense and limited control which lead to development of antimicrobial resistant bacteria and antimicrobial/chemical residues findings in food products of animal origin. Moreover, adulteration and frauds are common practice in milk to increase specific gravity and volume of milk, and misbranding of meat (buffalo meat instead of beef). Bangladesh is by far way to attain the international quality standard for its exporting of livestock products. Bangladesh is still away to reach the full capacity to meet the recommended safety level of quality standard for livestock products for sanitary measures regulated by the world organization for animal health (OIE) and Codex Alimentary Commission.

Human being receive infection from animal by direct contact and by consumption of animal originated food products which is termed as zoonoses. Therefore, prevention and control of animal diseases at the primary production is highly important to reduce production cost and to reduce zoonotic disease transmission. Disease like Foot and Mouth Disease (FMD), hemorrhagic septicemia (HS), anthrax, brucellosis , tuberculosis, black quarter, fascioliasis which are fatal for animals and some of which are transmissible to human. Therefore, it is highly important to follow recommended practices on preventing and controlling of microbial and parasitic diseases. on the other hand, poultry diseases like Ranikhet disease (New castle disease) causes conjunctivitis in human while avian influenza (H5N1) in some cases are considered to be deadly agents for human being.

Milk

In reality, adulteration in milk is a common practice in Bangladesh which is a public health threat. Moreover, antimicrobials and chemicals are widely used in dairy industry in the primary production level. This has resulted in occurrence of antimicrobial resistant bacteria and antimicrobial resistant genes and residue findings in milk and milk products. Adulterants like formalin, melamine and fortified with poor quality milk powder is a threat to public health. By and large, unhygienic handling creates opportunities for the entry of pathogens in milk and milk products and pass to the human body through consumption.

To mitigate this detrimental issue it is essential to establish methodological approaches to monitor antimicrobial resistance phenotypically by using single bacterial indicators of antimicrobial renitence and genotypically though detecting antimicrobial detecting those chemical adulterants on dairy products e.g. raw milk pasteurized milk, UHT milk, butter, cheese, yoghurt etc.

Meat and meat products

In recent times, Bangladesh has attained self-sufficiency in meat production. There is lack of good production practices at the primary production system. Meat market is dominated by informal marketing system i.e. raw meats are sold in the traditional slaughterhouses. Bengal meat is the only beef processing company in Bangladesh, while poultry meat is processed by I.G. Foods, CP Bangladesh Limited, BRAC poultry etc. Raw frozen meats are the major market share in contrast to meat products like chicken nuggets, meat balls etc. But still meat market is dominated by raw meat in every corner of Bangladesh. Raw meat is harvested through slaughtering of animals in the very early morning at traditional slaughter house which lack of proper hygienic condition. Also butchers have limited methodological training. Misbranding (buffalo meat instead of beef or sheep meat instead of goat meat or sheep meat mixed with beef or goat meat respectively), dead animals' meat or spoiled meat mixed with fresh meat). These are conflict with ethical trade, nutritional security and surely public health concern.

In addition, poor production practice has imposed more dangerous issues in contrast to misbranding. Meat value chains are quite informal and mixed animal production system are predominant where animals are normally self-fed and graze around homestead. Grains are common ingredients for commercial and backyard food animal production (dairy, beef and poultry). Additionally, food animal production is mainly relied green grasses. Grain production is enhanced by application of pesticides and insecticides which are fatal chemicals for animals. Animals fed with these ingredients which have resulted in occurrence of pesticides and heavy metal residues in meat and milk. Furthermore, chemicals and antimicrobials are used indiscriminately at primary production level to treat diseases which has resulted in occurrence of antimicrobial resistance bacteria. Antimicrobial resistance gene and antimicrobial residue findings in meat and meat products. Another striking issue is that steroid and hormones are frequently used in beef fattening practices which also resulted in hormones and steroid residue findings in red meat that is directly associated with public health. More concerning issue is poultry feed in which antimicrobials, steroids and other chemicals are used as growth promoter. In addition, in poultry feeds manufacturers use tannery waste as protein source. Generally, tannery wastes contain lead and chromium which resulted in residue findings in the poultry meat - a clear threat to public health.

Egg and egg products

Bangladesh is self-sufficient in egg production and egg value chains are mostly informal where whole egg dominates major market share in contrast to egg products like pasteurized egg, mayonnaise etc. In the traditional market, egg handling and preservation are not hygienic. Spoilage of fresh eggs are started soon after laying if not stored at recommended temperature (55 °F– 68 °F). Inappropriately stored eggs are spoiled and sold in the market in Bangladesh. There are reports that baking industry often uses rotten eggs in baking products (e.g. cakes). Eggs harvested from diseased flocks used to be source of Salmonella spp. and E. coli. These are associated with public health. Poultry feed are prepared with tannery waste which is the predominant source of chromium, lead and other heavy metals. Feed contaminated with such toxic chemicals resulted in residue findings in eggs. Furthermore, chemicals and antimicrobials are widely used in primary poultry production which resulted in occurrence of antimicrobial resistant bacteria, resistant genes and residue findings in egg products.

Regulatory functions of the government through relevant academic disciplinary department should be strengthen. Food Quality and Safety Laboratories through Department of Livestock Services are needed to establish and appoint highly skilled scientists in those laboratories to enhance analytical capability of chemicals and microbial food safety from the point of harvest to label products to attain highest quality food products. To attain such goal, law enforcement with due importance is highly required.

Slaughterhouse and meat marketing

Meat market in Bangladesh is dominated by the butchers who handled meat in the market under unsatisfactory sanitary conditions. Slaughtering and dressing system are not scientifically organized. Most of the abattoirs in rural and urban markets where slaughtering, flaying and dressing operations are carried out indiscriminately in the open place. Mary slaughterhouses have been constructed in cities, towns and Upazilla level by the local government authorities. Bangladesh is housing 192 slaughter house at district level, 1215 at Upazilla level and more than 3000 slaughtering points in rural market places, cities and towns. Unskilled manpower working with the slaughtering and flaying process cause a considerable damage to the quality of leather and meat. The most strikingly, they are not aware of hygienic practices during meat processing, the steps when meat acquire contaminants. There is no modern slaughterhouse in public sector in Bangladesh. Bengal meat, a private sector enterprise has been operating mechanized slaughtering settings and marketing of meat (beef) and meat products in Bangladesh. Local Government Authorities in Upazilla, municipalities and city corporations are managing the cleanliness and sanitation of the slaughterhouses. There are poultry meat processing plants in Bangladesh operated by BRAC, IG foods, CP Bangladesh and Kazi farms etc, and they are marketing raw frozen processed meat and other meat products e.g. chicken nuggets, meat ball etc.

Slaughterhouse and meat marketing

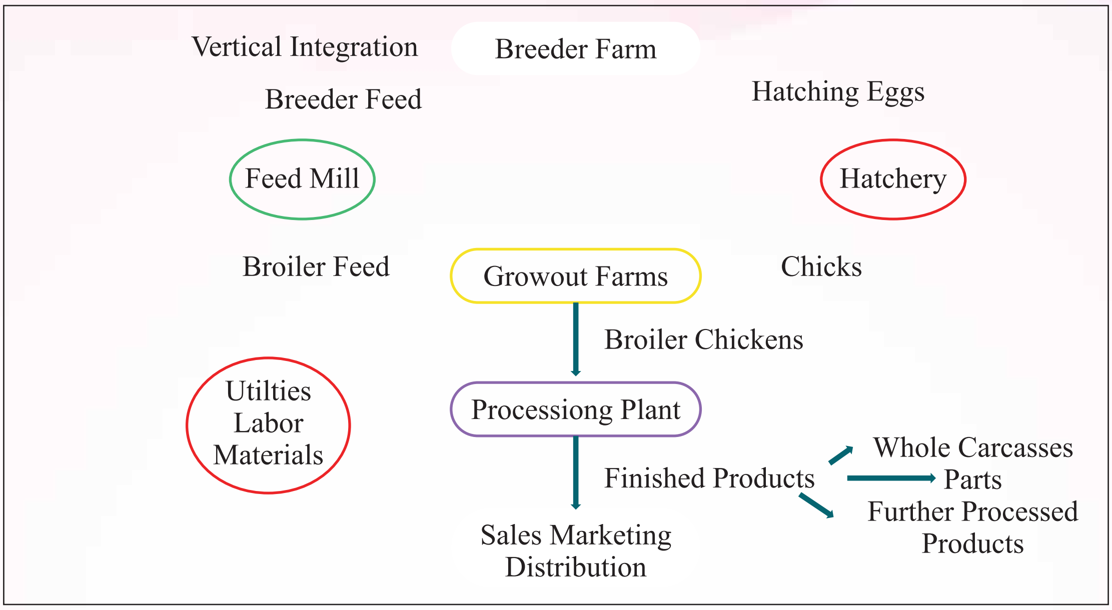


Figure: Diagram of the material flow between the components of a vertically integrated poultry company.

Quality control of animal originated food products

Food quality is mainly associated with nutritional security and ethical trade. The central point is food composition which is highly connected with food security and ethical trade. Still today, the Bangladesh Standard Testing Institute (BSTI) is the only regulatory body to control the quality of animal originated food products. Additionally, the Local Government Institution such as city corporations and municipalities are performing the ante-mortem examination of animals and post-mortem examination of slaughtered animals. This job is done by the veterinarians of the Department of Livestock Services (DLS) deputed to work in those organizations. In the small administrative unit (Upazilla level), the Upazilla parishad is responsible to carry out this work with the help of DLS officers. Sanitary Inspectors belonging to health department has the mandate to evaluate all food items including milk and milk products but in fact, the inadequate regulatory functions to ensure quality standard of animal originated food products is a serious problem associated with consumer's interest. For the preservation of food products chemicals and adulterants are added to increase the profit margin of commercial products which is supplied against the ethical trade. Additionally, out dated milk products are being sold in the market.

Milk and milk products

Bangladesh is home to 160 million people and one of the ancient civilization in the world with vibrant food culture including dairy foods. Pasteurized milk, UHT milk, yoghurt diverse type of sweet-meat, butter, cheese. Dairy production in Bangladesh has been increasing rapidly with the use of chemicals and antimicrobials to treat dairy animal diseases like FMD, Rinderpest, Hemorrhagic Septicemia (H.S), Black Quarter (B.Q) etc. This has resulted in development of antimicrobial resistant bacteria, resistance genes and residue findings in dairy products. These issues are not addressed due to lack of technical expertise and technologies which called for immediate national initiation.

Despite the fact of using chemicals and antimicrobials at the primary production level of Dairy industry, in Bangladesh where limited regulation of control of use of those chemotherapeutics, milk harvesting practice is unhygienic. Microbial contamination in milk starts from the teat canal of diseased animal and in case of healthy animals. Milk receives microbes from udder, milker's hand, animal's coat, air and environment of the house. As milk is rich in nutrient for microbial growth, spoilage starts soon after drawing milk is stored in chilling temperature.

Milk value chain is quite informal and due to limited regulation, milk adulteration is out of control till to date which is directly associated with public health. During milk processing, milk composition is adjusted with poor quality and out dated powdered milk and butter oil. Additionally, lethal chemical like formalin in a very low concentration is suspected to use to prevent spoilage. Moreover, urea, sugar and water are added to increase configuration in terms of density, sweetness and volume respectively. The presence of melamine has been reported in powder milk few years before. All of these malpractices are associated with public health.

Bangladesh is close to self-sufficient in meat, milk and egg production but there is lack of capacity in the area of food quality and food safety. This is urgently needed to establish infrastructure to conduct food quality control practice and ensure food safety through surveillance nationwide.

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CHAPTER 2

Feed and feed technology

Summary

Livestock of smallholding farms mainly raised on crop residues and most of the animals in the smallholdings are deprived of balanced ration leading to poor production performance. But in the commercial production system, animals are reared with formulated balanced ration to achieve optimum production. Intensified livestock industry has been growing exponentially in Bangladesh with the application of formulated feed, antimicrobials and other advance technologies. Hundreds of feed mills have been operating in Bangladesh with limited practice of feed quality control system. These feed mills used to collect feed ingredients from the local and international sources. The major feeds and fodder resources produced locally and used in feed formulation in those industries to enhance animal production. Overall, the local ingredients include crops, rice, maize, wheat, minor cereals, pulses, sesame, rape and mustard, ground nut, coconut, cotton, sugarcane, potato, mango, pineapple, banana, jackfruits, vegetables and green grass. Besides the addition of vitamins, minerals and enzymes, antimicrobials are added as growth promoter which is strictly prohibited in many countries of the world including Bangladesh. Addition of antimicrobials in feed as growth promoter leads to development of antimicrobial resistance bacterial population of feed some which are public health significant bacteria directly. Moreover, contamination of feed by Salmonella spp., E. coli, Campylobacter spp. is closely associated with public health issues.

Key words: Growth promoter, feed additives, antimicrobial resistance, feed quality

Introduction

Food animal production industry has emerged as one of the most influential income generating sectors for the rural people. Not only that, it has been appearing as large scale industry in many parts of the world. In rural condition, the contribution and relevance of livestock towards income of the rural people is mere, particularly in the underdeveloped and dry zones in livestock farming. In animal production, feed has the most of the cost involved in production which accounts for 70%. It is true in case of industrial production but in the rural areas where livestock production is the more family business where labour and other inputs are minimum.

The animal requires feed for maintaining themselves. Such as basic physiological process as well as restoring wear and tear of various body tissue. This involved in their maintenance requirement. The other requirements associated with growth and production which include reproduction, production of milk, meat, egg and wool. Proper understanding of the nutritional requirements of the animals, value of various feeds and fodders and their optimum utilization can result in saving on production expenses. Optimum feeding of superior animals justifies especially the crossbreds to exploit their potential.

The major feeds and fodder resources produced locally and used in animal production are crops, rice, maize, wheat, minor cereals, pulses, sesame, rape and mustard, ground nut, coconut, cotton, sugarcane, potato mango, pineapple, banana, jackfruits, vegetables, green grass.

Dairy animals and other ruminant animals in Bangladesh are mostly raised on fibrous crop-residue and areal milking by-products. It is estimated that the total roughage production in Bangladesh in 51056 x 103 ton in 2012 of which comes from cut and carry and roadside grazing. Around 54% of the fibrous biomass produced in Bangladesh is available to animals as feed and the rest part is used for other proposes.

Currently animal farming mainly relies on manufactured feed due to shortage of roughage, and concentrates, conventional feeding might lack of balanced nutrition in contrast to manufactured feed which is fortified with balanced ration. Particularly, commercial poultry industry highly relied on manufactured feed while backyard poultry feed on grain. There are hundreds of feed mills are manufacturing balanced ration for beef cattle, dairy cattle, broiler, layer and fish animals. The main raw materials such as roughage (green grass), concentrate grain like maize, whole rice, grind rice are mainly collected from local sources but some protein of these are also imported from Brazil, China , India from other countries. The protein source includes meat and bone meal, fish meal, protein concentrate, soybean meal which are mostly imported. The mineral sources are also collected locally and by importing from other countries.

The expansion of animal farming has been accompanied with transformation and intensification in Bangladesh during last 30 years. This process has diveded to increasing reliance on a wide-range of manufactured feed products used as food for animals destined for human consumption. Different types of firms are involved in animal feed manufacturing. These include process animals, meat trimming, and other slaughter by products into animal protein from various sources and mix or redistribute it as animal feed. In the feed mills, ingredients of animal and plant origin are being combined to produce feed mix suited to animals of a specific species and/or age. As for example, for older animals, the combined products of feed mills, occasionally termed ration, may be used as feed.

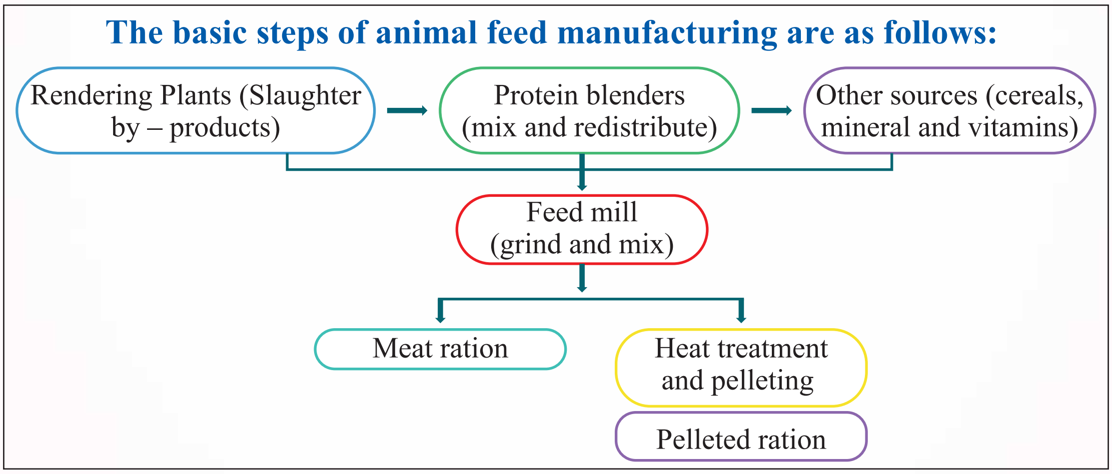

Fig 1: Outline of the steps in animal feed manufacturing

Animal feed might contain pathogens like Salmonella species. Currently, the appearance of variant Creutzfeld Jackob disease has made concern as about the significance of contaminated animal feed. But the issue of bacterial contamination of animal feed is still neglected in Bangladesh. As it is regarded that animal feed is at the first point of the food safety chain in the “Farm-to Fork” approach. Report showed that enteric pathogens cause major illness, hospitalization and deaths in the USA, EU and elsewhere (EFSA, 2006). Major food animals like cattle, poultry pig and turkey are the major reservoirs for these many of the organisms, including campylobacter species, and non typhi serotypes of Salmonella enterica, Shiga toxin producing strains of Escherichia coli and Yersinia enterocolitica (EFSA, 2006). These organisms reach to food animals through feed lead to infection or colonization.

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These bacteria lead to contaminate animal carcasses at slaughter or cross- contaminate other food items, causing to human illness. Though tracing contamination to its ultimate source is difficult, several large outbreaks have been traced back to contaminated animal feed (Crump et al., 2002). Modernization in the safety of animal feed should include strengthening the surveillance of animal feed for bacterial contamination and integration of such surveillance with human food borne disease surveillance systems. A hazard analysis and critical control point program can be instituted for the animal feed industry, and a Salmonella negative policy for feed should be enforced (Crump et al., 2002).

Intensification of animal production

Intensification in animal production has been accompanied with the use of chemicals, antimicrobials, manufactured ration feeding leads to emergence of antimicrobial resistant bacteria and food pathogens that subsequence by affecting human being.

There are hundreds of feed mills have been operating in Bangladesh. These include RRP Agro feed industries, Kazi feeds Ltd. CP feeds (Bangladesh), Aftab feed mill, Pacha feed mill, Paragon feed mill, Nourish feed mill, Aman feed mill, Provita feed mill, BRAC feed mill which are conducting large scale animal feed production. Moreover, many other small scale feed mills are operating small scale production and selling feed throughout the country. Despite of heavy levy burden, these feed mills contributing, relentlessly in animal production especially to the poultry industry. Their quest to meet the nutritional demand, create new employment opportunity and earn foreign currency through production of safe egg and chicken meat with low production cost. Feed mills are importing feed ingredients including fish oil, vegetable fat, vitamin and mineral premix, lime stone , choline chloride, Cibenza DP-100, Chicken Boost, DCP, MCP, L-Lysine, L-Threonine, L- valine and DL-Methionine; 99% enzymes with heavy levy for manufacturing feed in Bangladesh.

Antimicrobial use in feed as growth promoter leading to development of antimicrobial resistance in gut microbiota of animals. In this case both pathogen and normal gut micro biota develop resistance to antimicrobials and loose some beneficial microbiota associated with animal growth and production. Thus use of antimicrobials as growth promoter increase production cost and give rise of antimicrobial resistance microbes. In a study (Islam et al., 2012), report showed that 78% respondents among the interviewed farmers used feed additives for cattle fattening purpose for a duration of 3 to 6 months In the same study, 58% respondents used anabolic steroids for the duration of 3 to 6 months long cattle farming program. This practice led those farmers to low animal revenue return from cattle fattening program in contrast to those farmers who used neither growth promoter nor anabolic products in beef cattle fattening program. Generally, antibiotics are highly important in animal production where it has therapeutic significance. It is believed that sub therapeutic use of antibiotics in animal production helps in animal growth and production. There is evidence in Denmark, Norway, Sweden and other European countries where hygienic and disease control regimes are of a high standard has no reduction in production if growth promoting antibiotics are not used (Chowdhury et al., 2009). In Bangladesh, the use of antimicrobials and hormones are band from using as growth promoters.

Conclusion

To ensure food security, sustainable ethical trade, food quality and safety, it is essential to establish feed quality and safety monitoring system. This effort will also prevent transmission of antimicrobial resistance bacteria, resistance genes and pathogens to animal body through consumption of feed.

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CHAPTER 3

Quality of meat and meat products

Summary

Meat, especially poultry meat is one of the cheapest sources of protein. Therefore, meat has gained popularity among the people in every corner of the world. In Bangladesh, poultry is the cheapest source of animal protein. Due to increasing demand from the domestic market, beef and poultry production has been increasing rapidly by using antimicrobials, chemicals and other potential techniques. But poultry and beef industries are still vulnerable due to presence of weak value chain. Processed meat marketing facilities are insufficient. Meat quality control system is merely present and even does not exist in most of the places. Graded meats are not available to offer choice of option for consumers in meat purchasing. Overall, meat produced in the industry reached to the consumer's dining table travelling through the informal marketing channels. In this study, spoilage microbiota of processed meat, grading of meat which are associated with meat quality are reviewed.

Key words: Meat quality, spoilage microbiota, Meat grade

Introduction

Since the inception of human civilization, human have been enjoying meat and meat products. Our teeth have evolved in a fashion that they are able to tear apart and to chew meat. Apart from protein, meat and meat products are store house of enormous highly valuable vitamins, minerals and trace elements which are stored in a concentrated form. Therefore, meat and meat products have occupied the place and become a part of human balanced and healthy diet today.

The quality of raw meat and products made from raw meat is a topic of frequent discussion. Misbranding is a common practice in the countries where limited control and regulations on public health is available. This is highly relevant in Bangladesh, where informal meat market operated by few meat processing industries. In the raw meat market, consumers/customers are being frequently cheated by the meat seller. They used to sell buffalo meat instead of beef; sheep meat instead of mutton etc.

Apart from misbranding, there is currently limited or consensus on what the term 'quality' really stands for. In general, 'quality' is seen as a combination of two major elements. Primarily, total quality of meat and meat products which includes characteristics that can be measured, such as microbiota profile, tenderness, color, juiciness, shelf life, pH value and residue levels of antimicrobials, pesticides and heavy metals. In contrast, total quality also includes an aspect which is a bit difficult to measure: the consumer's personal perception of the value of meat and meat products. The quality judgment will vary from person to person and from product to product. However, chemical properties of meat are highly related to human health development. These include amino acids, proteins, fat, carbohydrates, vitamins and minerals. There are variations in chemical composition among meats from different animal origin. Results of chemical composition analysis are related with the species of animal origin. Moreover, some chemical parameters like pH of meat linked with freshness property of meat. Therefore, analysis of chemical composition of meat ensure fair trade, nutritional security and state of the products.

Microbiota of meats and poultry

The term “microbiota” is perfect to use in lieu of “microflora” because dates back to the time scientists believed that bacteria is kind of primitive plant. But now bacteria are not plants, therefore the ‘bacterial biota’ or ‘micro biota’ is preferred to “microbiota” of “bacterial flora”.

The predominant genera of bacteria, yeasts and molds that are found in these products before spoilage are summarized in the following table 1 and 2.

Table 1: Genera of bacteria most frequently found on meats and poultry

Genus	Gram Reaction	Fresh Meat	Fresh Livers	Poultry
Acinetobacter	-	XX	X	XX
Aeromonas	-	XX		X
Alcaligenes	-	X	X	X
Arcobacter	-	X		
Bacillus	+	X		
Brochothrix	+	X	X	
Campylobacter	-	X		X
Carnobacterium	+	X		X
Caseobacter	+	X		
Citrobacter	-	X		
Clostridium	+	X		
Corynebacterium	+	X	X	XX
Enterobacter	-	X		X
Enterococcus	+	X	X	X
Erysipelothrix	+	XX		X
Escherichia	-	X	X	
Flavobacterium	-	X	X	X
Hafnia	-	X		
Kocuria	+	X	X	X
Kurthia	+	X		
Lactobacillus	+	X		
Lactococcus	+	X		
Leuconostoc	+	X	X	
Listeria	+	X		XX
Microbacterium	+	X		X
Micrococcus	+	X	XX	XX
Moraxella	-	XX	X	X
Paenibacillus	+	X		X
Pantoea	-	X		X
Pediococcus	+	X		
Proteus	-	X		X
Pseudomonas	-	X		XX
Psychrobacter	-	XX		X
Salmonella	-	XX		X
Serratia	-	X		X
Shewanella	-	X		
Staphylococcus	+	X	X	
Vagococcus	+	X		X
Weissella	+	X	X	XX
Yersinia	-	X		

N. B. X= Known to occur; XX= most frequently reported

Source: James et al., 2005.

Table 2: Genera of fungus most frequently found on meats and poultry

Genus	Fresh and Refrigerated Meats	Poultry
Molds	X	X
Alternaria	X	X
Aspergillus	X	
Aureobasidium	X	X
Cladosporium	XX	
Eurotium	X	
Fusarium	X	
Geotrichum	XX	X
Monascus	X	
Monilia	X	
Mucor	XX	X
Neurospora	X	
Penicillium	X	X
Rhizopus	XX	
Sporotrichum	XX	X
Thamnidium	XX	
Yeasts		
Candida	XX	XX
Cryptococcus	X	X
Debaryomyces	X	XX
Hansenula	X	
Pichia	X	X
Rhodotorula	X	XX
Saccharomyces		X
Torulopsis	XX	X
Trichosporon	X	X
Yarrowia		XX

N. B. X= Known to occur; XX= most frequently reported

Source: James et al., 2005.

Generally, the biota is reflective of the slaughter and processing environments with Gram-negative bacteria being predominant. Among the Gram positives, the enterococci are the biota most often found along with Lactobacilli. Because of their ubiquity in meat processing environments, a rather large number of mold genera may be expected, including Penicillium, Mucor and Clostridium. The most ubiquitous yeasts found in meats and poultry are members of the genera candida and Rhodotorula (Table 2).

Enterococci is gut commensal and high prevalence of Enterococci has been observed in meats. In a study, 255 pork samples were analyzed and 97% were positive for these organisms with 54% of isolates being Enterococci facials and 38% E. faecuim (Hayes et al., 2003). Additionally, 262 beef samples were analyzed and found that 65% of samples contained Enterococci which are E. faecium, 17% E. fecalis and 14% E. hirae (Hayes et al. 2003).

All types of meat products were found to contain diverse types of microbiota. Among them the predominant genera include Paenibacillus, Bacillus, Erysipelothrix rhusiopathiae, Clostridium botulinum, C. perfringens, E. coli, Serratia liquefaciens, Pantoca agglomerans, Citrobacter freundis, Klebsiella pneumoniae, Enterobacter cloacae, E. hafniae, Acrobacter spp., Campylobacter spp. Salmonella spp. Listeria monocytogenes etc. the predominant genera identified from different types of meat and meat products are summarized in the following table 3.

Table 3: Microbiota found in different meat and meat products and poultry

SI.	Meat and meat products	Ref.
1	Pork, Beef, turkey meat, broilers, chicken	Jay et al., 2005
2	Fresh chicken, Frozen chicken, chicken meats, pork liver, lamb's liver, ox liver, retail pork broilers, sheep carcasses, ground beef, swine sample, turkey carcasses, pre-chilled meat, frozen meats, red meats, beef cuts	Jay et al., 2005
3	Broilers, Egg yolks, frozen ground turkey raw rolls, chicken carcasses, ground beef, butcher shop beef, chilled hogs	Jay et al., 2005
4	Broilers, chicken. broiler parts (raw), poultry parts, turkey meat, , ground beef, beef, beef carcasses, steer/heifer carcasses, lamb carcasses, pork corollas, poke	Jay et al., 2005
5	Pork, hog, ground meat	Jay et al., 2005
6	Red meat, poultry beef, ground beef, beef carcasses, lam/mutton, lamb carcasses, lamb products, sheep creases, (frozen) , pork, retail meats, beef carcass, beef products,	Jay et al., 2005

Source: James et al., 2005.

Microbiology of processed meat and meat products

Food habit of human society has been changing with the progress of industrialization. Additionally with the advent of modern technologies, it became possible to distribute meat products long distance from the primary production. Therefore, meat processing technologies are widely practiced to produce meat products which include smoked, cured, cooked and semi-cooked products. The predominant microbiota associated with these meat products are summarized on the following table 4.

Bacteria Genus	Gram Reaction	Relative Prevalence	Fungi Genus	Relative prevalence
Acinetobacter	-	X	Yeasts	
Aeromonas	-	X	Candida	x
Alcaligenes	-	x	Debaryomyces	xx
Bacillus	+	x	Saccharomyces	X
Brochothrix	+	x	Trichosporon	X
Carnobacterium	+	x	Yarrowia	x
Enterobacter	-	x		
Enterococcus	+	x	Molds	
Hafnia	+	x	Alternaria	X
Kocuria	+	x	Aspergillus	Xx
Lactobacillus	+	xx	Botrytis	X
Lactococcus	+	x	Cladosporium	X
Leuconostoc	+	x	Fusarium	X
Listeria	+	x	Geotrichum	X
Microbacterium	+	x	Monilia	X
Micrococcus	+	x	Mucor	X
Moraxella	-	x	Penicillium	Xx
Paenibacillus	+	x	Rhizopus	X
Pediococcus	+	x	Scopulariopsis	X
Pseudomonas	-	xx	Thamnidium	x
Serratia	-	x		
Staphylococcus	-	x		
Vibrio	-	x		
Weissella	-	x		
Yersinia	-	x		
Carnimonas	-	x		
Clostridium	+	xx		
Macrococcus	+	x		
Shewanella	-	x		

Note: X= Known to occur; XX= Most frequently reported

Source: James et al., 2005.

Grading of beef cuts

In the meat retail market, the grade of a beef cut is an important selection factor for many consumers. Similarly, the grade of a beef carcass is critically important to the beef producer, since the price value received is directly dependent upon the grade. Still the consumers and producers alike often are confused as to what grades mean, and how they are determined.

Purpose of Beef Grading

Standards for Grades of Slaughter Cattle and Standards for Grades of Carcass Beef (USDA, 1996) has established by the U.S. Department of Agriculture (USDA), which are designed to facilitate beef marketing through separating a highly variable population of live cattle and/or beef carcasses into groups which are more uniform in quality and composition. USDA provide voluntary service for beef carcass grading, and the user (the packer) is charged a fee for the service. An employee of the USDA determines grades of beef carcass, working independently of both the producer and packer. The USDA Standards include two separate grade designations such as Quality Grades and Yield Grades and are designated by the stamps shown in Figure 1. A carcass may be either Yield graded or Quality graded or both Yield and Quality graded at the same time.

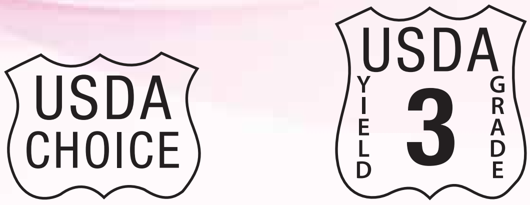

Figure 1: Qualily and Yield Grade Stamps for Beef Carcasses

USDA Prime	USDA Commercial
USDA Choice	USDA Utility
USDA Select	USDA Cutter
USDA Standard	USDA Canner

"Prime beef" holds most desirable eating quality and "Canner beef" is least desirable. Physiological maturity and marbling are the carcass indicators for determining the quality of grade of a beef carcass, as reflected in the Official USDA Grading Chart (Figure 2).

Maturity

The age of a beef animal has a direct effect on tenderness of the meat and its produces. With the increase of age cattle, their meat becomes progressively tougher. In order to account for the effects of the maturing process on beef tenderness, carcass maturity evaluations are used in determining USDA Quality Grades. USDA has established five maturity groupings, designated as A through E. Approximate ages corresponding to each maturity classification are as follows:

A — 9 to 30 months

B — 30 to 42 months

C — 42 to 72 months

D — 72 to 96 months

E — more than 96 months

Relationship Between Marbling, Maturity and Carcass Quality Grade’

Degrees of Marbling					
	A[3]	B	C	D	E
Slightl Abundant	PRIME				
Moderate			COMMERCIAL	COMMERCIAL	
Modest	CHOICE				
Small					
Slight	SELECT		UTILITY	UTILITY	
Traces					
Practically Devoid	STANDARD			CUTTER	

1. Assumes that firmness of lean is comparably developed with the degrees of marbling and that the carcass is not a “dark cutter”
2. Maturity increases from left to right (A through E).
3. The A maturity portion of the Figure is the only portion applicable carcasses.

Figure 2: USDA Beef Grading Chart

Determining USDA Quality Grade

Following maturity and marbling are determined, these two factors are combined to determine USDA quality grade. The association between maturity and marbling used to determine the Quality grade of a carcass are presented in Figure 2. For example, a carcass in the A maturity group which have a Small degree of marbling will be graded as USDA Choice. In general rule, the Prime, Choice, Select and Standard grades are limited to beef from young cattle (A or B maturity; but B maturity cattle are not eligible for the Select grade). Likewise, the Commercial, Utility, Cutter and Canner grades normally are comprised of carcasses produced by cattle of advanced maturity (C, D and E maturity). Carcasses produced by bullocks (A maturity bulls) are eligible only for the Prime, Choice, Select, Standard and Utility Grades, while mature bulls are ineligible for quality grading.

Beef carcass maturity is determined by considering some parameters including (a) the size, shape and ossification of the bones and cartilages in the carcass, and (b) the color and texture of the ribeye muscle. In young animals, the top of each bone in the vertebral column (backbone) used to house a “button” of cartilage. With the progress of maturation, these regions of cartilage gradually change to bone which is called ossification. This ossification process use to occur in a definite pattern. The sacral vertebrae located in the rump portion of the carcass show first signs of ossification. The ossification process gradually moves toward the head through the lumbar (loin) and, finally destined to the thoracic (rib and shoulder) regions of the backbone (Figure 3).

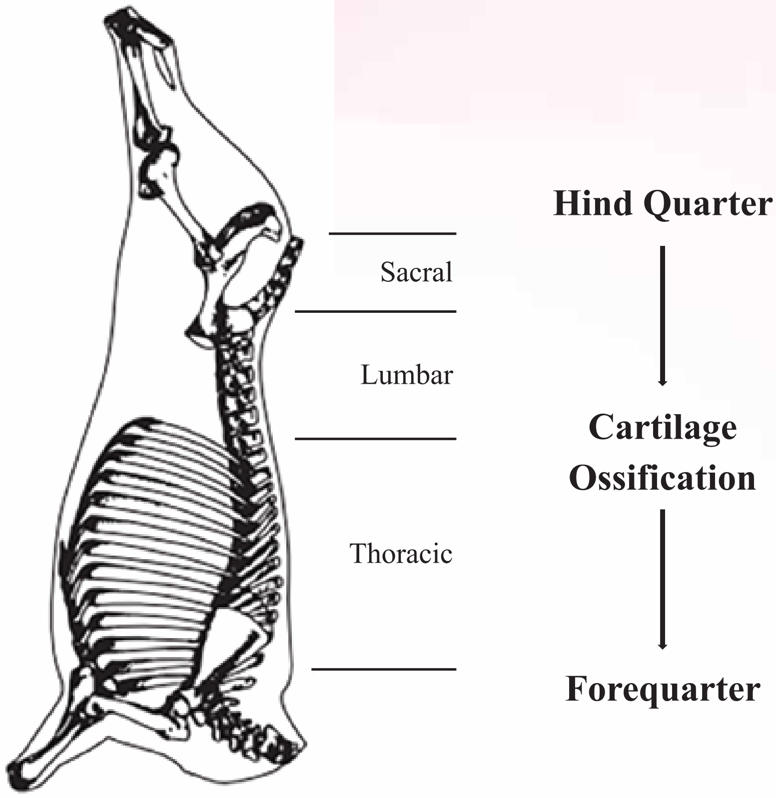

Figure 3

Skeletal Structure of Beef Carcass Showing Progression of Cartilage Ossification in Backbone

In the advance age changes in skeletal characteristics also include a gradual change in shape and appearance of the rib bones. The young animal has narrow and oval-shaped ribs which are red in color. With the progress of animal maturation, the ribs become wider and flatter, and become grey in color. Appearance of the lean tissue also changes with the progress of maturation. In the young animals, the lean tissue is fine-textured and light pinkish-red in color. The texture of the lean becomes progressively coarser and the muscle color becomes darker with the progress of maturation.

Marbling

Marbling is the amount and distribution of intramuscular fat within the ribeye which is the primary determinant of USDA Quality Grade. Marbling in the ribeye is evaluated visually (at the 12th rib cross-section) which are related to differences in eating quality of beef. Beef cuts with high levels of marbling score are considered to be more tender, juicy and flavorful in contrast to cuts with low levels of marbling. Results of studies showed that beef from carcasses grading at least USDA Select is seems to be acceptable in eating quality for most of the consumers. Photographic standards for USDA marbling scores are available from the National Cattlemen's Beef Association. Twelve marbling scores are used to determine USDA quality grades for beef, some of which are shown in Figure 2 and 4.

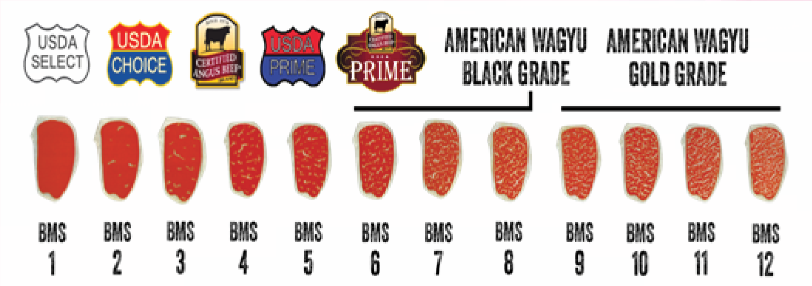

Figure 4: Marbling score of beef cuts

During maturation process the appearance of the lean tissue also changes. In young animals, the lean tissue is fine-textured and light pinkish-red in color. With the progress of the maturation process, the texture of the lean becomes progressively coarser and the muscle color becomes darker. Color is associated with the accuracy of the predictions of overall carcass fatness, the fat thickness measurement usually is adjusted up or down by the grader to account for visible differences in the distribution of external fat in other areas of the carcass.

Yield Grading

USDA Yield Grades is defined as the combined yield of closely trimmed, boneless retail cuts (%CTBRC) from the loin, round, rib and chuck. The Yield grade is a mean to estimate beef carcass cutability. This is a measuring process to estimate the relative amount of lean, edible meat from a carcass. The five Yield Grades for slaughter cattle and beef carcasses have been established which are mentioned below:

USDA Yield Grade 1

USDA Yield Grade 2

USDA Yield Grade 3

USDA Yield Grade 4

USDA Yield Grade 5

The lower the numerical value of the USDA Yield Grade, the higher the yield of closely trimmed, boneless retail cuts (Table 1).

Yield Grade	%CTBRC
1	> 52.3
2	50.0 to 52.3
3	47.7 to 50.0
4	45.4 to 47.7
5	<45.4

Table 1: Expected Yields of Closely Trimmed Boneless Retail Cuts (%CTBRC) for Each USDA Yield Grade

The Yield Grade of a beef carcass is fixed by assessing several factors like (1) external fat thickness over the rib eye, (2) ribeye area, (3) estimated percentage of kidney, pelvic and heart fat (%KPH), and (4) hot carcass weight.

Fat Thickness

Fat thickness is determined at a point of three fourths of the distance of the length of the ribeye from its chine bone side (Figure 4). This single measurement is an accurate indicator in the prediction of overall carcass fatness. But in order to improve the accuracy of the predictions of overall carcass fatness, the fat thickness determination usually is adjusted up or down by the grader to account for observable and measurable differences in the distribution of external fat in other areas of the carcass.

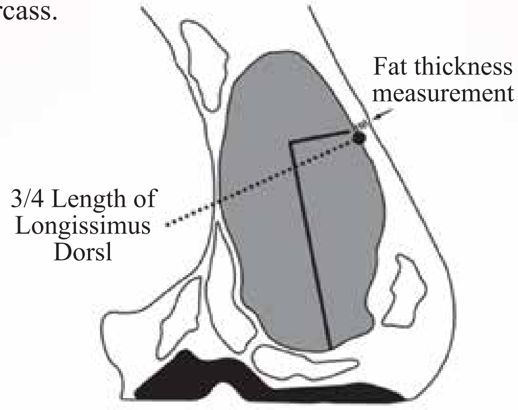

Figure 4
The Location Where Fat Thickness Over the Ribeye is Measured

Ribeye Area and Carcass Weight

The strong association between ribeye area and carcass weight is used in Yield Grading beef carcasses to elucidate differences in cutability stemming from carcass muscularity. Ribeye area generally ranges from about 9 to 17 square inches among carcasses of common weights and can be determined by using a plastic grid (Figure 5).

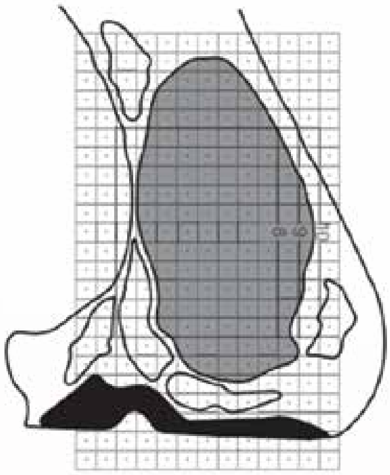

Figure 5: Method of Measuring Ribeye Area

In using the grid to measure a ribeye, place it on the cut surface of the ribeye and count all squares in which iean surrounds a dot. divide the number of squares counted by 10. The resulting number is the area of the ribeye in square inches.

Kidney, Pelvic and Heart Fat Percentage (%KPH)

Fat deposits around the kidney and heart, and in the pelvic cavity, typically are left in the carcass during the slaughter process and affect carcass cutability. Most carcasses have 1% to 4% of the carcass weight represented as kidney, pelvic and heart fat.

Determining USDA Yield Grades

The formula for calculating Yield Grade is:

YG = 2.5 + (2.5 x adjusted fat thickness, in.) + (.20 x KPH%) – (.32 x ribeye area, sq. in.) + (.0038 x hot carcass weight, lbs.)

While the USDA Grader may use this equation occasionally, most determinations are based upon the Grader's experience and training, checking occasionally with the formula when requested to do so. The same holds true for the Grader's determination of the USDA Quality Grade.

Conclusion

Consumers and producers often do not have a clear understanding of beef grading. Beef grades are of two types, Quality Grades and Yield Grades. Most consumers are familiar with the names of several Quality Grades and may use them as a selection criterion when purchasing at retail. However, Yield Grades have less direct impact on consumer selection decisions. Producers, on the other hand, depend greatly on both Quality and Yield Grades as a marketing tool for beef cattle and carcasses. USDA Quality Grades are used to predict the palatability of meat from a beef animal or carcass, using carcass physiological maturity and marbling to determine the USDA grade. USDA Yield Grades are used to estimate the expected edible lean meat, with a USDA YG 1 being the leanest and a USDA YG 5 being the fattest.

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CHAPTER 4

Microbiota of milk and milk products

Summary

Milk contains high level of nutrients and therefore, ideal food for child and old and even the first food of all mammalian species. Due to high level nutrient content, milk is ideal environment for microbial growth and multiplication. It is regarded that freshly drawn milk is sterile but contamination of milk starts from the teat canal. Milk receives microbes from teat, udder, coat of the animal, environment, milker's hand. Thus milk contains normal and pathogenic microbiota. Normal microbiota includes beneficial microbes like Pseudomonas spp. The main pathogens include Salmonella spp., E. coli etc. Microbiota of milk might change after processing and process microbiota comes from the processing environment. Therefore, controlling of constant microbial activity is essential to ensure milk self-life and food safety as well.

Key words: Spoilage microbiota, Pathogens, Normal microbiota, milk

Introduction

Milk is the nature's single most complete food for infants and the old people. Microbes grow and multiply very fast in milk due to its high nutritive value. Cow's milk is home to a diverse range of bacterial species. Although freshly drawn milk from healthy udder is considered to be sterile (Tolle, 1980), bacteria can be reached into the milk during and after milking (Gleeson et al., 2013). Sources of contamination include the resident bacteria within, the udder and the teats and the transient bacteria as well as the milking and storage equipment (Giffel Te. et al., 2002). Primarily, these bacteria are the commensal flora of the cow, or the environmental organisms available in the dairy farm environment.

Despite bovine milk from healthy cow considered to be sterile, some bacteria may enter the udder and cause infection leading to mastitis. Pathogens frequently associated with mastitis include Streptococci, Enterococci, Aerococci as well as Klebsiella spp. (Munoz et al., 2007), Escherichia spp. (Burvenich et al., 2003) and Pseudomonas aeruginosa (Robinsson, 1990). More others include Staphylococcus aureus, E. coli, Streptococcus agalacticeae, Streptococcus dysagalactiae, Streptococcus uberis, Corynebacterium pyogenes. Some other human pathogens including Salmonella spp., Listeria monocytogenes, Mycobacterium bovis and Mycobacterium tuberculosis are occasionally associated with mastitis. Primarily milk receives bacteria from milk handling equipment such as teat cups, pipework, milk holders and storage tank. Typically fewer than 102-103 CFU ml-1 in aseptically drawn milk from healthy cows and milk drawn from some quarters found to be sterile (Adams and Moss, 2008). Milk drawn from early stage of acute mastitic animal the bacterial count can exceed 108 CFU ml-1 and microscopic changes often visible in the milk.

Normal Microbiota associated with milk

The common microbial species associated with milk have extensively been reviewed by (Robinson, 1990). The common microbial species associated with milk listed by Robinson are summarized in the table 1. Milk is the animal produce and are produced in different conditions and approaches, resulting contamination by variety of bacterial species (Robinson, 1990). Therefore one should not consider any bacteria to be normally present in milk. Rather that some bacterial species are more commonly found to contaminate milk that has others. Some of the key milk contaminants are described. Pseudomonaceae and the Neisseriaecae families, Gram-negative aerobes are often seen in raw milk (Robinson, 1990). Some of the members of the Pseudomonads are able to grow in the psychrotrophic temperature range, and can produce heat-stable enzyme

Table 1: Microbiota of milk and milk products

Genus	Species
Spirochetes	
Leptospira	interogans
Campylobacter	jejuni
Pseudomonas	fluorescens fragi putida aeruginosa
Xanthomonas	maltophilia
Acinetobacter	Spp.
Moraxella	Spp.
Shewanella	putrefaciens
Flavobacterium	Spp.
Alcaligenes	Spp.
Brucella	abortus melitensis
Facultative Anaerobic Gram-negative Rods	
Escherichia	coli
Salmonella	Typhimurium
Citrobacter	aerogenes
Yersinia	enterocolitica
Aeromonas	Spp.
Chromobacterium	Spp.
Coxiella	burnetii
Gram-Positive Cocci	
Staphylococcus	varians
	aureus hyicus chromagenes epidermidis caprae
Streptococcus	pyogenes
	agalactiae dysagalactiae uberis salivarius equisimilis zooepidemicus
Enterococcus	fecalis
Lactococcus	lactis
Leuconostoc	mesenteroides paramesenteroides lactis

Spore-forming Gram-positive Rods and Cocci	
Bacillus	cereus Subtilis licheniformis stearothermophilus coagulans
Clostridium	butyricum tyrobutyricum sporogenes perfringenes
Non spore-forming Gram-positive Rods	
	lactis
Lactobacillus	helveticus
	acidophilus
	casei plantarum
	brevis
	fermentum
Listeria	monocytogenes
Kurthia	Spp.
Corynebacterium	Spp.
Arthrobacter	Spp.
Brevibacterium	Spp.
Caseobacter	Spp.
Microbacterium	Spp.
Aureobacterium	liquefaciens
Propionobacterium	freundenrichii
Actinomyces	pyogenes
Mycobacterium	tuberculosis
	bovis

Source: Peter de Vegt.2015. Microbiota of raw milk and the impact of milk on their survival at Low pH. PhD thesis. Massey University, New Zealand.

leading to spoilage of raw and pasteurized milk (Robinson, 1990). Pseudomonas aeruginosa is known to cause bovine mastitis (Watts, 1988). Of the bacteria in the Moraxellaceae family (formerly Neisseriaecae), that are commonly associated with milk is limited to the Acinetobacter and Moraxella-like bacteria (Robinson, 1990).

Enetrobacteriaceae are commonly associated with raw milk except Erwinia, Obesumbacterium, Xenohabdus, rahnella, Cedecea, and Tatumella (Robinson, 1990). The predominant Enterobacteriaceae found in milk, according to this author, are the Escherichia, Salmonella, Citrobacter, Enterobacter and Yersinia. These bacteria are the citizen of the lower intestine and known as indicators of fecal contamination (Tollan et al., 2005). The Enterobacteriaceae known to cause coliform mastitis, which might become acute (Hogan et al., 2003). Raw milk is implicated as vehicle of Enterobacteriaceae, Salmonella or E. coli O157:H7

(Claeys et al., 2013, Langer et al., 2012).

Gram-positive cocci are another dominant group of bacteria including Micrococcaceae and Staphylococcaceae (formerly Micrococcaceae), Streptococcaceae and Enterobacteriaceae (Robinson, 1990). The Staphylococcus is popular to cause serious food poisoning due to the production of enterotoxin (Balban et al., 2000). The major Staphylococcus species found in cow's raw milk include S. aureus, S. hyicus, S. chromagenes and S. epidermis (Robinson, 1990). The Streptococci, remarkably S. uberis are often associated with bovine mastitis (Leigh, 1999). The genus Enterococcus is occasionally isolated from milk. Their presence in milk is the indication of mastitis (Robinson, 1990). The other genera of cocci group as for example Lactococcus and Luconostoc can be isolated from milk and both have importance to the dairy industry as part of starter culture in production of dairy products like butter or cheese (Correler et al., 1998; Robinson, 1990).

The other Gram-positive rods which are non-sporulating agents. These are found in raw milk including lactobacillus, Listeria and the Coryneform bacteria (Robinson, 1990). The Lactobacillus spp. are widely used as mesophilic or thermophilic starters and may also cause spoilage of some cheese (Robinson, 1990). The lactobacilli have also probiotic properties (Spanhaak et al., 1998). Listeria monocytogenes is a well-known pathogen and may be isolated throughout the farming environment, including soil, silage, sewage and milk (Farber et al., 1991).

Raw milk is also the home to yeast, molds and viruses. Yeasts belonging to the genera Debryomyces, Kluyveromyces, Saccharomyces and Candida are associated with products and of these, only Kluyveromyces is likely to be isolated from milk. The remainder are more closely associated with cheese, or other fermented dairy products such as kefir (Robinson, 1990). Molds such as Geotrichum spp., Sporendoma spp., or Penicillum spp. are also often found on cheeses (Robinson, 1990). The double strand DNA cow-pox virus may sometimes be transmitted to human via milk are polio virus and Rubella (Robinson, 1990). Milk can also contain a range of bacteriophages that infect the lactic acid bacteria, i.e. member of the Lactococcus, thermophilic Streptococcus, Lactobacillus and Luconostoc genera (Robinson, 1990).

Bacterial Diversity in Milk

Reports from several studies over time has provided us an insight of the types of bacteria that may be present in raw milk. However, it is a must to investigate the microbiota of raw milk, to reveal what one is exposed to when raw milk is consumed. Before the advent of high throughput sequencing (DNA based) methods, plating method was the only method for microbial or bacterial population study in the old days which allowed to observe the viable but culturable bacterial population in milk. DNA based method like metagenomics study provide greater insight into the total Microbiota (viable but not culturable microbes also) not just those can be cultivated on agar plates.

The diversity of bacteria in raw milk was published in 1962 (Thomas et al., 1962) and the authors found that the type of bacteria isolated changed with total concentration of bacteria in the milk samples. Milk samples that showed low total counts on Yeastrel-Milk agar (<5 x 10^3 CFU/ml) contained predominantly Micrococcus spp. (68.7% of isolates). It was found that the total proportion of micrococci decreased as the total plate count increased. But micrococci were still detected as prevalent biota in 90% of all samples taken. In contrast, milk samples that contained higher total plate counts on Yeastrel-Milk agar (>1 x 10^6 CFU/ml) revealed Gram-negative rods as dominant biota (51.2% of isolates). (Thomas et al., 1962).

The milking practice carried by each farmer may contribute to the diversity of bacteria found in the milk they produce (Verdier-Metz et al., 2009). The results of this study showed that after comparing milk from 67 different dairy farms and surveying farmers about milking habit, that certain methods generated in a significantly different set of bacteria being prevalent in the milk. Following the use of single strand conformational polymorphism analysis to identify distinct groups of bacteria and found that bacteria of one group had a low diversity index and consisted mainly of skin bacteria. It was found to be consistent with good hygiene practices such as washing of teats and good animal husbandry. Another second group contained much diverse range of bacteria, and originated from farms where udder hygiene was less strictly observed (Verdier-Metz et al., 2009).

To figure out raw milk microbiota for cheese production, Mallet et al. compared the microbiota of raw milk produced in the French winter, when cows are housed in indoors with spring where they are grazed outside. The loads of the Lactobacilli were two fold higher in the spring, in contrast to the winter. This is due to seasonal variation. In addition, this study reported a high variation in the standard plate counts of individual farms, and that a high degree of diversity was observed (Mallet et al. 2012). Mallet et al also show that milking and farm management practices such as predipping of teats or herd size also affected the level of diversity seen in raw milk (Mallet et al. 2012).

Higher bacterial loads were found in raw milk in Bangladesh which ranges from 5.2 x 10^8 to 1.3 x 10^7 CFU/ml. This indicate poor milking practices and hygiene was not strictly regulated during milking practices (Banik et al. 2014). Similar results were generated by another study in Bulgaria where raw milk sample were found to contain very high level of total count (1.25 x 10^3-8.03 x10^2 CFU/ml). Coliform count 3.17 x 10^3-2.87 x 10^3 CFU/ml). This is the indication of poor sanitary condition.

In recent time several studies on microbial diversity in raw milk were carried out. The key microbial species found in raw milk are summarized (Quigley et al., 2013) which are presented in the table 2. In culture dependent microbial diversity the predominant genera include the genera Mycobacterium, Lactobacillus, Lactococcus, Enterococcus and Chryseobacterium. While the less dominant genera include Staphylococcus, Streptococcus, Pseudomonas, Corynebacteria and Acinetobacter. Moreover, many other bacterial species are occasionally detected. The exact set microbial population structure present in a sample are mainly relies on the environmental conditions and the milking practices employed at the farm from which milk is obtained.

Table 2: Bacterial diversity detected in raw cows' milk using culture-dependent diversity studies

Prevalent Populations	**Occasionally detected populations**
Microbacterium liquefaciens/oxydans/	Rhodococcus erythropolis
lacticum	Serratia liquefaciens/odorifera
Lactobacillus casei/curvatus/mindensis/	Enterobacter gergoviae
animalis/	Kelbsiella ozaenae/oxytoca
coryneformis/	Kocuria carniphila/kristinae/rhizophila
curvatus/	Frigoribacterium species
Delbruceckii/johnsonii/paracasei/	Paracoccus species
paraplantarum/	Micrococcus species
plantarum/	Orchobacterium anthropic/tritici
Rhamnosus/amylovorus	Pantoea agglomerans
Lactococcus lactis/garvieae	Proprionobacterium freudenreichii/jensenii
Enterococcus faecalis/gallinarum	Providentia stuartii
Saccharominimus	Psychrobacter maritimus
Chryseobacterium species	Pseudoclavibacter helvolus
Less prevalent population	Rahnella aquatilis
Staphylococcus capitis/cohnii/	Renibacterium salmoninarum
Saprophuticus/equorum/xylosus/	Sphingomonas species
aureus/hemolyticus/hominis/epdrmidis	Achromobacter delicatus
Streptococcus uberis/parauberis	Aeromonas hydrophila
Pseudomonas alcalophila/stutzeri	Arthrobacter arilaitensis/ psychrolactophilus
/synxantha/fluorescens/putida	Brachybacterium nesterenkovii
Corynebacterium	Deinococcus species
ammoniagenes/freneyi/glutamicum	Leuconostoc mesenteroides
/variabilis/casei	Escherichia coli
Acinetobacter johnsonii/junii/haemolyticus/lwoffii	Aerococcus viridans
	Bacillus cereus
	Brevibacterium helvolum/linens

Source: Peter de Vegt. 2015. Microbiota of raw milk and the impact of milk on their survival at Low pH. PhD thesis. Massey University, New Zealand.

The new insight into the diversity of microbes prevalent in milk has revealed with the use of DNA based technologies that do not require cultivation of microbial isolates. There are several culture independent methods including Denaturing Gradient Gel Electrophoresis (DGGE) (Gianni et al., 2009), single-strand conformation polymorphism (SSCP) analysis (Verdeir Metz et al., 2009), and 16S rRNA bacterial clone libraries (Delbes et al., 2007; Rasolofo et al., 2010) and Next generation sequencing method. These technologies have been used in recent years to analyze the bacterial population that may be prevalent in milk. There are some limitation should be considered in case of DNA based methods despite these methods promise great insight on the bacterial diversity prevalent in a given sample (Schloss et al., 2011). Importantly, bias may be introduced within some 16S sequences amplify more readily than others (Kindwarth et al., 2013). Additionally, chimeric sequences may produce after several rounds of replication where sequences anneal during the PCR cycles (Haas et al., 2011).

The composition of two 16S clone libraries have been generated from raw milk in such manners is shown in table 3.

Table 3: Microbial genera observed in raw milk based on two independent clone libraries

Group	Genus	Composition (%) Delbes et al., 2007	Composition (%) (Rasolofo et al., 2010)
Bacilli	Staphylococcus	1.6	32.7
	Turicibacter	4.8	-
	Jeotgaicoccus	0.8	-
	Facklamia	2.4	5.4
	Lactobacillus	4	3.6
	Lactococcus	4	3.0
	Streptococcus	4.8	4.8
	Enterococcus	0.8	??
	Trichococcus	-	3.0
	Aerococcus	-	1.8
	Other Bacilli		8.3
Clostridia	Clostridium	24	9.5
Unaffiliated Firmicutes		10.4	-
Actinobacteria	Corynebacterium	5.6	6.0
	Arthrobacter	6.4	-
	Other Actinobacter	4.8	6.0
Proteobacteria	Pseudomonas	0	2.4
	Enterobacter	0.8	-
	Acinetobacter	1.6	6.5
	Ralstonia	12	-
	Other Proteobacteria	7.2	5.4
Bacteroides		4	1.2

Source: Peter de Vegt. 2015. Microbiota of raw milk and the impact of milk on their survival at Low pH. PhD thesis. Massey University, New Zealand.

The milk samples from one farm in France was obtained to analyze and from which a clone library with 125 individual clones were constructed (Delbes et al., 2007). Another study conducted a 16S RNA analysis of raw milk from a milk processing plant in Canada which contained milk from several farms. A clone library with 168 clones was constructed. Surprisingly similar bacterial profile was revealed in raw milk from both studies despite being geographically separated. Firmicutes was the predominant genera among the bacterial population identified in both studies. In the second showed higher proportion of Staphylococcus than in the first study (32.7% and to 1.6% respectively). Anaerobic Clostridia consist of 24% and 9% of the bacterial genera found, revealed, respectively, which would not have been detected by using traditional aerobic culture approaches. The other portion of bacteria ranged between 15% and 20% of bacterial species detected and it appears as though there is some variation in the genera detected between the two studies. The both studies showed the prevalence of Bacteroides which is a common fecal macrobiotic (Mariat et al., 2009). The result of these studies revealed that anaerobic microbiota might be prevalent in raw milk.

Even though DNA based bacterial identification is stronger but there is limitation as these methods may identify only dominant bacteria in a given sample. The bacterial population available in milk is row being analyzed by using 454 pyro sequencing methods, which allows for the detection of rare species by analysis of a high volume of sequences. By using 454– pyro sequencing, the microbiota in Danish milk used for cheese making were analyzed. In two samples 211 and 256 different species were identified (Masousd et al., 2011). Only those comprising at least 0.1% of the total population were presented. Some of the rare isolates identified included the genera of Alistipes, Caulobacter, Carnobacterium, Kurthia or Ruminococcus. Interestingly, other bacteria more commonly isolated in milk were found to be present in low numbers, including Bacillus subtilis and Enterococcus fecalis (Masoud et al., 2011). Next generation sequencing method was applied to determine microbiota in milk of water buffalo from mastitic and from the healthy buffalo. In this study V1-V2 regions of 16S rRNA gene were amplified for each sample. The result of this study revealed that water buffalo milk microbiota consist of 9, main phyla, namely Actinobacteria, Bacteroides, Corynebacteria, Firmicutes, Fusobacteria, Proteobacteria, Spirochetes, TM7 and Tenericutes. In the milk drawn from healthy cows microbiota dominated by Firmicutes, representing the 57.70% of the bacteria, followed by Proteobacteria (23%), Actinobacteria (12%), Bacteroides (6%) and Fusobacteria (1%). In contrast to milk from healthy animals, subclinical mastitis milk present a decrease of Firmicutes (48%) and Actinobacteria (6%) and a relative increase in Bacteroides (11%) and Proteobacteria (33%). In case of clinical mastitic milk, the relative abundance of Bacteroides increases to 24% and Fusobacteria to 8%, whereas, Proteobacteria, Tenericutes and Actinobacteria were detected (Catozzi et al., 2017).

Milk processing and the microbiota of processed milk

Raw milk is home to diverse type of microbes including human pathogens like Mycobacterium bovis, Salmonella Spp., E. coli and Brucella Spp. Therefore, consumption of raw milk is a public health threat. Processing of raw milk reduces the threat of infection through consumption of milk. Pasteurization is one of the effective methods of inactivation of bacteria found in raw milk. This method allows to improve the safety and shelf-life of milk sold to consumers. To understand how bacteria may survive processing, this process will be addressed in the following section.

The processing of raw milk

The processing of fluid raw milk include few steps namely cream separation and skim milk fractions, pasteurization, and homogenization. Generally in the dairy processing plants fluid raw milk is pasteurized by heating to 72 °C for 15 seconds. Even though this step is effective but does not result in production of sterile milk.

In England and wales, the bacterial concentration in pasteurized milk considered unacceptable if the bacterial count was 30,000 CFU /ml (determined by total bacterial count) (Robinson, 1990). Milk and cream is generally recombined into a standardized product in a predefined formulation, depending on the milk–fat required in the final product. Homogenization is the reduction of fat globule in size by mechanical action under pressure.

Microbiota prevalent in processed milk:

Regarding microbiota of processed milk, much attention is paid on the identification and control of spoilage organisms. Spoilage bacteria of pasteurized milk can be categorized into two categories; those that are thermotolerant and those that contaminate milk post-pasteurization. Both kind of these bacteria may, together or in isolation, contribute to the spoilage of a given pasteurized milk sample (Ternstrom et al., 1993).

The survivor bacteria after pasteurization are known as thermoduric or thermo tolerant. They are capable to withstand periods of high temperature yet are not necessarily thermophiles (Egdell et al., 1950). Survivor thermotolernat bacteria found in milk after pasteurization include Micrococcus, Mycobacterium, Streptococcus, Lactobacillus, Bacillus, Clostridium, the Coryneform bacteria and some Gram-negative rods (Thomas et al., 1967).

Some of the member of these, e.g. the Streptococcus, Micrococcus and the Corynebacterium bacteria grow slowly at refrigeration temperatures (Robinson, 1990; Seiller et al., 1984). Other psychrotrophic spore forming bacteria can manage to survive pasteurization and also proliferate at low temperatures (Griffiths et al., 1990). Bacillus spp. are the predominant genera belonged to this group (Meer at al,1991) which are commonly found in pasteurized milk. This is due to their spores' ingenious ability to withstand high temperatures (Meer et al., 1991). One of the members of Bacillus genera e.g. Bacillus cereus can reach spoilage levels of 1 106 CFU/ml in enhanced shelf–life (UHT) milk (Schmidt et al., 2012). B. cereus is also responsible to cause enzymatic spoilage of milk, and produce food borne toxins (Schmidt et al., 2012).

Spoilage of milk occurs from thermo sensitive bacterial species even after prompt pasteurization studies on spoiled pasteurized milk revealed that was caused by heat labile Gram-negative rod and indicated that they are post-pasteurization contaminants (Schroder, 1984).

The microbiota of pasteurized milk included some post process contaminants potentially Pseudomonas fluoresces, P. Putida and Janthinobacterium levidium (Enroth et al., 2000) It is not fully understood how these bacteria are re-introduced, but one key area where this may occur is during bottle filling in the milk plant (Eneroth et al., 1998).

Pasteurized commercial milk might contain pathogenic bacteria even after processing Enterobacteriaceae has been isolated from 7% pasteurized milk or cream after storage at 7 °C (Lindberg et al., 1998). Serratia liquifaciens, Hafnia alvei and Rahnella aquatilis were most dominant bacterial species found in milk or cream. Additionally, milk might contain spores of the Clostridium and Bacillus genera. Fortunately, the Clostridia are not able to sporulate in fluid milk due to the high redox potential (Robinson, 1990). However, they might be able to grow in mascarpone cheese made from pasteurized milk (Franciosa et al., 1999). The predisposing cause is that it is stored under oil, creating an anaerobic environment.

Mycobacterium avium subsp. paratuberculosis is the bacterial pathogen cause Johne's disease in cattle, and can be found in infected cows (Taylor et al., 1981). It is also linked with Crohn's disease in human (Feller et al., 2007). It was controversial that whether this bacterium is capable of surviving pasteurization (Ellingson et al., 2005).

However, this has largely been refused by the work of Pearce et al., 2001. However, the live bacterium has been identified from the commercial milk in the United Kingdom and the United States of America (Ellingson et al., 2005). Most likely due to and in adequate pasteurization.

Microbiological Hazards made from raw milk

The microbiological hazards possibly present in raw milk dairy products, particularly cheese, butter, cream and butter milk. The major bacterial hazards of raw milk cheese (mainly fresh cheese and soft cause) are associated with Listeria monocytogenes, Vero toxin producing Escherichia coli (VITEC), Staphylococcus aureus, Salmonella and Campylobacter. Listeria monocytogenes, VITEC and S. aureus have been revealed as microbial hazard in raw milk butter and cream of choice to a lesser extent because of a reduced growth potential in contrast to cheese. Dairy products produced from raw milk may also be contaminated with Bacillus spp. Mycobacterium avium subsp. paratuberculosis have also been detected in dairy products made from raw milk.

Conclusion

Milk is prone to rapid microbial spoilage due to its high nutrients content. Milk is home to normal and pathogenic microbiota. Spoilage microbiota is responsible to cause rapid spoilage of microbes under improper chilling condition or under inconvenient environmental condition or entry of pathogens and other spoilage microbes in the processed products through leakage. Pathogens are responsible to cause food-borne diseases. Therefore, constant control microbial activity is recommended until consumption.a 1991) which are commonly found in pasteurized milk. This is due to their spores' ingenious ability to withstand high temperatures (Meer et al., 1991). One of the members of Bacillus genera e.g. Bacillus cereus can reach spoilage levels of 1 106 CFU/ml in enhanced shelf–life (UHT) milk (Schmidt et al., 2012). B. cereus is also responsible to cause enzymatic spoilage of milk, and produce food borne toxins (Schmidt et al., 2012).

Spoilage of milk occurs from thermo sensitive bacterial species even after prompt pasteurization studies on spoiled pasteurized milk revealed that was caused by heat labile Gram-negative rod and indicated that they are post-pasteurization contaminants (Schroder, 1984).

The microbiota of pasteurized milk included some post process contaminants potentially Pseudomonas fluoresces, P. Putida and Janthinobacterium levidium (Enroth et al., 2000; Dogan et al., 2003). It is not fully understood how these bacteria are re-introduced, but one key area where this may occur is during bottle filling in the milk plant (Eneroth et al., 1998).

Pasteurized commercial milk might contain pathogenic bacteria even after processing Enterobacteriaceae has been isolated from 7% pasteurized milk or cream after storage at 7 °C (Lindberg et al., 1998). Serratia liquifaciens, Hafnia alvei and Rahnella aquatilis were most dominant bacterial species found in milk or cream. Additionally, milk might contain spores of the Clostridium and Bacillus genera. Fortunately, the Clostridia are not able to sporulate in fluid milk due to the high redox potential (Robinson, 1990). However, they might be able to grow in mascarpone cheese made from pasteurized milk (Franciosa et al., 1999). The predisposing cause is that it is stored under oil, creating an anaerobic environment.

Mycobacterium avium subsp. paratuberculosis is the bacterial pathogen cause Johne's disease in cattle, and can be found in infected cows (Taylor et al., 1981). It is also linked with Crohn's disease in human (Feller et al., 2007). It was controversial that whether this bacterium is capable of surviving pasteurization (Ellingson et al., 2005).

However, this has largely been refused by the work of Pearce et al., 2001. However, the live bacterium has been identified from the commercial milk in the United Kingdom (Grant et al., 2002) and the United States of America (Ellingson et al., 2005). Most likely due to and in adequate pasteurization.

Microbiological Hazards made from raw milk

The microbiological hazards possibly present in raw milk dairy products, particularly cheese, butter, cream and butter milk. The major bacterial hazards of raw milk cheese (mainly fresh cheese and soft cause) are associated with Listeria monocytogenes, Vero toxin producing Escherichia coli (VITEC), Staphylococcus aureus, Salmonella and Campylobacter. Listeria monocytogenes, VITEC and S. aureus have been revealed as microbial hazard in raw milk butter and cream of choice to a lesser extent because of a reduced growth potential in contrast to cheese. Dairy products produced from raw milk may also be contaminated with Bacillus spp. Mycobacterium avium subsp. paratuberculosis have also been detected in dairy products made from raw milk.

Conclusion

Milk is prone to rapid microbial spoilage due to its high nutrients content. Milk is home to normal and pathogenic microbiota. Spoilage microbiota is responsible to cause rapid spoilage of microbes under improper chilling condition or under inconvenient environmental condition or entry of pathogens and other spoilage microbes in the processed products through leakage. Pathogens are responsible to cause food-borne diseases. Therefore, constant control microbial activity is recommended until consumption.

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CHAPTER 5

Microbiology and safety of egg and egg products

Summary

Eggs are considered to be sterile at the time of laying and eggs contain high nutritive value. Therefore, eggs are daily part of human dine in all over the world. Eggs might contain pathogens like Salmonella spp., Campylobacter spp., E. coli and molds as those pathogens get access into egg through vertical transmission, through cracks on shell, through pores. Furthermore, following longer storage period, eggs receive spoilage microbes like Pseudomonas, Acinetobacter, Flavobacterium, Aeromonas and molds which are closely associated with egg spoilage. However, intensified egg production system has been advancing through application of antimicrobials and chemicals leading to occurrence of antimicrobial resistance bacteria in egg and egg products which is associated with public health issues.

Key words: Spoilage microbes, Pathogens, antimicrobial resistance, eggs, egg products

Introduction

Egg is maintaining the carriage of species like birds, reptiles, amphibians, mammals and fishes. Eggs have been eaten by humans for thousands of years. Particularly. The eggs from chicken are the daily part of human dine in every corner of the world due to its high nutritive value.

The eggs of birds are generally regarded as being sterile during laying unless it has been infected congenitally, especially by certain Salmonellas. Following laying of eggs contamination might occur and the access of microorganisms into the egg through cracks in the shell. The egg shell is covered with a water repelling cuticle which acts as a mechanical barrier if intact but there is alternative means of entry for microorganisms. Such as microorganisms enter through pores which perforate the egg shell. There are two membranes beneath the shell which further retard invasion by bacteria for limited periods but probably offers no barrier to the infiltrating hyphae of molds (Board and Fuller, 1974). Therefore, egg might contain pathogens like Salmonella, Campylobacter, E. coli, molds etc.

Moreover, spoilage bacteria other than public health significant pathogens are associated with shelf life of the egg and egg products. The predominant genera of eggs include Acinetobacter, Pseudomonas, Aeromonas, Proteus, Alcaligenes, Escherichia, Micrococcus, Salmonella, Serratia, Enterobacter, Staphylococcus and Flavobacterium. The member of genera of mold include Mucor, Penicillium, Hormodendron, Cladosporium and others while “Torulo” is the only yeast found with any degree of consistency.

Poultry production in Bangladesh has been increased exponentially and therefore gained self-sufficiency in egg production. This has been attained by using modern technologies and application of antimicrobials to combat diseases which has resulted in antimicrobial residue finding in the egg and egg products. Additionally, occurrence of antimicrobial resistant bacteria and resistant genes in the egg and egg products does associated with food safety issues. The acceptability of a product to the potential customers mainly relied on the quality and safety of the products. This study reviewed physical examination method of eggs, pathogens and spoilage microbes of eggs and antimicrobial resistance in eggs and egg products.

Physical examination of eggs

Physical examination of eggs allows us to determine the viability of the embryo. Candling is one of the most effective methods to conduct physical examination to determine fertile and non-fertile eggs. In the candling process a strong light is used to hold above or below the embryo. A candling lamp is made up of a strong electric bulb masked by a plastic or aluminum container appending with a handle and an aperture. The egg is brought against the aperture and illuminated by the light. Candling is done in a dark room.

Determining the viability of embryo

The embryo reveals as a dark shadow and the head come up with a dark spot under the candling lamp. Under the candling lamp, the healthy embryo will found to be moving. When the movement is sluggish and it would like to take 30 to 40 seconds for the embryo to move seen under the candling lamp. This is the indicative of unhealthy condition of the embryo and suggested to discard the embryo. The blood vessels should be examined carefully which are well defined in a healthy embryo. Following the death of an embryo, the blood vessels start to break down and appearing as streaks under the shell when viewed under the candling lamp. Candling used to allow to view the cracks in the egg shells and eggs with cracked shell should be selected for discarding.

Determination of infertile eggs: Under candle and light infertile eggs can easily be detected by observing clear egg. Infertile egg is selected for marketing as table egg.

Determination of early death: The embryo used to develop by passing few days. A small dark area and disrupted blood vessels are visualized by Candling method. Often deteriorating blood vessels are seen as a dark ring around the egg. In that case the egg should be discarded.

Determination of late deaths: It is difficult to tell a viable embryo at the developmental stage. It is needed to notice for the absence of movement and the breakdown of the blood vessels.

Detection of Viable Embryos: The live embryo will move in response to light and well defined blood vessels will be observed.

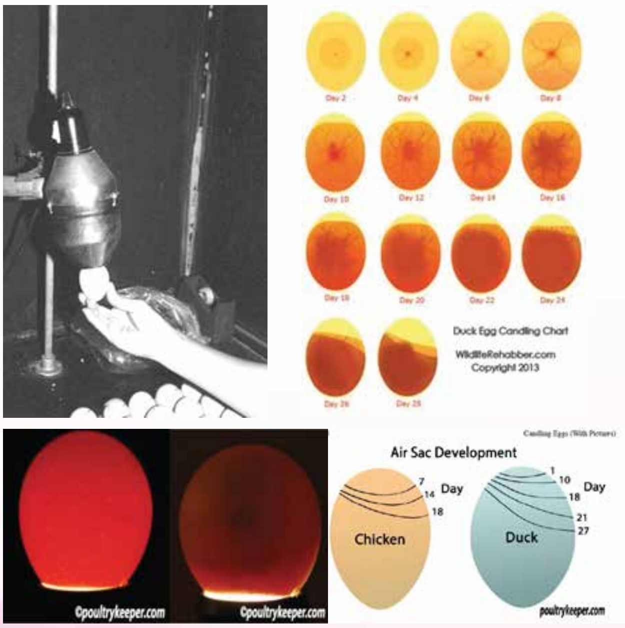

Fig 1: Candling of duck eggs

Table 1: Analysis of parameters of egg and egg products

Organoleptic evaluation	Physical/Texture analysis	Chemical analysis	Microbiological analysis	Identification/Genomics
Using eyes, nose and hand	• Whole egg weight measured to ± 0.1 g • Shell thickness and strength measured to ± 0.1 g • Shell deformation measured to ± 0.1 g • Egg albumen height measured to ± 0.1 mm • The height of the inner thick albumen • Calculation of Haugh's unit • Yolk index calculation • shell breaking strength • Yolk/albumen ration • Egg force reading • Egg Yolk color • Thickness of albumen • The height of the air cell • Egg mass • Diameter and height of the egg • Albumen height • Yolk index • Viteline membrane strength (yolk quality) measured to ± 0.1 g • Dry shell weight measured to ± 0.1 g • Destruction strength of sell • Gel strength of egg white	• Total solids • Crude fat • Moisture • Ash • Protein • Water% • Total Nitrogen • Energy (K Cal) • Energy (KJ) • Cholesterol • Nitrogen Conversion factor • Antimicrobial residue determination **Chemical Residue:** • Antimicrobials • Parasiticides • Hormones • Pesticides • Heavy metals • Nitrate, Nitrite, Nitrosamines • Mycotoxin • Persistent polyhaogenated environmental chemicals • Detergents and disinfectants	Bacterial population studies through microbiome analysis: a) Total viable count b) Total indicator bacterial count c) Bacterial species identification using MALDI-TOF and 16S rRNA gene sequence analysis d) Beneficial bacterial population studies e) Spoilage organism studies f) Antimicrobial resistant bacteria determination g) Bacterial zoonotic pathogen determination **Target Microbes:** • Bacillus spp. • Campylobacter spp. • E. coli • Salmonella spp. • Streptococcus spp. • Listeria spp. • Shigella spp. • Staphylococcus spp. • Pseudomonas spp. • Plesiomonas spp. • Yersinia spp. • Vibrio spp.	• Meat species identification using Min-Ion Nanopre sequencing/ Illumina MiSeq sequencing • Antimicrobial resistance gene determination by Whole genome sequencing (WGS) • Virulence gene determination by WGS

Microbiology of egg

Eggs and egg products are significant part of human diet. Eggs have inbuilt protection against the microbial entry into the inside of eggs. It has two layer of protection system against the microbial entry. External layer and internal layer. The external layer of protection consists of three structures, each of which are effective to some degree in regarding the entry of microorganisms: the outer waxy shell membrane; the shell; and the inner shell membrane. The internal layer mainly consists of egg white which contain lysozyme is known to effective against Gram-positive bacteria. Moreover, egg white contains avidin which forms a complex with biotin and thereby making this vitamin unavailable to microorganisms. The protection system is further enhanced due to high pH (9.3) and presence of conalbumin, which forms a complex with iron, thus rendering antimicrobial system in the eggs. In contrast to this inbuilt antimicrobial system in eggs, the nutrient content of the yolk material and its pH in fresh eggs (about 6.8) create an excellent environment for the growth of most microorganisms (Forsythe et al., 1998).

The existing intrinsic antimicrobial system in eggs has created a solid ground to believe that freshly laid eggs are sterile. The entry of microbes into eggs starts in a relatively short period of time after laying rendering to spoilage of eggs. The entry speed of microorganisms in eggs is associated with temperature of eggs, grow and age of eggs and level of contamination. One day old eggs are more resistant than 4-weeks old eggs for microbial entry and the speed of microbial entry depends on level of contamination and storage condition (Forsythe et al., 1998).

The dominant genera of eggs include Pseudomonas, Acinetobacter, Proteus, Aeromonas, Alcaligenes, Escherichia, Micrococcus, Salmonella, Serratia, Enterobacter, Flavobacterium and Staphylococcus. The member of genera of mold include Mucor, Penicillium, Hormodendron, Cladosporium and others while “Torulo” is the only yeast found with any degree of consistency (Salihu et al., 2015).

Spoilage of eggs

High humidity is the main favorable factor for the entry of microorganisms and under high humid condition their growth is favored on the surface eggs and thereby penetrate through the shell and inner membrane. The inner membrane is the most important barrier to the penetration of bacteria into eggs, followed by the shell and outer membrane. The egg yolk contains more bacteria than the egg white as the egg white contains antimicrobial substances. Moreover, following storage condition the egg white loses water to the yolk, resulting a thinning of yolk and shrinking of the thick white. This process makes it possible for the yolk to come into direct contact with the inner membrane, where it may be infected directly by microorganisms. Once microbes enter into the egg yolk, multiplying rapidly due to presence of nutritious components and produce by products of protein and amino acid metabolism such as H_2S and other foul-smelling compounds. The egg yolk become “runny” discolored which is the effect of significant growth of microbes. Mold grow in the air sac where available oxygen favors their growth. Under high humid condition molds grow outer surface of the eggs. On the other hand, under low humid condition molds does not grow on the egg surface but the eggs loss water at a faster rate rendering eggs unsuitable for commerce (Jay et al., 2005; Forsythe et al., 1998).

“Rotten” egg is the most common form of spoilage. Pseudomonas spp. Cause “Green Rots”.

Especially P. fluorescens, colorless rots by Pseudomonas, Acinetobacter and other species; Proteus, Aeromonas and Pseudomonas cause black rots; Pseudomonas causes pink rots; Serratia spp. causes red rots and Proteus vulgaris and Proteus intermedius cause custard rots. Spoilage of eggs caused by mold is called pin spots e.g. Penicillium and Cladosporidium. Mustiness of egg caused by Pseudomonas graveolens and Proteus spp. and P. graveolens produces most spoilage pattern in eggs (Jay et al., 2005; Forsythe et al., 1998)

Pathogens in Egg and egg products

It is expected that freshly laid eggs are sterile and eggs might receive pathogens like Salmonella enteritidis through vertical transmission. Moreover, Campylobacter spp., E. coli, Coliform (Citrobacter, Enterobacter and Pantoea) have been isolated from the surface of chicken eggs (Salihu et al., 2015).

Antimicrobial resistance in eggs and egg products

Freshly laid eggs are considered to be sterile but eggs might receive bacteria via vertical transmission, from the environment, from the hen itself and from the handlers. Egg production in Bangladesh has been increasing exponentially and Bangladesh is self-sufficient in egg production. In the primary production level, diseases are the major challenges in egg production. Antimicrobials and chemicals have been used to combat poultry diseases. Ciprofloxacin, azithromycin, tetracycline and Colistin are commonly used at the primary production level. There is limited regulation and control on use of antimicrobials in the primary production level. In the absence of antimicrobial resistance monitoring system in egg and egg products, it is not clear about the types and level of antimicrobial resistance prevailing in egg and egg products. Limited data on types and level of antimicrobial resistance are available.

Prevalence of Salmonella was observed in the chicken eggs collected from farms and marketing channels. The predominant serovar was S. typhimurium. All of the isolates were resistant to bacitracin, polymyxin-B and Colistin (Singh et al., 2010). One hundred and thirty one isolates of spoilage bacteria have been identified from the table eggs in Trinidad and 125 out of 131 isolates were resistant to one or more antimicrobial agents and resistance was high to streptomycin (90.1%), tetracycline 51.9%) and kanamycin (30.5%) (Adesiyun et al., 2005).

Antimicrobial resistance of non-typhoidal Salmonella isolated from egg layer flocks and egg shells in New South Wales and South Australia. Following phenotypic and genotypic characterization of antimicrobial resistance, the majority of Salmonella isolates (91.72%) were susceptible to all antimicrobials tested in this study. Those isolates showed limited resistance to amoxicillin and ampicillin (5.51%), tetracycline (4.13%), cephalothin (2.06%) and trimethoprim (0.68%). None of the isolates were resistant to cefotaxime, ceftiofur, ciprofloxacin, chloramphenicol, gentamycin, neomycin, chloramphenicol, or streptomycin. Low frequency of integron was found in the most commonly detected antimicrobial resistance genes among the Salmonella isolates were blaTEM (2.07%), tetA (1.38%) and dhfrV (0.69%). By and large, Salmonella enterica isolates exhibit a low frequency of antimicrobial resistance and represent a minimal public health risk associated with the emergence of multidrug resistant Salmonella spp. from Australian layer industry (Pande et al., 2015).

High prevalence of multiple antimicrobial resistance was observed in Salmonella enteritidis isolated from egg in the city of Combitore, South India which possible prior to selection by the use of antimicrobials in egg production (Suresh et al., 2000). Higher prevalence and antimicrobial resistance level were observed in Salmonella spp., Campylobacter spp., E. coli, Coliform in eggs of conventional farm and organic farm.

Conclusion

Eggs are generally regarded as sterile during laying, but egg might receive microbes through vertical transmission, cracks on shell, and pores in shell. Moreover, egg surface contamination occurs through exposure to environment, handlers and hen itself and with the progress of storage period spoilage of eggs happen by the activity of spoilage microbes. Fertile egg determination and embryo viability can be evaluated by egg candling methods. Pathogens in eggs and antimicrobial resistance in pathogen and normal microbiota is associated with public health issues.

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CHAPTER 6

Available chemicals, antibiotics and their use in animal production in Bangladesh

Summary

Intensified food animal production uses antimicrobials, chemicals to combat animal diseases. In Bangladesh, food animal production has been increasing in a fast pace by using antimicrobials, chemicals, probiotics, vaccines and other chemicals. This has been resulted in occurrence of antimicrobial residue and antimicrobial resistant bacteria in the harvested products. Following a survey based on a standard questionnaire, many antimicrobials, chemicals have been found to be used in the primary animal production. These include, ampicillin, penicillin, amoxicillin, tetracycline, cephalosporins, third generation cephalosporins, potassium permanganate, chlorine, FMD vaccines, Newcastle Disease vaccine etc. Most of the cases, farmers determine the dose and administer the drug themselves and farmers handle the drugs in bare hand and do not wash hand after handling of drugs and chemicals which is associated with occupational health. Moreover, occurrence of antimicrobial residue and resistant bacteria in food is associated with public health issues.

Key words: Antimicrobials, vaccines, antimicrobial resistance, antimicrobial residue

Introduction

Animal production has been growing exponentially in Bangladesh by using latest technologies including antimicrobials, chemicals, probiotics, vaccine etc. Particularly poultry production has been growing fast to meet up the domestic demand. Intensive animal farming practices has been using antimicrobials and chemicals which has resulted in residue findings in the final products and occurrence of antimicrobial resistant bacteria which is public health concern globally.

Antimicrobials are using for treating the animals and also for the prevention of diseases. In order to prevent diseases, antimicrobials are used in feed as growth promoter in many countries of the world where limited regulations and control are prevailing. Developed countries has developed regulations on use of antimicrobials and chemicals which is merely present in many countries of the world and even does not exist in many corners of the world.

Bangladesh is home to 160 million people and therefore, there is a huge demand of animal protein and poultry is the cheapest source of protein in Bangladesh. Since last 30 years, poultry production in Bangladesh is growing fast to meet the demand from the domestic market. Currently, Indian Government has stopped live cattle export to Bangladesh which has resulted in shortage of beef in local market of Bangladesh. Thus the domestic demand of beef production has been increased drastically. Therefore, dairying and beef production has been increasing through intensified production system aided by using antimicrobials, chemicals and other biological inputs. Both poultry and beef production is mainly relied on antimicrobials and chemicals. Even though, there is rules and regulations on the use of antimicrobials but in practice there is no lack of antimicrobials and chemicals at the primary production level.

Bangladesh is a big market of drugs, antimicrobials, chemicals, vaccines, anthelmintic and huge volume of products have been trading in every corner of Bangladesh. Available antimicrobials, chemicals, vaccines and anthelmintic have been summarized in the Table 1, 2, 3 and 4.

Two major livestock production zones were selected and a survey on antimicrobial and chemical use was carried out in 18 dairy farms and 1 poultry farm. These two zones included Savar, Dhaka and Jalalabad and Sikalbaha of Chottogram district. Farms were selected randomly and active surveillance was carried out. The main goal of the surveillance to learn about the trend of use of antimicrobials, chemicals, vaccines and anthelmintics. The overall list of antimicrobials, chemicals are summarized in the Table 5.

Among the interviewed farmers, 100% of the farmers have been found to use antibiotics to treat the animals. They use gentamycin, 3rd generation cephalosporins, tetracycline, oxytetracycline, penicillin, streptomycin, sulfanilamide, trimethoprim and combinations of antibiotics to get synergistic results with or without prescription from the registered Veterinarian.

All of the farms used anthelmintics including albendazole, trichlabendazole, mebendazole, ivermectin, nitroxinil, metronidazole routinely. Nutritional products like Vitamin B 12, vitamin ADE3, Vitamin dairy and Bull, Dicalcium Phosphate, vitamin b-complex were found to be used in every farm.

For the prevention of infectious diseases, diverse types of vaccines have been applied to animals against the infectious diseases. These vaccines included FMD, Black Quarter, Anthrax, and Mastitis. Chemicals and disinfectants like Potassium Permenganate, Bleaching powder, Iodine are commonly used in those farm for cleaning and disinfection purposes.

It was noted that the farmers determine the dose of administration as the instructed dose mentioned on the label of the vial is not effective. They usually administer higher dose than dose level mentioned on the label of the products. Therefore, it is doubtful about the quality of drugs available in Bangladesh which does require to conduct systematic investigation on the quality of drugs available in Bangladesh. Moreover, people in the farm use the drugs and chemical by bare hand which lead to direct exposure to chemicals associated with occupational health hazard.

Table 1: Drugs and antimicrobials available in Bangladesh

Types of animals/farms	Types of chemicals/antibiotics
Large animals/Large animal farms	Penicillin, Crystalline penicillin, Neomycin + Bacitracin, Neomycin + Bacitracin + Polymyxin B Sulphate, Penicillin + Streptomycin, Amoxicillin + Cloxacillin, Amoxicillin, Ampicillin, Cephalosporin, Quinolones, Tetracyclines, Macrolide antibiotic, Aminoglycoside antibiotic, sulfonamide, Sulphadimidine, Combined Sulphadiazine + Sulphadimidine + Sulphapyridine, Potentiated Sulphonamide, Sulphadiazine + Trimethoprim, Combination of sulpha drugs with streptomycin, Cefuroxime, Flucloxacillin, Suppository NSAID, Metronidazole, Griseofulvin, Imidazole, Flucytosine, Nystatin, Benzimidazole, Albendazole, Trichlabendazole, Mebendazole, Imidazothiazole, Levamisole, Hexachlorophene, Trichlabendazole + Levamisole, Niclosamide, Oxyclozanide + Tetramisole, Ivermectin, Pyrantel pamoate, Imidocarb Dipropionate, Diminazine aceturate, Pheniramine Maleate, Promethazine, Paracetamol, Ketoprofen, Meloxicam, Tolfenamic acid, Dexamethasone, Dexamethasone, Dexamethasone + Prednisone, Mucolyte, Expectrorant, Antitussive, Bronchodilator, Diuretics, Nerve tonic, Digestive stimulant, Drug for tympany or bloat, Stomachic and other digestive stimulants, Appetizer, Prebioitcs and Probioitcs, Butaphosphan, Toldimphos, Cyanocobalamine, Vitamin B complex, DB (Dairy and Bull Vitamin, Hematinic mixture, Calcium preparation, Vitamin, Mineral, Amino acid, Multivitamin preparation, Some other nutritional products, Toldiphos, Zink preparation, Vitamin E + Selenium, Liver tonic, Atropine, Haemostat, Electrolytes, Prostaglandin (PG F 2 α), Oxytocin, GnRH, detergents, Antiseptic, Disinfectants,

Small Animal	Amoxicillin, Ampicillin, Cephalosporin, Cefradine, Cefixime, Cefradine, Cefixime, Cefotaxime, Ceftriaxone, Ciprofloxacin, Quinolone, Clindamycin, Erythromycin, Gentamycin, Metronidazole
Poultry/Poultry farm	Amoxicillin, Colistin, Amoxicillin + Colistin, Cephalosporin, Pefloxacin, Enrofloxacin, Flumequine, Tetracycline, Chlortetracycline, Doxycycline, Doxycycline + Oxytetracycline, Neomycin, Doxycycline + Colistin, Gentamycin, Gentamycin + Colistin, Norfloxacin, Sulfanilamide, Sulfachloropyridazine + Trimethoprim, Sulfadiazine + Trimethoprim, Sulfamethoxazole + Trimethoprim, Sulfur drugs + Erythromycin, Sulfaclozine Na, Amprolium HCl and combined with others, Toltrazuril, Diclazuril, Salinomycin, Maduramycin, Pyremethamine + Sulfaquinoxalin, Tiamulin, Tylosine tartrate, Tylosine tartrate + Doxycycline, Tilmicosin, Levamisole, Piperazine citrate, Ivermectin oral solution, Diuretics, Ammonia Controller, Acidifier, Glucose + Vitamin C, Enzyme, Antioxidant, Tozin Neutralizer, Toxin Binder + Mold Inhibitor, Vitamin B complex, Vitamin A, D, E preparation, Vitamin E + Selenium, Vitamin K, Vitamin C, Choline, Zink preparation, Liver tonic, Antiseptics, Disinfectants, Phytase, Pepsin, Pancreatin, Lipase, Cellulose, Cellulase, Xylanase, β-Glucanase, α-amylase, Pectinase, Aminoglycosidase, Hemicellulsae, Pentosanse, Amylase, Arabinase, Xylanase, α- and β Galactosidase

Table 2: Vaccines available in Bangladesh

Types of Animals/Farms	Types of vaccines
Cattle/ Goat / Sheep / Dog	HS Vaccine, Anthrax Vaccine, Black Quarter Vaccine, FMD Vaccine (Bivalent, Trivalent), PPR vaccine, Rabies vaccine (LEP and HEP), Goat Pox vaccine
Poultry	Marek's disease vaccine, Baby Chick Ranikhet Disease vaccine, Fowl pox vaccine, Pigeon pox vaccine, Fowl typhoid vaccine, Fowl cholera vaccine, Infectious bursal disease vaccine, Duck plague vaccine, Haemovax- vaccine against Haemophilosis, MGVAX- Vaccine against Mycoplasma gallisepticum, Avian Influenza virus vaccine, GALLIVAC LT- Fowl laryngo-trachitis vaccine), GALLIVAC AE+ FP- Avian Encephalomyelitis and Fowl pox vaccine, Infectious bronchitis vaccine, Coccidiosis vaccine, Pasteurellosis vaccine, Salmonellosis vaccine, Avian viral Arthritis plague vaccine

Table 3: Antiseptics and disinfectants available in Bangladeshs

Types of farms	Types of antiseptics, disinfectants
Poultry farm	Quaternary ammonium compound, Gluteraldehyde, Isopropanol, Iodine, Benzyl conium, Sodium hypochloride, formaldehyde, Povidine Iodine, Iodine Isopropanol

Table 4: Nutritional compounds and antioxidants available in Bangladesh

Types of Chemicals	Poultry Farm
Antioxidant	Butlated Hydroxy anisol, Ethoxyquin, Citric acid, phosphoric acid, Fatty acid, Silica and calcium
Feed additives	Citric acid, Phosphoric acid, lactic acid, Formic acid, Propylin glycol, Yeast extract, Organic acid, Minerals, Amino acid, Biotin, Yeast Extract Propionic acid, Capropionate, Pumeric acid, Sorbic acid, Yeast Culture, Charcol, Organic acid, Cytoxin, Aluminium silicate, Lecithin
Nutritional compounds	Vitamin B1, B2, and B 6, Thiamin hydrochloride, riboflavin, Pyridoxin Hydrochloride, Nicotinic acid, Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin C, Choline, Zink Sulphate, Zink Sulphate monohydrate, Colin Chloride, Innositol, Nicotinamide, Pantothenic acid, Niacin

Table 5: Use of drugs, chemicals, vaccines, hormones and antibiotics in Bangladesh

Farms from Chottogram Zone	Types of chemicals/antibiotics/anthelmintic
Chittagong 1-10	
Farm 1	Caprofen, Gentamycin, Trichlabendazole, Ivermectin, Ciprofloxacin, Nitronex, Acef, Cefronvet, Renacef, Renamycin, DB Vitamin, Calcium, Catasol, Vitamin B12, Saline, Bleaching Powder,
Farm 2	Acigen, AM-Cox, Kitovet, Renamycin, Metro-IV, Tricef, Trizol, Potassium Permenganate
Farm 3	Trizon-Vet, Cough Vet, Kito-Vet, Acigen, Bleaching Powder
Farm 4	Histavet, Renamycin, Acigen, Amoxicillin, Vita ADE3, Apthoacare, Metro-IV, Encos, Trizon-Vet, Ceftron Vet, Eracef, Renacef
Farm 5	Kito-Vet, Trizon Vet, Pronapen, Bleaching Powder, Endex, Anorexon, Digitop
Farm 6	Endex, Vermic, FMD vaccine, Black Quarter, Gentamycin, KitoVet, Ciprofloxacin, Amoxicillin, Eracef, Ketoprofen, Antihistaminic, Bleaching, Potassium Permenganate
Farm 7	Endex, Triclazol (Levamisole + Trimisol), Vermic, Gentamycin, Trimethoprim, Sulphadimidine, Ciprocin-vet (Ciprofloxacin BP), Ketoprofen, Renamycin, Oxytetracycline, Streptomycin, Penicillin, Potassium Permenganate, Bleaching Powder
Farm 8	Endex, Nitronex, Ivermectin, Eracef, Acigen, Hypomox, Gentamycin, Ceftriaxone, Diadin, Streptomycin, Derma Spray (Chlortetracycline + Methylene Blue + Excipient q. s. p.), Phenol, Potassium Permenganate, Renamycin, Ceftron, Metro-IV, Milk boost, Auyomin, ADE3
Farm 9	Endex, Renadex, Antiworm, Vermic, Gentamycin, Amoxicillin, Kitovet, Eracef, Ceftriaxone, Zymovet, Rumen E, Rasterzyme, Streptomycin, Penicillin, Ciprofloxacin, Kitovet, Diadin, Sulfanilamide, Patilacca, Renavit DB, Auyomin, GPC- 8 (Gluteraldehyde, Quaternary ammonium nonionic surfactants and phosphoric acid), Farm-30 (Iodine)
Farm 10	Triclazol, Nitronex, Gentamycin, Eracef, Tricef. Kitovet, Diavet, Ketoprofen, Antihistaminic, Cal-D-mag, Cal-C Max, Catafos, DCP, DB, Bleaching, Farm-30 (Iodine)
Farm 11	Endex, Vermic, Gentason plus, Kitovet, Eracef, Calvit-P, Pecatrin, Renacal P, Bleaching, Potassium Permenganate
Savar, Dhaka (11-	
Farm 12	Atrovet pure (FMD Vaccine), B-50, Anthrax vaccine, Cataphos, Calcium forte, Streptopen, GnRH Hormone, Digitop, Sulfa-3 plus
Farm 13	Micronid, Tylosine, Ciprofloxacin, Calphos, Triclazol, Neotrex vet, Bleaching Powder
Farm 14	Bleaching Powder, Streptomycin, Gentamycin, Kitovet, Calcium, Zymovet, Bovivet DCP, Nixel, Endex, Ivermectin, Vita AD, Toxinil, Cataphos, Vitamin B-complex, Ultra DB, DC plus
Farm 15	FMD vaccine, Penicillin, Streptomycin, Zinger, Bovicare, No Bloat, Sulfa drugs, Calcium DB, Masti-4, LT- Zole, Livamisole, Triclabendazole, Ivermectin, Potassium Permenganate, Bleaching Powder
Farm 16	Ace Cef-3, Combillin LA, Gentamycin, Doxycycline, Nurelle (Anti-mosquito), Doxycycline, FMD vaccine, Napa, Pronapen, Streptopen, Endex, LT Vet, Ivermectin, Niloxin, Arriah FMD, Soybean oil, Picotin
Farm 17	Endex, Renamycin, Penicillin, FMD vaccine, Napa, Combillin-LA, Ace Cef-3, Doxycycline, Bleaching Powder, Potassium Permenganate, Iodine
Farm 18	Iodine, Gentamycin, Ktoprophane, Albendazole, Endex, Trichlabendazole, Gentamycin, Marbafloxacin, Sulfa-3 (Sulfanilamide + Streptomycin + Metronidazole), Amoxicillin, Ceftriaxone, Mastivac (Vaccine for mastitis)

Table 3: Antiseptics and disinfectants available in Bangladeshs

	Banned antibiotics	
Single antibiotics	**Combined antibiotics**	**Order publication date** This list was published in daily Ittefaq on 07.05.2019
	Amoxycillin + Cloxacillin	
	Amoxycillin + Bromohexin + Vitamin A	
	Doxycyline + Oxytetracycline powder	
	Doxycillin + Neomycin powder	
	Doxycillin + Tylosin	
	Doxycycline + Trimethoprim	
	Doxycycline + Gentamycin	
	Neomycin + Sulphadimidine + Trimethoprim	
	Neomycin + Procaine penicillin	
	Oxycillin + Tetracycline	
	Procaine Benzyl Penicillin + Streptomycin	
	Streptomycin + Sulfadiazine + Sulfadimidine +	
	Sulfapiridine + Sulfaclozine	
	Sulfachloropyridazine sodium + Trimethoprim +	
	Vitamin K	
	Erythromycin + Sulphamethazine + Trimethoprim	
	injection	
	Tylosin + Roxythomycin	
	Norfloxacin + Trimethoprim + Lopramaide	

Source: Published in daily Ittefaq on 07.05.2019.

Conclusion

In Bangladesh, all classes of antimicrobials and chemicals are used to combat diseases. The most striking issue is that farmers themselves determine the dose of antibiotics and administer themselves too. It indicates that indiscriminate use of antibiotics might lead to development of antibiotics resistance bacteria in the primary production. Therefore, it is highly needed to impose regulation and control on the use of antimicrobials in the primary production to protect public health.

CHAPTER 7

Antimicrobial residues in animal source food products

Summary

Intensified animal farming has been using wide array of antimicrobials, chemicals and other latest inputs, leading to residue findings in the livestock products like meat, milk and eggs. Antimicrobial residues like sulfonamide, tetracycline, quinolones, ampicillin, streptomycin, aminoglycosides, fluoroquinolones, neomycin, gentamycin, Oxytetracycline, ciprofloxacin, doxycycline, enrofloxacin have been reported by many scientists from many parts of the world. Occurrence of antimicrobial residues in livestock products pose threat to public health. The detrimental impact include autoimmunity, carcinogenicity, antimicrobial resistant bacteria transfer to human, mutagenicity, nephropathy, hepatoxicity, reproductive disorders, bone marrow toxicity and allergy. Codex Alimenterius Commission (CAC), World Health Organization (WHO) and Food and Agriculture Organization (FAO) have provided regulations on using drugs in the primary production level of farm animal production. Following the regulations, it is mandatory to maintain strictly drug withdrawal period. Moreover, therapeutic use of antibiotic must ensure the use of proper dose and the proper time and use of antimicrobials as growth promoter should be restricted. To sum, the detrimental impact of antimicrobial residue found in livestock products can be minimized by using scientific guidelines and precautions.

Key words: Antimicrobials, residue, Antimicrobial resistance, livestock products, allergy

Introduction

Animal production has been increasing rapidly globally to meet up the demand of food production. This has been achieved by using modern technologies including use of wide array of antimicrobials and chemicals (Pryanka et al., 2017). There has been two-fold increase in per capita milk consumption of developing countries since the early 1960s. Similarly, most of the animal protein demand is met by poultry meat in the world, mainly in the developing countries. As poultry meat is the cheapest animal protein source. Therefore, animal farming has been intensified by using wide range of antimicrobials, chemicals and other advance inputs at the primary production level. Antimicrobials are indiscriminately used as therapeutic and sub-therapeutic in the countries with limited control and regulation. Particularly, use of antimicrobials as feed additives causes dysbiosis in the animal gut, development of antimicrobial resistance in human pathogens and lead to residue findings in the final products. Residues of these antibiotics in milk, meat and eggs have been determined in many of the studies across the world and was found to be one of the possible causes of antimicrobial resistance in human pathogens normal microbiota. The presence of residues of antibiotics in milk, meat and their products beyond maximum permissible limits is clearly associated with public health issues.

Human receives those unwanted antimicrobials through consumption of farmed food products like meat, milk and eggs. Consequently experienced dysbiosis in their gut and acquire antimicrobial resistance in the normal and pathogenic flora in the gut without having any antimicrobials formally. Food processing through heat treatments can reduce the risk of some tetracyclines, sulfonamides and fluoroquinolones without any guarantee of the complete degradation or elimination of these antimicrobial residues present in meat, milk and eggs and their products. Therefore, some of the developed countries like Denmark, Norway, Sweden and the European Union have already stopped the use of antimicrobials at the primary animal production either as growth promoter or sub-therapy in since 1995.

Antimicrobial residues have inhibitory effects in animal product technologies, impairing product quality as well as on human health. Therefore, presence of any residue in animal products are illegitimate and leading to cause economic loss to the industry (Nisha, 2008). Training of farmers to monitor drug withdrawal periods, banning the use of antibiotics as growth promoters, and adopting the veterinary feed directive of the U.S. Food and Drug Administration (US FDA) are salient parameters to mitigate the emergence of antibiotic resistance in bacteria related to poultry production.

Antibiotic used in food animals at the primary production level

In modern animal farming system, antimicrobial drugs are used for both therapeutic and prophylactic purposes. Pencillins, tetracyclines, sulphonamides and aminoglycosides were most frequently used in lactating animals, which led to occurrence of their residues in milk. Drugs are extensively used to promote the animal health, control and treat the infection and to step up the production. Mastitis is the most prevalent and economically important widespread disease in cattle and much of the veterinary treatment of dairy cattle involves intramammary infusion of antibiotics to control mastitis. The most likely cause of violative drug residues is the failure to observe prescribed withdrawal times. The withdrawal time is defined as the time required for the residue of toxicological concern to reach safe concentration as defined by tolerance. However, the extra label use of antibiotics (whenever a drug is used in a manner other that which it is licensed for), mainly dosages deviating from recommendations of the drug manufacturer fall under the main reason for occurrence of antibiotic residues in milk after the end of the withholding period in India. The inappropriate use of veterinary drugs and negligence regarding withholding periods of milk can lead to the presence of residues of these compounds or their metabolites. Usage of antibiotics as preservatives and as growth promoter has also been reported. Other major reasons for occurrence of drug residues in milk are incorrect milking order of cows and insufficient cleaning of milking cluster or milking installation. Few cases of prolonged occurrences of residues in milk are related to veterinary error and insufficient cleaning of milk contact surfaces after milking of treated cows. Education on prudent use of antibiotics has been observed to be particularly lacking amongst dispensers and prescribers of antibiotics (Gaurav et al., 2014; Vishnuraj et al., 2016).

Residue in the animal originated food and food products

Animal husbandry practices mostly relied on use of chemicals and antimicrobials and other modern technologies to combat the disease occurrence and to achieve high production. This practice has resulted in residue findings in the harvested products and antimicrobial resistant bacteria in those intensive farmed products. Studies provided results on occurrence of residue in animal originated food and food products, mainly from the developed countries in contrast data is merely generated from the developing countries even there is no existence of residue testing laboratories.

Antimicrobial use in Dairy animals has resulted in residue findings in milk and milk products. A study was conducted in the USA on antimicrobial residue detection different brand of milk and milk products. Sixty-four samples of different brand company were collected from the supermarkets of USA and analyzed for residue findings. The qualitative results showed that 63% of milk samples contained one or more residues, 27% samples were found to contain two residues, 11% contained 3 or more residues. Sulfonamides and tetracyclines were the most frequent residue detected. Quantitative analysis was done using microbial assays. Among the 9 presumptive tetracycline-positive samples, all the samples were confirmed while 4 presumptive streptomycin-positive samples, 3 were shown to confirm (Brady and Katz, 1988).

A wide array of antimicrobials has been using in animal as therapeutic and non-therapeutic drug at the primary production level. The sub-therapeutic use of antimicrobials in animals has resulted in occurrence of antimicrobial resistance among the environmental bacteria (Bester and Essack, 2010). Among the antimicrobials used in farm animal production, fluoroquinolones, quinolones and are important antimicrobials are used in human and veterinary medicine (Veils-sariou, 2006, Andreu et al., 2007, Canada-Canada et al., 2009, Chafer-Pricas et al., 2010). Several studies has shown the impact like direct toxic effect or the emergence of drug-resistance microbiota predicting a potential risk to human health. These residues might cause allergic hypersensitivity reactions or toxic effects (Juan-Garcia et al., 2006; Canada-Canada et al., 2009). A study was conducted in Turkey to screen quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara. A total of 127 chicken and 104 beef meat samples were collected randomly from the market to conduct study. Result showed that 51.1% chicken meat and beef samples were shown to contain quinolone antibiotic residue. Among the chicken meat and beef samples, 45.7% chicken meat samples and 57.7% beef samples were positive for quinolones, respectively. Moreover, another study was conducted to detect antibiotic residues in poultry meat where kidney and liver samples were analyzed. The results showed that the highest concentration of antibiotic residue found in these tissues were tetracycline which is counted for 8%. Furthermore, ampicillin residue was found in 4% samples, streptomycin in 2% samples and aminoglycosides in 1% samples.

The use of antimicrobials and chemicals in animal farms has been increasing in Bangladesh to enhance the production. Therefore, regulatory enforcement initiative has been launched in the recent past and still there is limited control on the use of antimicrobial and other chemicals. The occurrence of antimicrobial resistance bacteria and residue findings in the final products pose public health threat. Recently, a study was conducted in Bangladesh to screen antibiotic residues in broiler chicken meat and liver collected from farms and commercial market. Results showed that liver samples were mostly positive for antibiotic residues followed by thigh muscle and breast muscle. The antimicrobial residue level was highest in liver in contrast to thigh and breast muscle. The residue level of ciprofloxacin was the top ranked in all samples. In breast muscle antimicrobial residue profile include: ciprofloxacin in 39% of samples; doxycycline in 26% of samples; amoxicillin in 24% of samples; Oxytetracycline in 23% of samples and enrofloxacin in 21% of samples. On the other hand, the residue profile in thigh muscle: ciprofloxacin in 42% of samples; Oxytetracycline in 29% of samples; doxycycline in 28% of samples; amoxicillin in 27% of samples and enrofloxacin in 24% of samples. Highest number of liver samples were found to contain residue of all kind of antimicrobials including ciprofloxacin in 52% of samples, Oxytetracycline in 46% of samples; doxycycline in 43% of samples; amoxicillin in 42% of samples and enrofloxacin in 36% of samples. The results of this study indicated that all of antibiotics are important human and veterinary drugs and their residue findings in chicken meat is a threat to public health (Sarker et al., 2018). The results of this study corroborates with another study which was conducted to determine the level of antimicrobial residue in broiler and layer meat in Chittagong district of Bangladesh. High level of antimicrobial residue was found in broiler and layer meat which include that residue of tetracycline were found in 48% of liver samples, 24% in kidney samples, 20% in thigh muscle samples, and 24% in breast muscles; Enrofloxacin residue were found 40% in liver samples, 34% in kidney samples, 22% in thigh muscles and 18% in breast muscles; Amoxicillin residue were found 42% in liver samples, 30% in kidneys, 26% in thigh muscles and 22% in breast muscles. In most cases, highest level of residue was found in liver such as tetracycline (48%), ciprofloxacin (44%), enrofloxacin (40%) and amoxicillin (42%) and almost lowest in breast muscle. The results of this study suggest that prudent use of antimicrobials in animal at the primary production level will reduce residue findings in the final products, emergence of antimicrobial resistant bacteria and will help to preserve those critically important drugs for veterinary and human use (Sattar et al., 2014).

Residue findings associated with antimicrobial use at the primary production level is not limited only in meat but also in milk and egg products. Boiling or processing has got some effects on limiting of antimicrobials residue levels in products. A study was conducted to screen antimicrobial residues in milk and eggs of commercial and local farm in Chittagong district in Bangladesh. The results showed that tetracycline, amoxicillin and ciprofloxacin residues were significantly higher in milk and egg from commercial farms in contrast to those from the local farms. But the boiling approach significantly reduced residue level in milk and egg. The mean concentration of amoxicillin residue in local milk, commercial milk, local egg and commercial egg were found to be 9.48 μg/ml, 56.16 μg/ml, 10.46 μg/g and 48.82 μg/g, respectively in raw milk samples and were reduced to 9.81 μg/ml, 55.54 μg/ml, 10.29 μg/g, and 48.38 μg/g, respective, after boiling (Chowdhury et al., 2015).

There are many methods to determine heat stability of antibiotics in foods are available. These include heating of liquid food products like milk, water, buffers and meat extracts and also solid such as buffered meat homogenates and various sausages. Inactivation antimicrobials in tissues and eggs can also be done using thermal process. Time and temperature can be more regulated in liquid but results in actual meat products are more indicative of true cooking process. Ordinary cooking procedure for meat, even to "well-done", cannot be relied on to inactivate even the more heat sensitive compounds such as penicillin and tetracyclines. Cooking with higher temperature such as for canning or prolonged cooking with moist heat can inactivate the more heat sensitive compounds. The issue of food safety is uncertain as the nature of degradation products is unknown in most of the cases (Moats, 1999).

Harmful consequences of antibiotic residues

Occurrence of antimicrobial residues lead to cause health hazard like allergic reactions in sensitive persons. A wide array of health effects posed by antimicrobial residues in food products like bone marrow aplasia (chloramphenicol), allergy (penicillins) and ototoxicity. The most significant concern regarding dietary exposure of antimicrobial residues is due to emergence of antibiotic resistant strains of pathogens, jeopardizing the treatment option for both human and animal diseases. Besides the health hazards, antimicrobial residues in milk have also been associated with major technological problem in the food technology industry. It is due to presence of minimum residual quantities of antimicrobials in milk and other food products are responsible for interference with starter culture activity which disrupts the manufacture process of milk products like cheese, yoghurt etc. Methylene Blue Reduction yields underestimated microbiota load in milk due to minimum presence of antimicrobial residue in the raw milk. By and large occurrence of Antimicrobial residues in animal source food products is clearly associated with the use of antimicrobials as growth promoter and for therapeutic and sub-therapeutic purposes and pose threat to public health (Kumar et al., 2013; Singh et al., 2014).

Public Health Aspect

Farmed animal food and food products are the major source of animal protein of mass population of the world. Human being of all ages ranging from infants to old people receive animal through consumption of meat, milk, egg and their products. Consumption of farmed animal food and food products containing antimicrobial residue might pose toxicological, pharmacological, microbiological and even immunological health risk for the consumers. These health hazards include autoimmunity, carcinogenicity (Sulphamethazine, Oxytetracycline), transfer of antimicrobial resistant bacteria to human, Mutagenicity, Nephropathy (Gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol) and allergy (Penicillin).

Depending on the exposure moments these hazards can be categorized in to two types as direct-short term hazards and indirect- long term hazards. As for example allergic hypersensitive reactions caused by immediately after consumption of antimicrobial residue (Penicillin) containing milk to the sensitive people; chronic toxic used to occur with prolonged exposure to low levels of antimicrobials include carcinogenicity, teratogenicity, reproductive effects, development of antibiotic resistance bacteria in treated animals and disruption of normal human flora in the intestine. Chronic exposure to OTC includes blood profile changes like leucocytosis, lung congestion, toxic granulation of granulocytes and thrombocytopenia purpura, and brown discoloration of the teeth. Antimicrobial agents including nitrofurans, tetracyclines and sulfonamides are used as feed additives in animal feed which passed through milk, meat, eggs and their products and sometimes directly linked with toxicological effects in human (Vragovic et al., 2012; Ram et al., 2000).

Method of detection of antibiotic residue

To ascertain the safety of consumers and quality of animal originated food products destined for export, food from farmed animal is needed to analyze regularly for the presence of antimicrobial residues. Detection of excess levels of antimicrobial residues in food from farmed animals by using residue screening and other qualitative tests will help to prevent the entrance of food containing antimicrobial residues in the human food chain. There are many methods to engage for screening of food from the farmed animals for the presence of antibiotic residues which can be categorized in two main groups like screening methods and chromatographic methods to detect as many antibiotics as possible at very low concentration. Microbial growth inhibition assays using Sarcina lutea and Bacillus stereothermophilus are the common methods of screening. The antimicrobial residue detection assays that are currently available use microbial growth inhibition tests using test microorganism. The other available methods for routine screening of antimicrobial residues include receptor assays, immunoassays and enzymatic assays. These methods can be opted for a preliminary identification of diversified classes of antibiotics. Most of these devises are highly expensive, and require technical skills and expensive instrumentation but have the selective advantages of reliability. The rapid assays include Penzyme test, Charm II, LacTec test, SNAP test, Beta Star test and Charm Safe Level test etc. Analytical method like Liquid Chromatography-Mass Spectrometry (LC-MS) coupling is another effective and sensitive system for the detection of antibiotic residue (Padol et al., 2015; Khaniki, 2007).

Solution to tackle antibiotic residues

Antimicrobial residues finding in foods of animal origin such as milk, meat and their products is horrible. Therefore a number of international organizations such as The European Economic Community (EEC), Codex Alimenterius Commission (CAC), World Health Organization (WHO) and the Food and Agricultural Organization (FAO) are involved in regulating the use of drugs in animal production activities. Their effort helps to tackle the occurrence of antimicrobial residues in foods of farmed animals such as meat, milk, eggs and their products to protect public health. With an aim to minimize the risk to human health, these organizations have proposed maximum residue levels (MRLs) for livestock products based on regular monitoring, controlling and surveillance programs. Livestock products containing higher concentrations of antibiotic residues than the MRLs are illegal which should be monitored to avoid this public health hazard.

Drug companies should mention the withdrawal period of antimicrobials and other drugs on the product labels. The drug withdrawal period should be strictly maintained and good hygiene and good management practices at the primary production site and in the food processing units will help to cut down the residues in livestock products. Establishing good farming practices and regulatory standards that reduce the risk of occurrence of antimicrobial residues in milk, meat and egg supply are essential components of food safety (Nolan et al., 2000).

Conclusion

Following the administration of antibiotics to animal, antibiotics remains at high concentrations in the tissues of the treated animals. Every antibiotic have withdrawal period by which time specific antibiotics are metabolized and ensure zero residue findings in the final products. Therefore, residue finding is mainly linked with the duration between administration time and withdrawal time. Therefore, the withdrawal time of different drugs is mandatory to strictly follow and during this period livestock product should not be used for human consumption. Therapeutic use of antibiotic must ensure the use of proper dose and for the proper time and use of antimicrobials as growth promoter should be strictly restricted. The harmful effects of antimicrobial residues can be minimized by using scientific guidelines and precautions.

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CHAPTER 8

Antimicrobial resistance in primary animal production and food safety

Summary

Animal agriculture including aquaculture has flourished in Bangladesh. Thanks to the application of advanced technologies along with intense use of diversified antimicrobials to treat and prevent diseases. The intense use, overuse and even misuse of antimicrobials, however, are undoubtedly associated with residue findings in farm environments and products as well as the development of antimicrobial resistance in normal and pathogenic microbiota, and antimicrobial resistance genes have the transferability capacity in the bacterial population. We discussed on the impact of over use of antimicrobials in animal agriculture. The published information on the usage and occurrence of antibiotics and development of antibiotic resistance in Bangladeshi animal farming including global situation are reviewed. A brief overview follows the occurrence of residue findings in the harvested produce and development of antimicrobial resistance in pathogens and normal microbiota. This includes dissemination of antimicrobial resistance genes among bacterial populations through the genetic transfer system as a negative impact of over use of antimicrobials in the primary animal production. The existence of antibiotic residue in water, sediments, soil and products may accelerate the development of antimicrobial resistance and transfer through mobile genetic elements such as plasmids in the bacterial population of the farm environment and products including human pathogenic bacteria. In sum, human exposure to antibiotic residue and antimicrobial-resistant bacteria via the food chain is injurious to health and warrants further study.

Key words: Antimicrobials, Antimicrobial-resistant bacteria, Antimicrobial residue, Food safety

Introduction

Animal originated food products like meat, milk, egg and fish are the major source of animal protein for human consumption. Animal agriculture has flourished almost exponentially in Bangladesh to satisfy the increased demand from domestic markets owing to a decline in wild sources caused by population growth, over-hunting, and chemical-toxin pollution in the environment and climate change.

There is significant variation in intensive animal production methods in different parts of the world such as tropical and subtropical, temperate and arid, land areas. For high production, the majority of animal production practices are highly dependent on different inputs such as formulated feed, agrochemicals, antibiotics, probiotics and other inputs. This is certainly true of Asian countries, where the majority of animal farmed products destined for human consumption. Recently documented information regarding food safety and the use of antimicrobial agents and probiotics in animal farming are discussed in this chapter.

Since the mid-twentieth century, global animal farming (intensive) has flourished, becoming major source of animal protein for human intake. Animal agriculture including aquaculture has helped in ensuring food security, nutritional supply, poverty alleviation and economic prosperity (Bondad-Reantaso & Subasinghe 2008). The key causes of the exponential growth of animal agriculture including aquaculture in this region include agriculture practices that have existed since prehistoric time, an available population, the growth of the economy, relaxed regulations and a wide range of domestic and export markets.

Animal farming in these areas has flourished, largely because of favourable natural conditions. But the Asian countries are a breeding ground for disease, and the havoc caused to industry by disease is increasingly being recognized as a major problem in the animal industry in particular in the Asian countries like India, China, Vietnam and Bangladesh. Consequently, huge quantities of chemicals and antibiotic products are frequently used to counteract disease (Le et al. 2005; Tu et al. 2008). It is well known that a wide array of antibiotics are commonly used in Asian animal production to prevent or treat diseases, but there is a scarcity of detailed information regarding usage patterns of antibiotics in animal agriculture including aquaculture, the persistence of antibiotics in the farm and aquatic environment, their products and subsequent occurrence of antimicrobial-resistant bacteria and the dissemination of antimicrobial resistance in normal and pathogenic microbiota.

The occurrence of antimicrobial-resistant bacteria and residue findings in animal originated and the subsequent possibility of the transfer of antimicrobial-resistant bacteria to human represent a major public health concern worldwide. Research has so far been undertaken in other animal husbandry practices, excluding seafood, where Escherichia coli and enterococci have been proven to be good bacterial indicators of antimicrobial resistance (Nicholls et al., 2001; DANMAP, 2010). These are normal gut flora in warm-blooded animals and are therefore widely released into the environment via faecal contamination. The presence of these bacteria in pork and poultry is a clear sign of direct or indirect faecal contamination, with the possible presence of other enteric pathogens (Krumperman, 1983). The occurrence of bacterial resistance in these products is the reflection of the use of antimicrobials at the primary production level. The significant properties of these two indicator bacteria, which make them the most suitable bacterial indicators of antimicrobial resistance in pork and poultry, include their easy isolation from human and animal faeces, their remarkable ability to develop resistance to all classes of drugs, and their potential ability to transfer antimicrobial-resistance determinants to any other bacteria and serve as sources or reservoirs of antimicrobial resistance.

Development and impact of bacterial resistance to antimicrobials

Intensive use of chemicals, disinfectants and antibiotics and the incidence of antimicrobial-resistant bacteria attributed to faecal contamination and probiotic application have resulted in an outbreak of antimicrobial-resistant bacteria in animal agriculture including aquaculture settings and also in cultured seafood. Different studies have demonstrated a significant association between the use of antimicrobials in the primary production of animal agriculture and the incidence of antimicrobial resistance in the bacterial population of the animal, farm settings and farm environment and cultured species (Alali et al., 2009; Jordan et al., 2009; Varga et al., 2009). Once the population of bacteria attains the resistance determinants, they are sustained for a long time after the cessation of selective pressure, especially when the encoding genes are associated with other genes from which the selection pressure is coming (Aarestrup et al., 2001; Maynard et al., 2003). Sometimes cross-selection between different drugs of the same class may occur, but co-selection takes place due to the location of various resistance mechanisms on the same genetic elements induced by antimicrobial exposure.

Antimicrobial resistance can broadly be categorised as intrinsic or acquired by mutation or as the transfer of resistance genes through transduction, conjugation or transformation (Fig. 2). Vertical transmission has been seen in the case of acquired resistance by mutation, while resistance determinants located on the mobile genetic structures including plasmids, integrins or transposons might be disseminated horizontally to other normal and pathogenic bacteria (Cavaco et al., 2008). Resistance is potentially mediated by mutations, which might arise as an effect of using antimicrobials. Subsequently, spontaneous mutations that are further chosen by selective pressure (Fig. 2) are conferred by the application of disinfectants, chemicals and antimicrobials (Cavaco et al., 2008).

It is likely that antimicrobial-resistant bacteria are found in most aquatic environments. A study reported that 50% of the isolates of aquatic Acinetobacter spp. showed resistance to chloramphenicol, 27% resistance to ampicillin, 26% resistance to oxytetracycline, 26% resistance to sulfamethoxazole and 7% resistance to gentamycin (Guardabassi et al., 1999). An increase in Acinetobacter spp. resistant to oxacillin was also demonstrated from a stream receiving effluent from a fish farm following oxacillin application on the farm (Guardabassi et al., 2000). A similar effect of oxytetracycline resistance in bacteria isolated from catfish and aquaculture ponds was observed where oxytetracycline was applied for treatment purposes (McPhearson et al., 1991). A further investigation of bacteria from water and catfish detected increased levels of resistance during and after treatment with oxytetracycline (Depaola et al., 1995). It was found that levels of resistance increased from below 20 % to around 40 %, with the premedication level of resistance reached again 21 days after the cessation of oxytetracycline application for treatment.

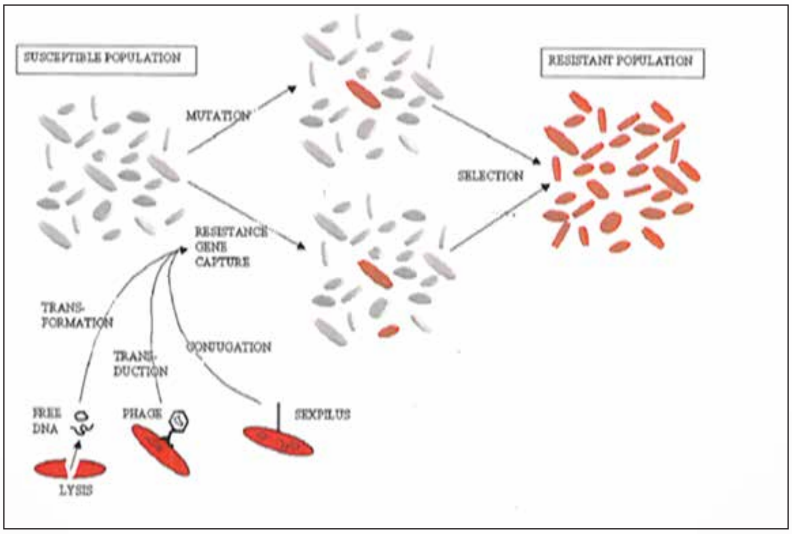

Figure 2. Resistance development in a bacterial population.

Source: (Bruun, 2001)

The intense use of antimicrobials in animal production may have a long-term and permanent potential to select for antimicrobial-resistant bacteria at multiple levels of the animal agriculture including aquaculture environment (Seyfried et al., 2010). This may pose a risk to human health, particularly in countries where antimicrobial use is heavy, prophylactic and uncontrolled, since archaea and bacteria in the animal agriculture environment share a large assortment of mobile genetic elements and antimicrobial resistance genes with a wide range of terrestrial bacteria (Taylor et al., 2011; Buschmann et al., 2012). Therefore antimicrobial resistance genes increasingly flow via the environmental or commensal flora of animals and humans to human pathogens (Cabello et al., 2013). This will enhance the dangers to public health if the flow results in a high-risk clone that can disseminate widely throughout the human population (Woodford et al., 2011).

However, plasmids (of the incompatibility IncA/C group) were shown to harbour antimicrobial-resistance genetic elements and metal resistance genes which have been found to be shared by pathogens of fish, e.g. Salmonella, Yersinia rukeri, Aeromonas and Vibrio cholera (Douard et al., 2010). Additionally, sharing of the tetG and floR-resistance determinants of an antimicrobial-resistant Salmonella genomic island 1 (SGI1) between P. damselae piscicida and epidemic S. typhimurium DT104 (Cabello et al., 2013), fish-transmitted Salmonella albany, S. agona and S. paratyphi B further demonstrate the ready distribution and transfer of antimicrobial resistance genes between bacteria in the aquatic environment and terrestrial bacteria and human pathogens (Smith, 2008b, a; Cabello et al., 2013). Salmonella genomic island 1 can be mobilised between many different bacteria through antimicrobial-resistant plasmids of the incompatibility group of IncA/C, which has been found in piscine (Photobacterium spp., Aeromonas spp.) and human pathogens (Vibrio spp., Salmonella spp. and Proteus spp.) (Douard et al., 2010). This indicates that the possibilities of horizontal gene transfer between bacteria of human pathogens and the aquatic environment are increased in settings where the uncontrolled use of antimicrobials in aquaculture leads to a release of large volumes of antimicrobials into the aquatic environment (Buschmann et al., 2012). In addition, the negative impact of the use of antimicrobials can be seen in areas where the marine aquatic environment is the source of epidemics of Vibrio parahaemolyticus in shellfish (García et al., 2013). Selected antimicrobial-resistant vibrios in the marine aquatic environment facilitate the transfer and mutagenesis of its chromosomal qnr-like loci and alternative antimicrobial resistance genes to other pathogens (Cabello et al., 2007; Cabello et al., 2013).

Another detrimental impact on human health can potentially be mediated by the selection and dissemination of antimicrobial-resistant bacteria and the excessive use of antimicrobials in animal agriculture including aquaculture (Abraham, 2011; Naviner et al., 2011). Fish products might contain antimicrobial residue that exceeds the maximum residue limit (Nogales et al., 2011). The consumption of such products can potentially alter the composition of normal gut flora in humans, select for antimicrobial-resistant bacteria, and facilitate infection with human pathogens which further facilitates the horizontal gene transfer of antimicrobial resistance (Silbergeld et al., 2008). However, scavenging (wild) shellfish, fish and crustaceans from the aquaculture surroundings can also result in the passage of antimicrobials used in aquaculture to human intestines through their consumption, since antimicrobials can reach other animals near this site and remain in their tissue for some time (Sapkota et al., 2008). Similarly, antimicrobial-resistant bacteria can contaminate marketed animal produce that has been selected in the animal farm site (Nawaz et al., 2012). The most neglected but reasonable issue is that workers in farm sites, feed mills and farmers engaged in medicated feed preparation are exposed to antimicrobial-resistant bacteria from aerosols and through direct contact with medicated feed, resulting in a shift in the normal flora in their mucosa, intestine and skin at antimicrobial-resistant bacteria (Neyra et al., 2012).

Antimicrobial-resistant bacterial strains can cause more prolonged or more severe illness compared to antimicrobial-susceptible bacteria. Therefore, they are somewhat more virulent than susceptible strains (Travers and Barza, 2002). Humans can receive antimicrobial-resistant bacteria through food (Fig. 3) originating from colonised animals by way of increasing antimicrobial resistance in the environment and food contamination during processing. Contamination of the environment with antimicrobial-resistant bacteria, such as surface water in the aquatic environment, is directly associated with human exposure (Shea et al., 2004). Adverse consequences on human health have been posed by the emerging antimicrobial-resistant bacteria associated with non-human usage of antimicrobials (FAO/OIE/WHO meeting in Geneva in 2006). Banning the use of antibiotics as a growth promoter in animals has been introduced in the EU to reduce the emergence of bacterial antibiotic resistance in the food chain.

Spread of antimicrobial-resistant bacteria and resistant genes

There is a positive correlation between the types and levels of antimicrobials used and resistance development in the gut flora of human and animals (EFSA, 2008) (Fig. 3). A similar effect can be anticipated from the use of antimicrobials and probiotics in shrimp culture and the antimicrobial resistance in the bacterial flora of the shrimp culture environment (Alderman and Hastings, 1998). In Vietnam, antimicrobials are generally used in shrimp culture for therapeutic and preventive purposes. Additionally, probiotics are used in shrimp culture to improve feed digestion and water quality. Moreover, in Vietnam a very high use of antimicrobials is observed in pangasius farms (100% of farms) in contrast to shrimp farms (2.9% of farms) (Rico et al., 2013). This is because the sharing of common water between both types of farms due to tidal web and flow may lead to the transfer of chemicals, resistant bacteria or resistance genes in shrimp farms. In addition, the use of antimicrobial resistance genes harbouring probiotic bacteria contributes to the dissemination of antimicrobial-resistant bacteria and resistance genes in the bacterial flora residing in the intestine of shrimp and the pond environment. Together these create selection pressure in the pond and shrimp intestine towards the development of bacterial resistance to antimicrobials.

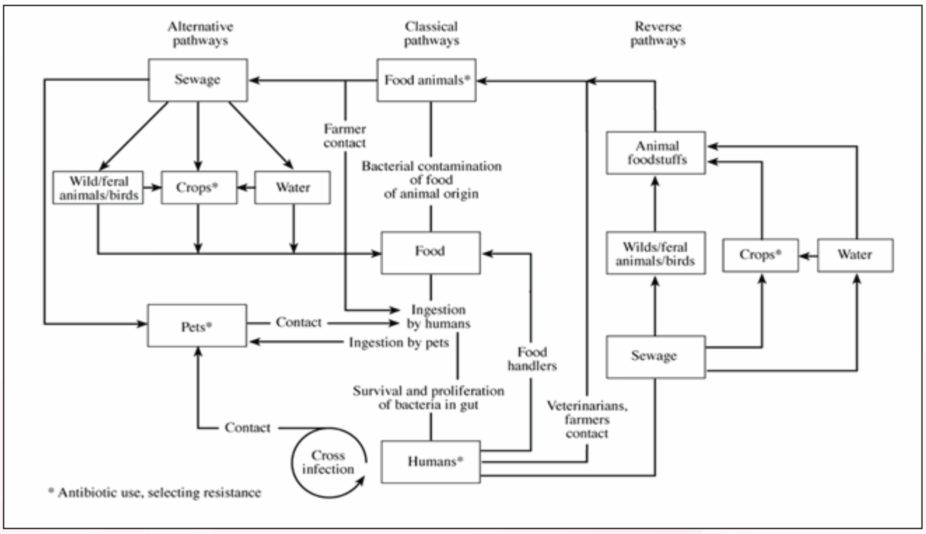

Figure 3. Transmission routes between animals and humans for antibiotic-resistant or susceptible gastrointestinal pathogens or normal intestinal flora. Source: Phillips et al. (2004)

Humans may come into contact with antimicrobial-resistant bacteria through the direct consumption of raw or uncooked meat, milk and eggs or through cross-contamination during the cleaning and preparation. Additionally, food from animals also vehicle of transfer of antimicrobial resistant bacteria to human body through consumption. There is also an occupational health risk as humans generally come into contact with antimicrobial-resistant bacteria when working in the animal farm or using water from water sources receiving effluent from farm, domestic or recreational purposes, including drinking, cooking, washing and bathing. This might result in a therapeutic problem when humans become infected by resistant bacteria associated with shrimp or antimicrobial-resistance genes that are transferred from normal bacteria to human clinical or pathogenic bacteria.

Antimicrobial resistance monitoring in animal food products

In contrast to seafood, Escherichia coli and enterococci have been proven to be good bacterial indicators of antimicrobial resistance in pork and poultry (Nicholls et al., 2001; DANMAP, 2010). These are the commensal flora of the intestinal tract of humans and animals, and are spread widely in the environment (secondary habitat) by means of contamination with the excreta of animals and humans. The occurrence of E. coli and enterococci is an indication of direct or indirect faecal contamination and therefore the possible presence of other enteric pathogens (Krumperman, 1983). E. coli is included as a bacterial indicator of antimicrobial resistance in the DANMAP programme since it is more easily isolated from faecal samples and meat than Salmonella, and therefore is considered to be a better indicator of the occurrence of antimicrobial resistance.

Indicator E. coli is selected as a typical representative of Gram-negative bacteria and is suitable for monitoring antimicrobial resistance in this group of bacteria. It provides insight into the selective pressure on other normal bacteria. In fact, the bacterial indicator E. coli in humans, animals and other surroundings can act as a donor and recipient of exchanging antimicrobial resistance development within the same genera and species and other bacteria including human pathogens (Hammerum and Heuer, 2009). Therefore, indicator E. coli is the standard organism of antimicrobial resistance as it is easy to cultivate and isolate from both healthy animals and humans. This gives a more representative estimation of the occurrence of antimicrobial resistance in the entire human or animal population even though it is considered to be a pathogen (Aarestrup, 2004). The significant properties of E. coli that make it the most suitable bacteria for monitoring antimicrobial resistance among Gram-negative bacteria in food, e.g. pork and poultry, includes its remarkable ability to develop antimicrobial resistance to all classes of drugs and its ability of work as a source or reservoir of antimicrobial resistance.

Indicator bacteria such as E. coli, E. faecalis and E. faecium have been widely used in the DANMAP programme since 1995. As these are the natural gut flora of animals and humans, and can easily be isolated from their primary habitat, they are included in this program. Meat can be contaminated with enterococci and E. coli during the slaughter of production animals. Furthermore, enterococci and E. coli have a remarkable ability to develop antimicrobial resistance in response to selective pressure. Previously most of the antimicrobial agents were used for growth promotion in Denmark and their ban in 1998 had an effect on Gram-positive bacteria such as enterococci and particularly E. faecium. Now a wide range of broad-spectrum antimicrobial agents are used in veterinary clinical therapy and are mainly active against

Gram-negative bacteria, e.g. Salmonella and E. coli.

In order to follow the persistence of resistance after the ban of growth promoters, enterococci are still included in the DANMAP programme. Isolation of Enterococcus faecalis and Enterococcus faecium was carried out from faecal samples collected from broilers and pigs. Sampling was undertaken at the time of slaughter for all samples used in the DANMAP surveillance programme. Enterococci were also isolated from food that originated from meat sold by wholesalers and retailers. These samples were randomly collected from wholesale and retail outlets in all regions of Denmark by the Danish Veterinary and Food Administration Regional Laboratories in centrally coordinated programmes. Identification and susceptibility testing was undertaken at the National Food Institute to observe MIC distributions and the occurrence of resistance among E. faecium and E. faecalis (DANMAP, 2010).

Methods to determine antimicrobial resistance

MIC breakpoints (Kirby-Bauer test) are an essential tool for phenotypic antimicrobial susceptibility testing of bacterial isolates (Wikler and Ambrose, 2005; CLSI, 2013). Different organisations are involved in fixing clinical breakpoints. They include two notable organisations: the Clinical and Standards Laboratory Institute (CLSI) in the USA and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in Europe (Wikler and Ambrose, 2005; Turnidge and Paterson, 2007). The CLSI determines clinical breakpoints for bacteria and antimicrobial agents in both veterinary and human medicine, while EUCAST publishes human breakpoints only. EUCAST provides wild-type cut-off values. The wild-type cut-off value is mainly epidemiological in nature, and can be used on its own for epidemiological and surveillance purposes.

Bacteria isolated from different sources, such as infections or food products, are categorised as susceptible, intermediate resistant or resistant to therapy (Wikler and Ambrose, 2005; CLSI, 2013). The susceptible category implies that treatment with the recommended drug at recommended dosages is likely to result in a clinical cure. This means that the recommended antimicrobial with a specific dose will work against the strain causing the infection. The opposite is implied by the resistant category, where treatment with the antimicrobial is likely to cause treatment failure. The intermediate category functions as a “buffer zone” to avoid errors where high concentrations of drugs are needed for treatment purposes (Wikler and Ambrose, 2005; CLSI, 2013).

Antimicrobial resistance can generally be measured using three main sampling strategies, of which the characterization of a single isolate per sample for detecting resistance is the most commonly practiced (Caprioli et al., 2000). This strategy is applicable in the monitoring of antimicrobial resistance in seafood using Acinetobacter spp. as CLSI provides breakpoints for this genus. On the other hand, it is not possible to provide a quantitative estimate of the proportion of antimicrobial-resistant bacteria from a seafood sample. This is because it is necessary to know the genus and species content of the bacterial population and more importantly there is a lack of MIC breakpoint values in both CLSI and EUCAST guidelines for bacteria of aquatic origin. Alternatively, in the absence of Acinetobacter spp., a genotypic method can be applied in the monitoring of antimicrobial resistance in seafood instead of phenotypic antimicrobial susceptibility testing (Helmuth et al., 2009). DNA microarray can be an alternative tool for antibiotic resistance determinations (Helmuth et al., 2009). This provides an interesting tool for risk assessment and is becoming increasingly important. To do this, whole DNA from a shrimp or meat sample can be extracted (Hölzel et al., 2011) and DNA microarray performed using the whole DNA.

DNA microarray can be used for antibiotic resistance determinations and offers a promising alternative to other methods, e.g. phenotypic antimicrobial susceptibility testing or the PCR method, because this enables simultaneous screening of large sets of target genes. In contrast, PCR only allows the detection of a limited number of resistant genes. Nevertheless a prerequisite of applying DNA microarrays to trace resistance along the food chain would be its ease of use, its rapidity and the accuracy of its results (Koser et al., 2012).

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CHAPTER 9

Major contaminants and toxins in animal feed

Summary

Animal feeds and forages used to house a wide range of toxins and contaminants generated from anthropogenic and natural sources across the world. In this report, the distribution of mycotoxins, plant toxins, antibiotics, heavy metals, radionuclides and microbial pathogens in complete feeds and forages have been discussed. The impacts on the productivity of the farm animals and on the quality and safety of resulting edible products are also included. It was revealed that feeds contain a variety of substances as co- contaminants that there varied from region to region in the nature of the compounds involved. In conclusion, the scope for the remedial action is limited. Therefore, it is highly needed to strengthen the appropriate legislation and develop laboratory capacities to control those unwanted contaminants.

Key words: Heavy metal, mycotoxins, contaminants, forage, feed

Introduction

Animal farms in Bangladesh use a varieties of inputs like formulated feed, antimicrobials growth promoters and other technologies. Formulated feed is the combined preparation using plant and animal originated ingredients in the feed mill. These ingredients include maize, wheat, rice, green grass, meat meal, bone meal, fish meal, enzymes, vitamins, minerals, amino acids etc. In simple word “feed” is the blended preparation of different ingredients and forage. Animal feed ingredients like maize, wheat, barley are commonly subjected to contamination from diverse sources, including environmental pollution and activities of insects and microbes. These may also contain some endogenous toxins arising from specific primary and secondary substances produced by fodder plants. Therefore, feed toxins consist of compounds of both microbial and plant origin. These toxins share several common underlying features even their origins are different. Therefore, specific compounds within both plant and microbial toxins may contains antinutritional factors and transferred to animals through feed leading to reduced reproductive performance in farm animals.

Moreover, the combined effects might be like feed additives or synergistic interactions between the two groups of compounds. The extent and impact of these interactions in practical livestock feeding regimen is not clearly understood which is needed to be quantified. The global scale occurrence of feed contaminants has distinct geographical differences in the relative impact of individual compounds.

This report is mainly focusing on those contaminants and toxins that represent significant risks to farm animal production. Feed contamination arising from insect fragments and excreta is ignored in this study, but the role of such vectors in the transmission of fungal spores and hyphae is not ruled out. Legal control of certain feed contaminants and toxins is in place and operating within a continually evolving framework; regular laboratory monitoring system related salient issues will be briefly discussed here.

Environmental Contaminants

Animal feedstuffs might receive a wide range of organic and inorganic compounds which include industrial pollutants, pesticides, radionuclides and heavy metals. Feeds might be contaminated with pesticides originated from major groups including organochlorine, organophosphate and pyrethroid compounds (van Barneveld, 1999). Reports showed that 21 percent of feeds contain pesticide residues in the United Kingdom. The compounds Pirimiphos-methyl which is an insecticide used in grain stores, was detected with the highest frequency. Pesticides are not only potentially toxic to farm animals but also lead to residue findings in animal originated products destined for human consumption. Feeds specially herbage are contaminated with industrial pollutants like dioxins and polychlorinated biphenyls (PCBs). Cows raised and grazing pasture nearby industrial area produce milk with higher content of dioxin in contrast to cows farmed the rural areas. In the late nineties, dioxin contaminated animal fat was used to prepare feeds destined for the farms located in France, Belgium and the Netherlands. Products like meat products and eggs produced from those farms were found to contain unacceptable level of dioxins.

Radionuclide pollution monitoring is paramount to protect human health. Caesium-134 and caesium-137 were released following the Chernobyl accident in 1986, leading to widespread contamination of pastures and conserved forages. Consequently, milk and sheep carcasses had been contaminated and restrictions were imposed on the movement and slaughter of sheep (MAFF, 1994). Application different types of fertilizers to crops and pastures causes contamination of feeds and herbage with cadmium. In contrast, lead contamination arises from industrial and urban pollution, while mercury in feeds arises from the use of fishmeal.

Bacterial contaminants

Bacterial contamination in feeds is potentially associated with feed as feeds are the first step in "Farm to fork" food safety model. Currently, much interest is growing in the occurrence of Escherichia coli in animal feeds following human illness outbreak caused by E. coli O157 in many places of the world. Results from studies showed that 30% of cattle feed samples secured from commercial sources and farms contained E. coli but none of the samples were positive for E. coli O157 (Lynn et. al., 1998).

Replication of faecal E. coli and E. coli O157 type was found to occur in a variety of feeds of cattle farms in the summer months. Widespread fecal contamination of feeds on farm is a potential route for exposure of cattle to E. coli and other pathogens of fecal origin. The potential for exposure to fecal pathogens also exists when poultry litters are offered as cattle feed, e.g. poultry waste products are commercially available for use as cattle feed in many parts of the world. However, prior to distribution products are adequately heat- processed to reduce the contamination with E. coli, Salmonella spp. and Campylobacter species or even eliminated those pathogens (Jeffrey et al., 1998). By It is worthy to mention S. enterica commonly occurs in cattle feeds in the Europe, South Africa and United States, with contamination rates ranging from 5 to 19 percent (Krytenburg et al., 1998).

The occurrence of Listeria monocytogenes have been reported to occur in poor-quality silages and big-bale silage. The ensiling process of grass is conducted under anaerobic condition and at lower pH which condition favors to exclude from the resulting silage. But in case of big-bale silage a degree of aerobic fermentation may occur, raising pH levels and allowing the growth of Listeria species. Listeria monocytogenes also survives at low temperatures and in silages with high levels of dry matter. The occurrence of Listeria species in silage is a concerning issue as this agent causes abortion, meningitis, encephalitis and septicaemia in animals and humans. The incidence of various forms of listeriosis has been increasing in recent times.

Fungal contaminants

Contamination of feeds with fungus and their spores have been reported constantly worldwide. In the tropics, Aspergillus is the predominant genera in dairy and other feeds in the tropics (Dhand, Joshi and Jand, 1998). There are other significant contaminants of cereal grains included Penicillium, Fusarium and Alternaria (D'Mello, Macdonald and Cochrane, 1993). Contamination of feed with these fungi are concerning because of their potential for mycotoxin production (will be discussed in the next section). Mycosis in animals might be caused by inhalation or consumption of spores from moldy hay, silage, brewers' grain and sugar-beet pulp. As for example, such conditions include ringworm and mycotic abortion. Abortion is generally occurred in cattle as a result of systemic transmission and subsequent proliferation in placental and fetal tissues.

Mycotoxins

Mycotoxins are mainly the secondary metabolites of fungi, e.g. Aspergillus, Penicillium, Fusarium and Alternaria those are known for their capacity to impair animal health and productivity (D'Mello and Macdonald, 1998). The diverse effects precipitated by these compounds are generally termed as "mycotoxicosis", and include distinct syndromes as well as non-specific conditions. A list of the main contaminant and the fungal species associated with production of those are listed in Table 1. The occurrence of mycotoxin contamination in forages and cereals in the field following is frequently associated with infection of plants with particular pathogenic fungi or with symbiotic endophytes. During processing and storage of harvested products and feed, contamination may also occur while environmental conditions are appropriate for spoilage fungi.

Moisture content and ambient temperature are key favorable factor of fungal colonization and mycotoxin production. It is conventional to subdivide toxigenic fungi into “field” fungi and “storage” fungi. Fusarium, Claviceps, Neotyphodium and Alternaria are classical representatives of field fungi while Aspergillus and Penicillium exemplify storage organisms. Mycotoxin producing fungal species may be further differentiated based on geographical prevalence, reflecting specific environmental requirements for growth and secondary metabolism. Aspergillus flavus, A. parasiticus and A. ochraceus were found to readily proliferate under warm, humid conditions particularly in the tropics and sub-tropics, in contrast to temperate fungi like Penicillium expansum and P. verrucosum. As a consequence, the Aspergillus mycotoxins predominate in plant products emanating from the tropics and other warm regions. On the other hand, the Penicillium mycotoxins occur widely in temperate foods, particularly cereal grains. Fusarium fungi are found to ubiquitous, but even this genus contains toxigenic species that are almost exclusively associated with cereals from warm countries.

An emerging feature is the co-production of two or more mycotoxins by the same species of fungus (Table 1). This observation has enabled a fresh interpretation of the causes of well-known cases recorded in the history of mycotoxicosis.

Mycotoxins	Fungal Species
Aflatoxins	Aspergillus flavus; A. parasiticus
Cyclopiazonic acid	A. flavus
Ochratoxin A	A. ochraceus; Penicillium viridicatum; P. cyclopium
Citrinin	P. citrinum; P. expansum
Patulin	P. expansum
Citreoviridin	P. citreo-viride
Deoxynivalenol	Fusarium culmorum; F. graminearum
T-2 toxin	F. sporotrichioides; F. poae
Diacetoxyscirpenol	F. sporotrichioides; F. graminearum; F. poae
Zearalenone	F. culmorum; F. graminearum; F. sporotrichioides
Fumonisins; moniliformin; fusaric acid	F. moniliforme
Tenuazonic acid; alternariol; alternariol methyl ether; altenuene	Alternaria alternata
Ergopeptine alkaloids	Neotyphodium coenophialum
Lolitrem alkaloids	Neotyphodium coenophialum
Ergot alkaloids	Claviceps purpurea
Phomopsins	Phomopsis leptostromiformis
Sporidesmin A	Pithomyces chartarum

Aflatoxin

Aflatoxin group consist of aflatoxin B1, B2, G1 and G2 (AFB1, AFB2, AFG1 and AFG2, respectively). Moreover, aflatoxin M1 (AFM1) has been identified in the milk of dairy cows which were fed on AFB1 contaminated feeds. The genus Aspergillus is generally known as storage fungi which proliferating under conditions of relatively high moisture/humidity and temperature. Therefore, Aflatoxin contamination is almost exclusively limited to tropical feeds such as oilseed by-products derived from cottonseed, groundnuts and palm kernel. Where, particularly in the warm humid regions, A. flavus infect the maize crop prior to harvest and remain viable during storage, there aflatoxin contamination of maize is also a significant problem. Because of the diverse forms of toxicity routine monitoring of aflatoxins in animal feed is a common practice in the developed countries where strong legislations and control are available (D'Mello and Macdonald, 1998). In the United Kingdom during 1987-1990, imported feedstuffs were analyzed to detect aflatoxin which totally complied with legislation in force for AFB1 levels. Higher levels of aflatoxin in certain feeds does pose threat to animal health and also associated with food safety. A ground nut cake sample was found to contain 3700 mg/kg of aflatoxin in India. Moreover, the contamination of maize samples in China and northern Vietnam with combinations of AFB1 and Fusarium mycotoxins is potentially significant. Studies revealed in China, 85 percent of maize samples were contaminated with both AFB1 and fumonisin B1 at levels ranging from 8 to 68 mg/kg and 160 to 970 mg/kg, respectively. Feed-grade maize in northern Vietnam had AFB1 levels ranging from 9 to 96 mg/kg, and fumonisin B1 levels in the range of 271 to 3 447 mg/kg (Placinta, D'Mello and Macdonald, 1999). Analyses of farmgate milk in the United Kingdom showed low levels of AFM1 contamination during the late Nineties. Additionally, more than 50 percent of milk samples in the United Republic of Tanzania were found to contain the mycotoxin (D'Mello and Macdonald, 1998). In the early sixties, more than 100,000 turkey pullets died from acute liver necrosis and hyperplasia of bile duct attributed to the consumption of ground nuts infected with Aspergillus flavus. Since then mycotoxin is significantly considered in animal health and food safety and leading to the discovery of aflatoxins.

This event marked a defining point in the history of mycotoxicoses, leading to the discovery of the aflatoxins. Studies showed that aflatoxins are acutely toxic to ducklings while ruminants are more resistant. However, the major impetus arose from epidemiological evidence linking chronic aflatoxin exposure with the incidence of cancer in humans.

Ocratoxin

Ocratoxins are produced by Aspergillus ochraceus which shares a property with at least two Penicillium species. There are two forms of Ocratoxins such as Ocratoxin A (OA) Ocratoxin B which are natural contaminants in feeds and of which Ocratoxin A is more ubiquitous. Ochratoxin occurs predominantly in cereal grains and in tissues of animals fed with contaminated feed.

Another mycotoxin known as citrinin which sometime co-occurs with ochratoxin. Studies showed that Bulgarian wheat was found to contain ochratoxin and citrinin ranging from <0.5 to 39 mg/kg and from < 5 to 420 mg/kg respectively. Higher levels of ochratoxins were detected (maximum 140 mg/kg) while citrinin was below the detection limits in oats (D`Mello, 2001). Both ochratoxins and citrinin are nephrotoxic to a wide range of animal species. Ochratoxin is frequently causing nephropathy in porcine and also known as Balkan nephropathy in human. The disease related role of citrinin is not clearly known yet.

Fusarium mycotoxin

Research data indicate that the global occurrence of contamination of cereal grains and animal feed with Fusarium mycotoxins (D`Mello and Macdonald, 1998). These mycotoxins include trichothecenes, zearalenone and the fumonisins which are significantly associated with animal health and food safety issues. The trichothecenes are further classified into four basic groups with type A and B being the most important. Type A trichothecenes consists of T-2 toxin, HT-2 toxin, neosolaniol and diacetoxyscirpenol (DAS). Type B trichothecenes consists of deoxynivalenol (DON, also regarded as vomitoxin), nivalenol and fusarenon-X. The production of the two types of trichothecenes is a particular characteristic of a specific Fusarium species.

However, the production of ZEN as a secondary metabolites is the common feature of these fungi. This ZEN used to occur as a co-contaminant with certain trichothecenes. Another distinct type of fumonisins are synthesized by another distinct type of Fusarium species (Table 1). Three members of this group (fumonisins B1, B2 and B3) often occur together in maize.

Virtually all the toxigenic species of Fusarium listed in Table 1 are known as major pathogen of cereal plants. These cause diseases like head blight in wheat and barley and ear rot in maize. Grains harvested from this diseased crop are likely to be contaminated with the appropriate mycotoxins generated by the infection causing fungi. There are ample of evidences. Fusarium mycotoxins surveillance in grain and animal feed has been the subject of many investigations over recent years (Tables 2 and 3).

Although the occurrence of mycotoxins is globally distributed but striking regional can be observed. Co-occurrence of various Fusarium mycotoxins in the same sample is further intensified the issue of mycotoxins contamination in feed and animal products. From a German study it was found that 94% of wheat samples were contaminated by two to six Fusarium mycotoxins and 20 percent of the samples were co-contaminated with DON and ZEN. The common combination included DON, 3-ADON and ZEN. T-2 and Ht-2 toxins were detected at levels ranging from 0.003 to 0.20 mg/kg, respectively, but these mycotoxins only occurred in combination with DON, NIV and ZEN.

Table 2: Global distribution of deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN) in cereal grains and animal feed (mg/kg)

Country	Cereal/feed type	DON	NIV	ZEN
Germany	Wheat	0.004-20.5	0.003-0.032	0.001-8.04
Poland	Wheat	2.0-40.0	0.01	0.01-2.0
	Maize kernels	4.0-320.0	-	-
	Maize cobs (axial stems)	9.0-927.0	-	-
Finland	Feeds and grains	0.007-0.3	-	0.022-0.095
	Oats	1.3-2.6	-	-
Norway	Wheat	0.45-4.3	max 0.054	-
	Barley	2.2-13.33	max 0.77	-
	Oats	7.2-62.05	max 0.67	-
Netherlands	Wheat	0.020-0.231	0.007-0.203	0.002-0.174
	Barley	0.004-0.152	0.030-0.145	0.004-0.009
	Oats	0.056-0.147	0.017-0.039	0.016-0.029
	Rye	0.008-0.384	0.010-0.034	0.011
South Africa	Cereals/animal feed	-	0.05-8.0	-
Philippines	Maize	-	0.018-0.102	-
Korea, Republic	Barley	0.005-0.361	0.005-0.361	-
	Maize	mean 0.145	mean 0.168	-
Vietnam	Maize Powder	1.53-6.51	0.78-1.95	-
China	Maize	0.49-3.10	0.6	-
Japan	Wheat	0.03-1.28	0.04-1.22	-
	Barley	61.0-71.0	14.0-26.0	-
New Zealand	Maize	max 3.4-8.5	max 4.4-7.0	-
USA	Wheat	up to 9.3	-	-
	Wheat (Winter), 1991	< 0.1-4.9	-	-
	Wheat (Spring), 1991	< 0.1-0.9	-	-
	Wheat, 1993	< 0.5-18.0	-	-
	Barley, 1993	< 0.5-26.0	-	-
Canada	Wheat (hard)	0.01-10.5	-	-
	Wheat (soft, winter)	0.01-5.67	-	-
	Wheat (soft, spring)	0.01-1.51	-	-
	Maize	0.02-4.09	-	-
	Animal feeds	0.013-0.2	0.065-0.311	-
Argentina	Wheat	0.10-9.25	-	-

Source: Adapted from Placinta, D'Mello and Macdonald, 1999.

Table 3: Worldwide contamination of maize and animal feeds with fumonisins (mg/kg)

Country	FB1	FB2	FB3	Total
Maize				
Benin	nd1-2630	nd-680		nd-3310
Botswana	35-255	nd-75	nd-30	35-305
Mozambique	240-295	75-110	25-50	340-395
South Africa	60-70	nd	nd	60-70
South Africa	max 2000			
Malawi	nd-115	nd-30	nd	nd-135
Zambia	20-1420	nd-290		20-1710
Zimbabwe	55-1910	nd-620	nd-205	55-2735
Tanzania, United Republic	nd-160	nd-60	nd	nd-225
Honduras	68-6555		nd-3 537	
Argentina	85-8791	nd-11300		85-16 760
Uruguay	nd-3688			
Costa Rica	1700-4780			
Italy	10-2 330	nd-520		
Portugal	90-3370	nd-1080		10-2850
Viet Nam	268-1516	155-401	101-268	90-4450
China	160-25 970	160-6770	110-4130	524-2185
Philippines	57-1820	58-1210		430-36870
Thailand	63-18 800	50-1400		
Indonesia	226-1780	231-556		
Animal feed				
South Africa	4 000-11000			
Uruguay	256-6342			
India	20-260			

1 nd = not detectable.

Source: Adapted from Placinta, D'Mello and Macdonald, 1999

Endophyte alkaloids

The endophytic fungus Neotyphodium coenophialum causes infection in perennial tall fescue and another related fungus like N. lolii, may be present in perennial ryegrass (D'Mello, 2000). Ergopeptine alkaloids namely ergovaline, occur in N. coenophialum-infected tall fescue, while the indole isoprenoid lolitrem alkaloids, particularly lolitrem B, are found in N. lolii-infected perennial ryegrass. The ergopeptine alkaloids have effect in animal husbandry like reduction of growth, reduce performance and milk production in cattle, while the lolitrem compounds cause neurological effects in ruminants.

Phomopsins

Lupin stubble is valuable fodder for sheep production in Australia but this fodder is infected by fungus like Phomopsis leptostromiformis and that cause production of Phomopsins. This cause toxicity in lupin fodder which is a limiting factor to use this fodder in sheep production. Mainly the stems, pods and seeds which are mature and sensing parts and prone to infection. Phomopsin A is a primary toxin which cause ill-thrift, liver damage, photosensitization and reduced reproductive performance in sheep (D'Mello and Macdonald, 1998).

Sporidesmin

Pithomyces chartarum is a ubiquitous saprophyte of pastures and known to have the capacity to synthesize sporidesmin A. Sporidesmin is a compound to cause facial eczema and liver damage in sheep leading to reduced production.

Plant Toxins

Many plant components potentially generate adverse effects on the productivity of food production animals (D'Mello, 2000). These compounds are adequately present in the foliage and/or seeds of plants that is used in practical feeding. The concentrations for selected toxins are displayed in Table 4. Plant toxins may be divided into two group such as heat-labile group and heat-stable group. Heat-labile group consist of lectins, proteinase inhibitors and cyanogens, which are sensitive to standard processing temperatures.

In contrast, the heat-stable group consist of antigenic proteins, quinolizidine alkaloids, condensed tannins, gossypol, glucosinolates, saponins, the non-protein amino acids S-methyl cysteine sulphoxide and mimosine, and phyto-oestrogens. These substances are known as antinutritional factors that has been considered at length by D'Mello (2000), but the salient points are worth reiterating.

Table 4: Plant toxins: sources and concentrations

Toxin	Principal sources	Typical concentrations
Lectins	Jack bean	73 units/mg protein
	Winged bean	40-320 units/mg
	Lima beans	59 units/mg protein
Trypsin inhibitors	Soybean	88 units/mg
Antigenic proteins	Soybean	-
Cyanogens	Cassava root	186 mg HCN/kg
Condensed tannins	Acacia spp.	65 g/kg
	Lotus spp.	30-40 g/kg
Quinolizidine alkaloids	Lupin	10-20 g/kg
Glucosinolates	Rapeseed	100 mmol/kg
Gossypol	Cottonseed	0.6-12 g/kg (free)
Saponins (steroidal)	Brachiaria decumbens; Panicum spp.	
S-methyl cysteine sulphoxide	Kale	40-60 g/kg
Mimosine	Leucaena leucocephala	145 g/kg (seed)
Phyto-oestrogens	Clover; lucerne; soybean	25 g/kg leaf

Source: Compiled from D'Mello, 1995.

Proteinase inhibitor

The proteinase inhibitors are burning examples of thermo-labile factors which pose antinutritional activity in the animal feeding regime. They consist of a remarkable class of proteins with the ability to react in a highly specific manner with a range of proteolytic enzymes in the digestive secretions of animals. The trypsin inhibitors of soybean (Table 4) are now well defined (D'Mello, 1995) and are an important determinant of nutritive value.

Proteinase inhibitors are found in different leguminous seeds like winged beans, pigeon pea field beans and cowpea. The effect in animal production in an ingenious way by reducing protein digestion and endogenous loss of amino acids, with the overall result that performance is impaired.

Antigenic proteins

Certain storage proteins of legume seeds have ability to crossing the epithelial barrier of the intestinal mucosa. This even helps to cause adverse effects on immune function in food production animals. The antigenic proteins in soybean are defined as glycinin and conglycinin. The antigenic proteins have potential property to resist denaturation by conventional thermal processing procedures and to enzyme attack in the digestive tract of mammals. Immune hypersensitivity syndrome happened in sensitized calves and piglets after feeding with heated soybean (D'Mello, 1991).

The component antigens cause extensive local and systemic immunological reactions together with severe intestinal damage. The resulting effects include abnormalities in movement of digesta, impaired nutrient absorption and a predisposition to diarrhoea.

Cyanogens

Cyanogens are found widely in plants and in diverse forms. The predominant cyanogens sorghum and cassava are dhurrin and linamarin (Table 4). Linseed was also found to contain linamarin. Cyanogens are known as glycosides that readily produce hydrogen cyanide (HCN), causing dysfunction of the central nervous system, respiratory failure and cardiac arrest (D'Mello, 2000). Due to presence of cyanogenic potential compound, metabolizable energy values for poultry tend to be lower in untreated cassava root meal.

Condensed tannins

Tannins are a group of phenolic compounds with a molecular weight in excess of 500 Daltons. Condensed tannins (CTs) are a subset belongs to this group and are widely distributed in sorghum (Table 4) and seeds and in leguminous forages. Cattle and sheep are known to sensitive to condensed tannins in contrast to goats which are more resistant. Adverse effects may be seen in sheep when condensed tannins are available in lotus or in browse legumes like Acacia species and comprise a significant part of their diets. The visible effects are impaired rumen function and depressed intake, wool growth and live-weight gain. However, at moderate levels (30 to 40g/kg legume dry matter), condensed tanins may result in nutritional advantages grading increased bypass protein availability and bloat suppression in cattle. It has been reported that at higher levels (100 to 120 g CTs/kg legume dry matter), reduced gastrointestinal parasitism in lambs (D'Mello, 2000).

Quinolidizine Alkaloids

The quinolizidine alkaloids are found in lupins which include sparteine, lupinine and lupanine. Bitter cultivars are found to contain relatively high levels of total alkaloids (Table 4) and therefore are not suitable as animal feedstuffs because of their negative effects on intake. Furthermore, cattle reared on lupin species during pregnancy resulted in calve birth with multiple congenital deformities.

Glucosinolates

Glucosinolates are glycosides, significant compounds, are reported to be found in brassica forage crops like kale (Table 4; D'Mello, 2000). The glucose removal from glucosinolates by plant or microbial enzymes (myrosinase), have resulted in the release of a wide array of compounds which undergo further breakdown to yield a number of toxic metabolites. The breakdown of products are stimulated by pH, temperature and metabolic ion concentrations and the most common breakdown products are isothiocyanates and nitriles and a number of other metabolites may also be produced. These products are known to cause organ damage, goitrogenic effects or reduced feed intake, particularly in non-ruminant animals.

Gossypol

Gossypol pigment are found in cottonseed either in free form in bound forms (Table 4). In whole seeds, gossypol is essentially in the free form, but variable amounts may bind with protein during processing to yield inactive forms. Free gossypol is highly toxic and known to causes organ damage, cardiac failure and death. Bulls reared on cottonseed can induce increased sperm abnormalities and decreased sperm production.

Saponins

Saponins are divided into two groups including steroidal saponins and triterpenoid saponins. Steroidal saponins occur as glycosides in certain pasture plants like Brachiaria decumbens and Panicum species. On the other hand, triterpenoid saponins are occurred in soybean and alfalfa (Table 4). Many hepatogenous photosensitization conditions in sheep have been found to be associated with the intake of forage plants containing steroidal saponins. In contrast, triterpenoid saponins from alfalfa reduce feed degradation in the rumen.

Amino Acids

A wide array of non-protein amino acids have been found in the foliage and seeds of plants. Forage and root brassica crops were found to contain S-methyl cysteine sulphoxide (SMCO), while mimosine is known as aromatic amino acid mimosine which is found in the foliage and seeds of the tropical legume Leucaena leucocephala (D'Mello, 2000). Heavy feeding of brassica forage to ruminants was found to cause organ damage with haemolytic anaemia, which is associated with the intake of SMCO. Sheep reared on Leucaena is known to causes reduced intake, shedding of fleece, organ damage and death. The effects observed in cattle are lethargy, loss of hair, excessive salivation, weight loss and enlarged thyroids are common features of Leucaena toxicity.

Phylo-oestrogens

Phyto-oestrogens are primarily in forage and grain legumes which are diverse group of isoflavonoid compounds found (Table 4). Formononetin is the major form of phyto-oestrogen compound found in clover. Phyto-oestrogens compounds are actively metabolized in the rumen and form products that vary in their biological activity. Formononetin is a type of oestrogenic compound which have been associated with "clover disease" in sheep, which is demonstrated by low ovulation and conception rates (D'Mello, 2000).

Weed Seed

Animal feeds contaminated with weed seeds is a major problem worldwide. The impact of weed seeds arises from the toxins that they were found to contain and from their diluent effects on nutrient density of feeds. The toxins include alkaloids, saponins, amino acids and proteinase inhibitors many of those cited in the previous section, particularly. Therefore, weed seeds are controlled in the border by legislation in many countries include those of Datura spp., common vetch, and castor - oil plant and Crotalaria spp.

Animal Toxins

Among the diverse types of naturally occurring animal toxins, the prion proteins of mammalian meat and bone-meal have recently emerged as a significant feed contaminants necessitating statutory control. Prion proteins are harmless animal tissue components but it has capacity to transform themselves into agents causing fatal neurological lesions in a wide range of species. The significance of prions has been highlighted following the occurrence of bovine spongiform encephalopathy (BSE) as a major disease of cattle in the United Kingdom. The onset of this disease was associated with the feeding of cattle with meat and bone-meal manufactured from the carcasses of scrapie-infected sheep.

Scrapie disease is known to be caused by prion proteins, as is the human equivalent new variant Creutzfeldt-Jakob disease (vCJD). The occurrence of vCJD in humans has been found to be associated with the consumption of BSE-infected beef. It is this association of disease outbreak that has led to extensive and stringent legislation in the European Union (EU) concerning the use of specified animal products in livestock feeding.

Undeclared additives

Residue findings in animal originated products is clearly associated use of drugs in animal feed as growth promoters. As for example, growth promoters or feed additives may be used with an aim to disease control and for the enhancement of livestock performance. Residues mainly arises through contamination of animal feeds with undeclared drugs. The occurrence of these drugs is mainly happened due to cross-contamination in feed mills (Lynas et al., 1998). For example, medicated feed residues have been found to be retained within equipment and then contaminate subsequent batches of feed. Under these conditions, levels of contamination might be low but good enough to cause detectable residues in animal products. In Northern Ireland, Lynas et al. (1998) examined the extent of feed contamination with undeclared antimicrobial additives. Out of 247 medicated feeds, 35 percent were found to contain undeclared antimicrobials; and of 161 unmedicated feeds, 44 percent were found to contain antimicrobials.

The contaminants were most frequently identified such as sulphonamides, chlortetracycline, penicillin and ionophores. Sulphadimidine in contaminated feeds was good enough to cause violative tissue residues if animals are reared on that feed in the finishing stages. It is possible that feed contamination with undeclared antimicrobials is a global problem which demands further investigation. Drug residues in animal products are undesirable because of human health implications concerning allergies and the development of antibiotic resistance in pathogens and normal microbiota.

Regulations

It is instructive and relevant to provide a brief review of the regulatory prospects for the control of undesirable substances. Currently, regulations are most comprehensive in Europe and North America in contrast the in developing countries statutory directives may not even exist. Thus, 50 countries in the world, mostly in Africa, have no regulations for mycotoxin control (D'Mello and Macdonald, 1998). This situation can be changed by the new rules imposed on feeds imported into the EU which came into force in August 1999. Non-EU feed manufacturers are now required confirm safety standards for exported animal feeds to EU what will be ensured by the EU representatives.

For heavy metals and aflatoxins, residue level limits are marked for straight, complete and complementary feedstuffs. More distinctions may be applied according to the destination of feeds for a particular class of animal. For controlled pesticides, separate regulations are prevailing for feedstuffs and for fats, with no distinction for the class of animal.

Among the wide variety of toxins originated from plants, only cyanogens, gossypol and certain glucosinolates are subjected to be under regulatory control in the EU. Special regulations are subjected to apply to control contamination of feeds with specific bacteria. For example, under United Kingdom regulations, positive identification of Salmonella in feeds must be reported to a "veterinary officer of the Minister" (HMSO, 1989). Specific regulations are subjected to apply to the control of BSE in cattle. Such as in the United Kingdom, it has been illegal to feed ruminants with any form of mammalian protein since November 1994. Rearing of farmed livestock on meat and bone meal (MBM) has been prohibited since April, 1996 in the United Kingdom. However, pigs and poultry may still be reared on diets containing mammalian protein in forms other than mMBM, e.g. processed catering waste. Surveillance results of animal feeds indicate widespread compliance with these regulations, with 99.7 percent of feeds found to be negative for mammalian protein.

Effects of processing

Heat processing is an effective method in feed manufacture, ensuring improved properties as regards the safety and nutritive value of feeds. For example, heat treatment of dried poultry litter appears to be an effective method for controlling, or even removing, contamination with Campylobacter, Salmonella and E. coli (Jeffrey et al., 1998). Thermal processing has found to be effective for denaturing proteinase inhibitors cyanogens and lectins. However, for antigenic proteins, more complex procedures are required which include hot aqueous ethanol (D'Mello, 1991).

The heat processing method needs ammoniation in aflatoxin-contaminated oilseeds destined for animal feed. The feedstuff is used to treat with either ammonium hydroxide or gaseous ammonia at high temperatures and pressure in commercial feed mills, or at ambient temperature and low pressure in small- scale operations in developing countries. If the ammoniation reactions are proceeded until the end of production, the detoxification process is irreversible and aflatoxin contamination will virtually be eliminated. Given that the residual ammonia is dissipated, diets containing the decontaminated meals are readily consumed by animals without any harmful effects. The residues of AFM1 in the milk of dairy cows are substantially reduced or absent altogether depending on the efficacy of decontamination. The adverse effects of tannin-rich forages may be overcome by treatment or spraying of foliage with polyethylene glycol, but the practical application of this procedure still has to be economically evaluated.

Conclusions

Contamination in animal feed and herbage can be seen by organic and inorganic compounds as well as with particulates. Organic chemicals demonstrate the largest group and consist of plant toxins, mycotoxins, antibiotics, prion proteins and pesticides. Inorganic compounds consist of heavy metals and radionuclides. The common particulate contaminants of animal feed include weed seeds and certain bacterial pathogens. The effects of feed contaminants and toxins range from reduced intake to reproductive dysfunction and increased incidence of bacterial diseases. Residues moved to edible animal products represents another reason for concern. Comprehensive legislation and control measure in feeds are available in the developed countries but the developing countries are rare and even not exist. However, in many developing countries, particularly in Africa, statutory control of contaminants is at merely present. The scope for decontamination of feeds is limited and generally not economic, and prevention strategy is the most effective practical option.

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CHAPTER 10

Non-biological contaminants in animal source food products

Summary

Millions of people in Bangladesh are involved in agriculture and livestock production for livelihood. Use of pesticide is a common practice in the agricultural production system. In general, in rural areas most of the family has cattle, goats and buffalos and people receive a major portion of income from these animals. The major share of milk comes from cows in Bangladesh and buffalos produce second largest share of milk next to cows. Pesticides reached inside of the human body through animal originated food products which are rich in lipid content. Reports from several studies showed that some animal originated foods are responsible for the entry of pesticides in the human body. Milk is rich with high nutritional contents and suitable food for different age group people. Therefore, this issue poses significant threat to public health. As milk is home to considerable amounts of fat and therefore presence of these lipophilic compounds in milk is beyond any suspicion. Occurrence of pesticide residues in milk were reported by many authors in different countries over the last few decades and use of most of these chemicals had been banned in many countries.

Key words: Pesticides, residue, milk, public health

Introduction

Meat, milk, egg and their products are the major sources of proteins, minerals, vitamins and trace elements which are essential part of balanced diet. Heavy metal and pesticides contamination in food from animals happened due to anthropogenic pollution, leading to threat to public health because of the toxicity, bioaccumulation and biomagnification in food chain (1). Heavy metals have both positive and negative impact on human health. Some members of the heavy metal group are essential including iron, zinc and copper, while some other metals such as lead, cadmium, mercury and arsenic have toxic roles in biochemical reactions of our body (2). There are non-essential elements including lead, chromium, cadmium and nickel are toxic and their occurrence in the body can cause profound biochemical and neurological changes in the body (3). All metals are toxic at certain levels of intake, however, in contrast to elements such as arsenic, chromium, copper, selenium and zinc that have useful biological functions and cadmium, lead and mercury doesn't useful role and their intake should be limited to avoid organ damage (4). Several recent studies showed contamination of heavy metals, pesticide and other toxin in raw food and environment (5). Calcium, copper, zink, iron, cadmium, manganese, magnesium, nickel and lead have been detected in veal, beef, pork, chicken and horsemeat (6, 7). Moreover, copper, manganese, zinc, cadmium, mercury and lead concentrations were detected in liver, kidney, and muscle of geese, ducks, chicken, rabbits, and sheep slaughtered in northern Poland (8). Heavy metals might accumulate up to toxic concentrations under certain environmental conditions. Acquiring heavy metals through food chain is the result of pollution and which is potential chemical hazards threatening to consumers.

The evolution of food safety practices has been substantially affected by incidents or controversies involving food contamination. Food safety practices are mainly mediated by implementation of food safety laws and regulations in the arena of food production, harvesting, food handling, food processing, packaging, storage, distribution and precautions before consumption. These puissant events has drew attention of the public and of policy makers.

Animal originated foods primarily meat, milk and eggs can potentially be contaminated with one or more of the thousands of artificial chemicals which are used in the society. These include antibacterial drugs, hormonal growth promoters or production adjuncts, polyhalogenated hydrocarbon pesticides, industrial chemicals and heavy metals. Moreover, as a matter of fact foods from animals may also be contaminated with naturally-occurring toxic substances, which include bacterial toxin (e.g. botulinum toxin, staphylococcal enterotoxin) and algal toxin (e.g. saxitoxin in shellfish), and that even some animal tissue are inherently toxic (e.g. livers of puffer fish), should be recognized (9).

Off course, a wide array of naturally-occurring substances in foods would be considered potential hazards if subjected to the range of toxicological tests and assays which are applied to man-made compounds. Among the synthetic chemicals which occur in foods are a range of additives used intentionally for production, processing or preservation purposes. Some of them, for example, nitrite, are of concern if toxic metabolites (e.g. nitrosamines) are allowed to form in foods prior to ingestion. Chemical residues like iodine, chlorine or bromine are also contaminants of sanitation procedures and other which occasionally mitigate from packaging materials. Furthermore, other non-biological contaminants in animal originated food include metals, wood, plastics or other materials from processing plant and equipment. Other external materials may also be introduced during animal production, mainly needles broken during injection of drugs and biological and subsequently not surgically removed.

Sources of pesticide residues in milk

In agriculture, crops like fruits, vegetables and cereals are treated with different types of synthetic chemicals, which are known as pesticides (10). Pesticides include insecticides, herbicides, rodenticides and fungicides etc. These pesticides are applied pre-harvest, post-harvest and storage stages. They have ability to transfer from lower plants and animals to the higher plants and animals among the food chain and can accumulate in the higher organisms (11). In addition to this, sometimes pesticides are directly sprayed to the animal accommodation to infest the pest (12). Ultimately, both routes (plants and animals) lead to the bioaccumulation of pesticides in the animal products like milk, meat, fat and eggs. Pesticides source in dietary rout is main way of chronic exposure to these substances (13, 14).

Analytical methodologies for pesticide residues

There are different techniques used for pesticide residues analysis in milk. Gas chromatography (GC) as well as liquid chromatography (LC) is being used as separation technique coupled with some detectors. Ideal detectors used for the detection and quantification of pesticide residues would respond only to target analyte, while other coextracted elements remain transparent (15).

Gas chromatography has been used with different detectors like electron capture detector (ECD), micro- ECD (μ-ECD), GC-NPD (nitrogen phosphorus detector) and Flame ionization detector (FID). Mass spectrometric detector (MSD) is termed as the universal detector on the basis of its non-specific properties. MSD being versatile and selective detector is preferred by analyst (16).

LC-MS and LC-MS/MS is an ideal, extremely specific and highly sensitive technique used for identification and quantification of pesticide residues. It provides information about analyte without derivatizing. It can compensate sample purity and it enables simultaneous analysis of the compounds with varying polarity (17).

Public health concerns

A wide range of milk and milk products are consumed by the peoples of all ages. From polluted grass, corn, silage and through pesticides direct application on cattle, these chemicals accumulate in the cattle milk. As human beings are on the top of tropic level or in the food chain, they are bigger consumers of pesticides. Products of animal origin: meat, fish, eggs and especially dairy products are main source of OCPs and OPPs intake in general public (18). These pesticides cause a wide range of toxic effects and pose very severe health risks, specifically in infants, who have less developed metabolic and enzymatic systems (19). Overall health effects on human by pesticides are not well defined but evidences are increasing for genotoxicity, carcinogenicity and hormonal disturbances (20, 21).

Milk has been analyzed as an indicator of the bio-concentration for the persistent organic pollutant like pesticides (22). A class of pesticides, organochlorines, which are lipophilic and have little metabolism in the body of living animals. Environmental exposure of organochlorines leads to their accumulation in fat tissues and magnify in living tissues through the food chain (23).

Status of pesticide residues in milk and milk products

This review covers the level of pesticides contamination in different countries especially in developing countries. In this regard, Pandit (24) monitored milk samples of different brands in Maharashtra, India to check the pesticide residues contamination. Analysis was done with GC technique with µECD. Hexachlorocyclohexane (HCH) and di-chlorodiphenyltri-chloroethane (DDT) were detected in trace amount in milk samples. Overall HCH level was lower than DDT. This may be due to anti-malarial sanitary activities. Results showed that butter have higher concentration of DDT than milk and cheese. However, Organo chlorine pesticides levels were below the FAO (Food and Agriculture Organization)/ WHO (World Health Organization) standards.

It was investigated in a study, cow and buffalo milk samples were collected from 6 different markets of Menia El-Kamh province of the Sharkia Governorate constitutes, one of the largest agricultural areas in Egypt. Thirteen different pesticides were analyzed by High performance liquid chromatography (HPLC) and DDT, Larvin, Anifose and methomyl were detected in milk samples and their concentrations as high as 67 µg/kg (larvin), 88 µg/kg (anilifos), 138 µg/kg (DDT), and 325 µg/kg (methomyl) were found in cow or buffallo milk (25). Another monitoring study was carried on to check the HCH and DDT. As chemicals are extensively being used in public health and livestock programs in the central tropical region in Mexico. Milk samples were collected from Tlalixcoyan and analyzed HCH and DDT. Results demonstrate the mean level of HCH was significantly higher than residues in milk samples from Medellin (0.049 mg/kg) and Paso San Juan (0.022 mg/kg). The DDT mean level from Medellin milk samples (0.089 mg/kg) was significantly higher than the levels detected in the other two areas. These results showed that infants are at more risk of exposure to pesticides residues. These findings indicate that those cattle exposed to DDT and HCH accumulates these chemicals in their milk and may pose health risk to the consumer (26).

Pagliuca et al. (27) described that OPPs, which are being used in agricultural system, they can accumulate in food chain and ultimately pose toxic effects on animals and human beings. In Italy a research was carried out conducted to determine the OPPs in dairy milk and to adopt the special procedure for risk management in the whole milk production chain. Milk samples were collected from tanks trucks of four dairy plants in Italy, which were representations of 920 tons of raw milk. The separation of the 8 OPPs (acephate, chlorpyriphos, chlorpyriphos-methyl, diazinon, methamidophos, methidathion, phorate, and pirimiphos-methyl) was done through liquid partition and then clean-up with solid phase extraction. Gas chromatography with Nitrogen phosphorus detector (NPD) was used for detection analysis. Total 135 samples were analyzed and 37 showed positive results and 10 were contaminated with OPPs (5-18mg/kg). Acephate and chlorpyriphos were main contaminant. However, OPPs level of contamination was lower than MRLs given by European Commission (EC).

Weber (28) stated that OCPs are not readily degradable and are lipophilic in nature. That is why OCPs have tendency to bio- accumulate in fatty foods like milk. After a long exposure to OCPs adverse type of health effects may develop. Although it is banned in some countries even then its residues are being found and OCPs accumulation in fatty foods is a major concern.

A method was developed for trichlorfon residue as dichlorovos analysis by GC-µECD. Dichlorovos confirmation was done by mass spectroscopy. In this protocol acetonitrile was used for milk extraction then centrifugation followed by freezing and partitioning in dichloromethane. Ethyl acetate was used for dissolution of residues for GC. Average recoveries were noticed from 92.4%-103.6%. No residue was detected in milk samples collected from seven major cities of Korea (29).

During the handling and processing, milk and milk products could be contaminated. Buffalo and cow milk were investigated for pesticides residues in Egypt. OPPs (profenofos, malathion, pirimiphos-methyle and dimethoate) were not detected in any milk sample. However, OCPs (lindane, aldrin, heptachlor, epoxide, HCB, eldrin, chlordane and DDT) were present above the recommended limit established by FAO/WHO (19).

Milk and feedstuffs of goats and sheep were monitored in a research. Total 200 milk samples were collected from 10 goats and 10 sheep farms. Milk samples were analyzed for 99 multi residues by GC-MS (GC- Mass Spectrometry) and LC- MS/MS (LC-Liquid chromatography) systems. Feedstuff samples were contaminated with pesticides residues; however, milk samples were contaminated but found under safe limits (30).

Another study was reported by Karabasanavar and Singh (31) for public health and plants defense against pest, pesticides are being used. Entrance of these pesticides to the food chain is very harmful. Application of chloropyrifos in the agricultural and associated fields leads to the pesticides accumulation in milk also. A study was designed to determine the chloropyrifos concentration in milk. For this purpose milk samples were collected from Kumaon and Tarai of Uttarakhand state. HPLC technique was used to analyze the milk samples. Total 170 samples were analyzed and out of which 4.7% milk samples were detected with chloropyrifos residues which were also above the MRLs (0.02 mg/kg). It was investigated OCPs in dairy milk produced by buffalos, cows and sheep. 21 different types of pesticides were present in milk and beta-HCH was more dominant in all collected samples. Some pesticides were above the MRLs recommended by European Union (32).

In another study (33) 30 raw milk samples were collected from 28 different dairy farms in August 2007 from Spain. Extraction of raw milk samples was done by following the protocol of Pagliuca et al. (27) and analyzed through gas chromatography. The main pesticide was fenthion, detected in four samples of 12 (33.33%), followed by dimethoate (25%), coumaphos (8.33%) and Malathion (8.33%). In Carbamate group, the pesticides detected were carbofuran (25%), aldicarb (16.67%) and carbaryl (8.33%). In some samples, two or more active principals were detected, what explains percentages over 100%. The frequency of pesticides found in this study is in agreement with Araujo et al. (34) that noted that the most pesticides commonly used in Pernambuco are from OP class, followed by CB and pyrethroids. The frequency of samples positive to OPPs and CB residues was lower than the one found by Nero et al. (35) that verified 196 (93.8%) positive samples among 209 raw milk samples in four Brazilian regions. Thin Layer Chromatography, which would justify the difference in the results, since this technique presents lower specificity when compared with GC.

Pasteurized and fresh milk samples were tested for OCPs by using GC with ECD in Kampala. Aldrin, Lindane, Dieldrin, DDT and Endosulfan were detected in milk. Results showed that most of the residues detected were above the residue limits set by the FAO/WHO (2008). Bioaccumulation of these residues is likely to pose health risks to the consumers of milk in Uganda (36).

Aslam et al. planned a research to analyze the OC residues and their chemical composition in milk of buffalo. Milk samples were collected from Dehli. Monitoring of milk could be useful for getting information about the type and quantity of OC residues in environment and in our daily life also. The results indicated that p, p/-DDT was exceeded in 70% of milk sample, p,p/-DDE in 80% of the milk samples of in Delhi state. Dichlorodiphenyldichloroethane (DDD), another metabolite of p, p/-DDT was also present in 65% of the milk samples. The results revealed that DDT was the main contaminant in Dehli state. Endosulfan was detected in 35% milk samples. Mixture of toxic compounds present in buffalo milk samples might possibly toxic for infants' health mainly nervous system, reproductive and immune system (37).

In another study, it was reported that 16 OPPs residues were determined in Tizayuca, Hidalgo, Mexico during 2008-2010. GC with ECD was used for OCPs determination and residues concentrations were found higher in wet season than dry season. Overall pesticides residues were below the MRLs proposed by Codex Alimentarius. This reduction in residues reflects that Mexican government has achieved the safety levels in response to persistent organic pollutants (POPs) agreement (38).

Ayoub and coworkers conducted a study in Egypt. They analyzed 72 buffalo milk samples for OCPs and the only detected pesticide was p, p/- DDE. Maximum concentration was found 4.714 ppb but overall samples were below the MRLs recommended by Codex Alimentarius Commission (2004) (39).

In Brazil CBs and OPPs were investigated in feedstuff, water and dairy milk. GC with NPD was used to analyze these compounds. Total 30 milk samples were analyzed out of which 17% samples were contaminated with OPPS. CBs were not present in milk however, they were present in feedstuffs and water samples. Same ingredient was noticed in 3 dairy farms (40).

A study was carried out to find the OCPs and OPPs residues in milk and fodder samples around the Musi river belt in India. Milk and fodder samples were collected from different six location of Musi river belt in Hyderabad, India. Collected samples were analyzed by GC with ECD for OCPs and pulsated flame photometric detector (FPD) was used for the detection of OPPs residues. Analysis of fodder showed dicofol concentration ranging from 0.071-0.077 (0.07).

Dimetheoate (OPPs) was found in milk samples ranging from 0.111-0.167 (0.13). Residues of other OPPs and OCPs were below the MRs specified by European Union (EU) and Codex. Whereas fodder dicofol and in milk dimethoate were above the MRLs values established by EU and CODEX (41). It was reported in a study that fresh milk of buffalo collected from agro-industrial zone in Upper Egypt was analyzed for OCPs and OPPs by using GC. Five different OCPs (alachlor, HCB dieldrin, methxychlor and linadane) and three different OPPs (Malathion, parathion-methyl and chlorpyrifos) were identified in fresh milk samples. It was found that Malathion and Lindane exceeded permissible limit set by EU established in 2008 (42). In addition to this, chloropyrifos, HCB and methoxychlor were exceeded in 33%, 88% and 66%, respectively. However, parathion-methyl, alachlor and dieldrin were below the MRLs established by EC. Overall it was concluded that Egypt peoples are at the risk of pesticides exposure. Based on this, it was recommended that pesticides monitoring programs should be established in all developing countries. Non- judicious use of pesticides leads to contamination of food commodities with pesticide residues. Although, pesticides consumption in India is 0.5 kg/ha. A survey was conducted to analyze the levels of OCPs residues in cow milk from different locations of Dhanbad city, Jharkhand, India. Milk samples were collected seasonally, and pesticide residues were assessed using gas chromatography with an electron capture detector. These results indicate that milk samples were contaminated with DDT and its metabolites (DDE and DDD). Seasonal variations of these pesticide residue levels were also observed in all the milk samples. Samples collected during winter season were found to contain higher residue levels as compared to other seasons (43). Ismail and Elkassas, carried out a survey to analyze the concentrations of OCPs, OPPs and Pyrethroids pesticides in milk of buffalos. The findings demonstrate that OCPs were present with high levels and their concentration exceeds the FAO/WHO and EU MRLs. OPPs detection levels were higher than MRLs established by FAO /WHO. Cypermethrin and pyrethroid concentrations were exceeded the FAO/WHO and EU limits. This survey suggests that proper monitoring of milk is required to keep consumers safe (44). Muhammad and his co-workers conducted a study in Faisalabad, where cattle milk was collected from different localities of Faisalabad, Pakistan and solid phase micro-extraction was done for pesticides residue analysis and residues were determined by using HPLC. The results of this study revealed that overall 40% samples showed pesticides contamination. The mean levels of cypermethrin, chloropyrifos, endosulphane and syhalothrin were 0.085, 0.072, 0.26 and 0.38 µg/mL, respectively. Pesticides residues risk analysis was calculated on the bases of provisional acceptable daily intakes and analyzed pesticides residues. The daily intake levels of pesticide residues including cyhalothrin, chlorpyrifos and cypermethrin in present study were 3, 11, 2.5 times higher, respectively in cattle milk. These results showed that pesticides residues present in the milk might pose health problems in the people of this vicinity (45).

Shahzadi and her co researchers identified and quantified the insecticide imidacloprid, pyrethroid (bifenthrin, deltamethrin and lambda cyhalothrin), and OPP (chlorpyrifos) in buffalo, sheep, cow, goat and camel milk. Milk samples were collected from different locations of Lahore in Pakistan. High performance thin layer chromatography (HPTLC), Ultraviolet–visible (UV) and GC-MS were used to analyze the residues contamination level. These pesticides residues were extracted with petroleum ether, ethanol and sodium oxalate. Results indicate 50 % of the milk samples were contaminated with pesticides residues. Most significantly present pesticides were Deltamethrin and maximum contamination was found in sheep milk. Milk consumption contaminated with pesticide might pose health hazardous to humans in this locality (46).

In dairy farms present in peri-urban areas, cattle are being fed on agro-industrial by products diet (cotton khal, sugarcane khal, wheat bran etc.). This activity may transfer chemicals to cattle milk. In a similar type of study (47) pyrethroid and OPPs were assessed. These pesticides when accumulated in the fat tissues and milk, they may pose adverse health risks to human's health. In the present study 30 diet and 80 milk samples were collected from different dairy farms. All the samples were processed through QuECHERS kit and analyzed by using GC coupled with mass spectroscopy. The results revealed that cypermethrin and chloropyrifos concentrations were above the MRLs in 40% milk samples proposed by WHO. Profenofos was exceeded in the 20% milk samples.

It was reported in a research in Pakistan in which pesticides' residue levels was monitored in milk of cattle from cotton growing areas of Punjab. Analyzed pesticides were aldrin, bifenthrin, cypermethrin, endosulfan, deltamethrin, permethrin, DDD and DDT. HPLC technique was used and findings showed that 70% milk samples showed exceeded level of pesticides residues. Maximum contamination was shown by Aldrin (0.68 µg/mL). By and large, the results indicated that 23%, 21%, 18% and 7% of the milk samples were contaminated with cypermethrin, bifenthrin, permethrin and detlamethrin, respectively (48).

Table 1: Status of pesticides residues in milk in different countries

Country	Year	No. of samples	No. of samples contaminated (%)	No. of samples above the MRLs	Pesticides detected	Analytical method	Reference
India	2002	54	-	60	HCH and DDT	GC with µECD	18
Mexico	2003	240	-	70	HCH and DDT	GC with ECD	20
Italy	2006	135	-	56	Acephate, chlorpyriphos, chlorpyriphos-methyl, phorate, pirimiphos-methyl, diazinon, methamidophos, methidathion	GC with NPD	21
India	2007	-	75	-	2,4 DDE , 4,4 DDE, Aldrin, Dieldrin, BHC, Endosulfan ,Methyl, Parathion, Malathion, Dimethoate	GC with ECD	3
Egypt	2015	120	-	100	OPPs	GC with µECD and FID	23
Korea	2011	12	-	-	Trichlorfon residue as dichlorovos	GC with µECD	23
Uganda	2011	101	90	80	Aldrin, dieldrin, endosulfan, lindane, DDT and its metabolites	GC-MS	30
Uttarakhand State	2013	170	-	4.71	Chloropyrifose	GC with µECD	25
Turkey	2011	-	-	5	16 OCPs	GC with µECD	26
Egypt	2012	72	50	0	p, p/-DDE	GC equipped with double electron	34
Pakistan	2012	-	50	25	Cypermethrin, chloropyrifos, endosulphane and syhalothrin	HPLC	40
India	2013	20	-	80	p, p/-DDT, p, p/-DDE, DDD and Endosulfan	GC with µECD	31
Pakistan (Lahore)	2013	140	50	25	Insecticides and OPPs	GC-MS	42
Pakistan	2014	80	-	40	Insecticides and OPPs		42
Mexico	2013	40	-	50	OCPs	GC-MS	32
Mexico	2015	-	-	0	16 OPPs	GC-ECD	33
Pakistan (Punjab)	2014	150	70	70	Aldrin, bifenthrin, cypermethrin, endosulfan, deltamethrin, permethrin, Dichlorodiphenyldichloro ethylene and Dichlorodiphenyltricthloro ethane	GC-ECD HPLC	34
Brazil	2014	30	-	16.67	OPPs and CBs	GC-NPD	35
Egypt	2015	200	-	44	5 OCPs and 3 OPPs	GC	37
Egypt	2016	-	-	100	OCP and OPP and pyrethroid	GC	39

Prevention and control of pesticide residues

Maximum residues levels (MRLs) have been set by the European Union and Codex Alimenterius to ensure that pesticides are present below the unacceptable risk limit. These MRLs are the upper legal limits of pesticides concentrations in feed and food. MRLs are established for a wide variety of plants and animal's origin based food commodities. MRLs are not simply set as threshold levels of toxicologically, but they are derived after a broad assessment of the active substance properties and their residue behavior on treated crops [44]. There was a need to investigate the pesticides residues in milk in order to provide a baseline to health department or governing bodies to make safety regulations. In addition to this, pesticides residues monitoring program is very essential for the safety of consumer health and to achieve the food safety in country.

Conclusion and recommendations

On the basis of above review worldwide including, it is concluded that human health is associated with exposure to OCPs, OPPs and pyrethroid pesticides via milk or milk products and this issue deserves more attention. Several OPPs and OCPs pesticide residues were detected in raw animal milk samples collected from different sources in Pakistan.

The results of this review demonstrate the need to establish pesticides residues monitoring programs for milk analysis for human consumption to improve food safety and decrease exposure risks for consumers. In addition to this, these findings suggest creating awareness in owner of dairy farms and general people regarding the avoidance of pesticides residues in milk.

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