Sub-Project ID: 007

Program Based Research Grant (PBRG)

Sub-Project Completion Report

on

Value Addition and Standardization of Nutritional Level in Selected Food Items from Animal and Plant Origin

Sub-Project Duration
28 March 2018 to 31 January 2022

Coordinating Organization
Nutrition Unit
Bangladesh Agricultural Research Council
Farmgate, Dhaka 1215

Project Implementation Unit
National Agricultural Technology Program-Phase II Project
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

November 2021

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Value Addition and Standardization of Nutritional Level in Selected Food Items from Animal and Plant Origin

Implementing Organization

Department of Poultry Science
Patuakhali Science and Technology University
Barisal Campus, Barisal-8210

Department of Dairy and Poultry Science
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Project Implementation Unit
National Agricultural Technology Program-Phase II Project
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

November 2021

Citation

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National Agricultural Technology Program-Phase II Project (NATP-2)
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Abbreviation and Acronyms

ANOVA	:	Analysis of Variance	Kg	:	Kilogram
µg	:	Microgram	L	:	Litre
<	:	Less than	LoA	:	Letter of Agreement
>	:	Greater than	LOD	:	Limit of Detection
AG-F	:	Agro Foods Ltd	LSD	:	Least Significant Difference
As	:	Arsenic	Mg	:	Milligram
BARC	:	Bangladesh Agricultural Research Council	mg	:	Milligram
BDL	:	Below Detectable Limit	Mm	:	Millimeter
BDS	:	Bangladesh Standard	MMT	:	Million Metric Ton
BDT	:	Bangladesh Taka	MRL	:	Maximum Residue Level
BLQ	:	Below Limit of Quantification	MR-VP	:	Methyl Red (MR) and Voges-Proskauer (VP) Tests
BPICC	:	Bangladesh Poultry Industries Central Council	MT	:	Metric Ton
BSTI	:	Bangladesh Standards and Testing Institution	NARI	:	National Agricultural Research Institute
Ca	:	Calcium	NATP	:	National Agricultural Technology Program
CAGR	:	Compound Annual Growth Rate	ND	:	Not detected
CF	:	Commercial Feed	ND	:	Not detected
cfu/ml	:	Colony-Forming Units per Milliliter	NRC	:	National Research Council
Co-PI	:	Co-Principal Investigator	P	:	Phosphorus
CP	:	Crude Protein	Pb	:	Lead
Cr	:	Chromium	PBRG	:	Program Based Research Grant
CRD	:	Completely Randomized Design	PCR	:	Project Completion Report
DL	:	Detectable Limit	PET	:	Polyethene Terephthalate
DOC	:	Day-Old Chick	PI	:	Principal Investigator
EU	:	European Union	PIU	:	Project Implementation Unit
FAO	:	Food and Agricultural Organization	PLDP	:	Second Participatory Livestock Development Project
FDA	:	Food and Drug Administration	PP	:	Project Proposal
G	:	Gram	ppm	:	Parts Per Million
GDP	:	Gross Domestic Products	PSTU	:	Patuakhali Science and Technology University
GENSTAT	:	General Statistical Package	PUFA	:	Polyunsaturated Fatty Acid
GL	:	Geographical Location	RFL	:	Rangpur Foundry Limited
GoB	:	Government of Bangladesh	SED	:	Standard Error Differences of Mean

H_2S	:	Hydrogen Sulphide	SGS	:	Societe Generale Surveillence
HACCP	:	Hazard Analysis Critical Control Point	SIM	:	Sulfur-Indole-Motility
HPLC	:	High performance liquid chromatography	SLDP	:	Smallholder livestock development project
HSTU	:	Hajee Mohammad Danesh Science and Technology University	STD	:	Standard Deviation
IDA	:	International Development Association	TBS	:	Turner Broadcasting System
IFAD	:	International Fund for Agricultural Development	USD	:	United States Dollar
IQF	:	Individual Quick Freezing	WB	:	World Bank
IRIN	:	Integrated Regional Information Networks	WHO	:	World Health Organization

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Executive Summary

Technological innovations along with immense efforts in extension activities and other development events in agricultural sub-sectors during last few decades result in a substantial growth in productivity to meet the growing demand in Bangladesh. It is estimated that agricultural sector is contributing 13.35% in our GDP where about 50% population are employed and about 70% people depend on this sector for their livelihood. Despite this huge contribution, a fear is always in consumers' mind that whether the food is safe or not. Among the major concerns related to this development are health issues threatening not only production, but also the people using the products. Numerous reports and findings indicated that basic and other food items in Bangladesh are adulterated and contaminated with various chemicals, biological and heavy metals those are of public health importance. Human foods are contaminated and adulterated in different stages of production, processing and marketing by mixing or adding non-food ingredients, preservatives, additives, colouring, flavouring and other chemical adulterants. Again, grains contaminated by heavy metal concentration, pesticides, fungal colonization and mycotoxin may affect the public health. The present research thus aims to generate new information on food contaminants, adulterants and other hazardous chemicals particularly applied in poultry products and to dissemination of ideas for awareness of producers, food processors, vendors and consumers and towards establishing food safety. Food safety is now a part of governments' strategies to ensure secure food for the consumers. The sub-project activities were designed to attain the set goal of ensure safe and nutrient enriched food production from poultry and plant sources for increasing human nutrition intake.

The entire activities of the sub-project were assigned in three components each with different specific objectives. The designed research activities were implemented in Dhaka, Gazipur, Barishal, Dinajpur and Chttogram districts. The components implemented the research programmes following different approaches and methodologies. In addition to coordination and monitoring by the respective component some research activities on contaminants and their level in plant and poultry origin foods were also conducted. A number of designed biological experiments on broiler, layer and sonali chickens including a detailed baseline survey were conducted. A study in Component-1 was further conducted in Component-2 for validation. Implementation of the HACCP system in various sectors of the poultry industry was monitored by evaluating the analytical findings. Awareness meetings and seminar were arranged to disseminate the new information among the stakeholders.

Findings of food contaminants reveals that Caffine concentration in local energy drinks are within the limit of BDS (145/L). In case of antibiotic, sulphur drugs and heavy metals in marketed milks of Dhaka city was observed below detectable level. While in case of different kinds of street foods under analysis for total *Coliform, E. coli, S. aureus* and *Salmonella* sp. it is found that most food items are loaded heavily with the pathogens except *Salmonella* sp. Analysis of sixteen marketed sugar samples revealed that 100% of them are contaminated with Sodium Cyclamate at different degrees (<1.0 ppm).

Baseline findings indicate that commercial DOC of broiler, Sonali and layer in 2019 were 70.0, 4.74 and 35.0 crores, respectively. Out of 6.57 MMT/year commercial feed poultry feed accounts for 4.45 MMT (38-40% broiler, 6-6.5% layer and 22-23% Sonali). About all farmers (92%) used antibiotics, anticoccidial drugs, enzymes and growth promoters following dealer's suggestions. More than 84% dealers unethically advice antibiotics and other drugs and about 75% of them sell drugs without prescriptions. Veterinarians' advised therapeutic drugs like ciprofloxacin, enrofloxcin, amoxicillin, doxycycline, oxytetracycline, colistin, tylosin and tilmicosin, toltazuril, salphaclozine sodium, and

additives, enzymes, probiotics and growth promoters. About 15 prominent players controlled over the value added frozen food market.

A number of antibiotics/anticoccidial drugs viz. avilamycin, virginiamycin, lincomycin, tylosin, oxytetracycline, cholotetracycline, flavophospholipol, salinomycin-Na olaquindox, maduromycin, diclazuril, etc. as sole or in combination were reported to use in commercial feed industry. Some of the compound feeds under trials contained lincomycin, oxytetracycline, cholotetracycline, tylosin or maduromycin as antimicrobial or anticoccidial drugs and heavy metals (Pb, Cr, As) in varying levels, although in most of the cases it was within permissible limit. But, house feeds were free from aforesaid antimicrobial drugs. Except a few, crude protein contents of commercial broiler and layer feeds were nearer to standard one. But all house feeds contained much lower amount of CP.

Although geographical location or feed had no influence, but seasonal variations were large ($p>0.01$) among final body weight of layers. Feed consumption of birds at 30-week age were significantly influenced by location, feed or season. Layers attained peak production at similar age, but it was influenced by feed types and rearing seasons. While egg weight at peak was affected by geographical locations or rearing seasons. Survivability of birds was almost similar. But, relatively higher mortality (due to egg bound and vent picking) at Dinajpur during summer interacted to produce significant results. Except yolk colour score, all other egg quality characteristics were found to beinfluenced by dietary treatments.

Geographical location, feed or season had profound influence on weight gain, feed intake and feed conversion of broilers except the feed consumption in various dietary treatment groups. Interaction effects were also significant. But, survivability was almost nearer to each other. Substantial differences were noted in body weight and feed consumption two Sonali genotypes (hybrid) reared in two different seasons up to 21-day. Standard broiler starter diet was provided to all the birds. However, Kazi Gold performed the best up to 21-day irrespective of season. Thereafter, Kazi Gold chicks gained market weight of 800g b^{-1} on 45-day against the local Sonali that achieved similar weight on 60-day age regardless of feed type and rearing season. None of the factors affect the survivability of birds. Results indicate that lowered CP contents of house feeds markedly influenced weight gain of broilers. Birds on standard broiler diet gained on an average of 499g compared to those of two house feeds of 127 and 194g. Harvesting age influenced a lot; heavier birds harvested on 35-day achieved higher ($p>0.01$) gain than those of 28-day (363 vs 184g) by consuming higher amount of feed. Again season had no significant influence on weight gain, though differences in body weights were large throughout the study period.

It was earlier reported that some of the commercial feed contained certain chemical hazards in varying levels. But none of these were reported in liver and muscle of broilers except maduramycin and lincomycin either in summer or winter. Maduramycin and lincomycin levels were far below the MRL value. Levels of heavy metals in either raw muscle or liver were also below the permissible limits. Chemicals and heavy metal residues in raw eggs were far below the MRL levels. Salmonella spp. and *E. coli* (cfu/ml) in and around farm premises and materials were determined through different microbial procedures. State of implementation of HACCP standard suggested reducing the use of chemicals in feed production, maintaining hygiene in broiler processing at wet market, applying technical knowledge in feed ingredient selection, and frequent monitoring for safe feed and food production in Bangladesh.

Keywords: Value addition, Nutritional level, Hazards, Residues, Plant & animal origin products.

PBRG Sub-Project Completion Report (PCR)

A. Sub-Project Description

1. Sub-Project title

Value Addition and Standardization of Nutritional Level in Selected Food Items from Animal and Plant Origin

2. Implementing Organization

a) Component-1
Department of Poultry Science, Patuakhali Science and Technology University (PSTU), Babuganj, Barishal

b) Component-2
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4. Sub-Project Budged (Taka) BDT **2,9638,872.00** (Two crore ninety six lac thirty eight thousand eight hundred seventy two)

a. Coordination Component (BARC):		Tk. 6200000.00
b. Component-1 (PSTU)	:	Tk. 16543232.00
c. Component-2 (HSTU)	:	Tk. 6895640.00

5. Duration of the Sub-Project: 03 Yrs 10 Months

5.1 Start Date (Basd on LoA signed):		28 March 2018
5.2 End Date	:	31 January 2022

6. Background of the sub-project

6.1 Statement on the problem and its significance

Agriculture has consistently been the largest sector in Bangladesh economy where animal agriculture has become the integral part. Tremendous efforts are given to the research and extension in agricultural sub-sectors during last few decades resulting in a massive growth in productivity to cope up with the growing demand of population in Bangladesh. About 50% population still are employed in this sector and about 70% people depend on agriculture for their livelihood. Agricultural sector is contributing 13.35% in our GDP out of which 1.43% is added by animal farming and 3.52% by fisheries sector in 2019-20 (Bangladesh Economic Review 2020). Report also indicates that sectoral growth in 2019-20 stood at 0.89, 3.04 and 6.10% in crops, animal farming and fishing, respectively over 2018-19. Bangladesh is now at about to reach food security. But, with the increment in agricultural productivity, food hazards become an alarming issue in Bangladesh over the last few decades. Important food hazards include microbial hazards, pesticide residues, misuse of additives, chemical contaminants, including biological toxins and adulteration. Although microbiological contamination and chemical

hazards have received most attention, food adulteration and food fraud should not be neglected considering their role in public health (FAO/WHO 1986). The Institute of Public Health Bangladesh has found adulteration in 43 consumer goods. In such a situation, awareness among the consumers regarding the hazards is also developed as a result of massive publicity using electronic and print media.

Basic food items in the market like cereals, meat, fish, fruits, oil, vegetables, spices, processed foods, etc. are being adulterated unscrupulously with hazardous chemicals and/or contaminated by drug residues, soil and water pollutions, etc. The poisonous residues in these food items are not only damaging vital human organs like liver, kidney, and heart along with affecting children's mental and physical wellbeing but also causing fatal diseases like cancer, hepatitis B (Khaled Bin Chowdhury 2019). About 45 lakh people in Bangladesh are now at health risks due to consumption of adulterated and contaminated food (Anonymous 2020a). Animal origin foods including processed ones are contaminated and adulterated in different stages of production, processing and marketing by mixing or adding non-food ingredients, preservatives, additives, colouring, flavouring and other chemical adulterants. Grain contaminated by heavy metal concentration, pesticides, fungal colonization and mycotoxin may affect the public health. The present research thus aims to generate new information on the basis of available information on food contaminants, adulterants and other hazardous chemicals and to dissemination of ideas for awareness of consumers and towards establishing food safety.

The way to address the situation for achieving the goal, through establishing of strong research support and linkage, as per NATP-II thoughts, all research and extension institutes need to make strong footing with team building holistic research culture to achieve desired output. With this consideration, as an effective approach, the program-based research grant of NATP-II which is particularly aimed to support coordinated research program amongst NARI to jointly combating national agricultural problems and strengthening the research and research management capacities of the institutes. Therefore, under the principal objective of NATP-II, the nutrition unit co-ordination component shall have to play the role to ensure smooth and efficient implementation of sub-project activities to achieve the desired project output through coordination of activities and strong and effective monitoring of research progress under an additional increased research support against each institute/organization.

Growing population and rising household income have led to rapid increasing demand for food products, especially meat, milk and eggs and processed food in Bangladesh over the last two decades. Together with innovations on the supply side, the search for the most viable protein sources has resulted in particularly rapid growth of industrial poultry production along with small-scale enterprises. Among the major concerns related to this development are health issues threatening not only animal production, but also the people using the products derived from these animals. Intensification has brought food-safety concerns into sharper focus (Blancou *et al.* 2005), and these concerns have been increasingly acknowledged, at least in developed countries, as information technology and medical science have advanced (Nelson, in FAO 2005). With the increment in demand and supply for meat and fish products, the new intensive production systems of the developing world are facing more and more pressure to comply with the regulations that prevail in the global market.

Food safety refers to all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer (FAO/WHO 2003). It, now a day, has become an important topic in Bangladesh as consumers have become victim due to serious adulteration in food. The emergence and discovery of new food-borne pathogens and other food-related hazards has increased the need for food-safety measures. Food security is a complex issue, where livestock and poultry products are generally regarded as high risk commodities with respect to microbial, chemical and other possible contaminants and adulterants (Yousuf *et al*. 2008 and Ukut I-OE *et al*. 2010). Food safety is now a part of governments' strategies to ensure secure food for the consumers.

Poultry meat and eggs are the two popular and easy-to-afford protein sources. This cheaper poultry especially chicken meat is an important part of the daily diet and accounts for 75% of the national demand for meat in Bangladesh (IRIN 2014). But recent reports revealed that these food items are to some unsafe as chickens are regularly and extensively exposed to various toxic chemicals, heavy metals, antimicrobial and some other drugs (The Daily Star 2014, Hossain and Hasan 2014) and some growth promoters/hormone derivatives. They found extremely higher levels of chromium in chickens. The excess amount of chromium can be transported from poultry feed to the human body leading to the carcinogenic effects. Some earlier findings indicated that presence of antibiotic residues in eggs and chicken were far beyond the acceptable limit (cited in The Daily Star 2014). These might contribute to the increasing trend in antibiotic resistance found among the people of Bangladesh. Continuous ingestion of higher level of antimicrobial drugs through animal food origin is contributing to the antimicrobial resistance problem by creating a reservoir of resistance bacteria (Bailar and Travers 2002 and O'Connor *et al*. 2002) constituting health risks to the consumers. In fact, most of the chemicals and veterinary drug residues in animal foods may have some noxious effects on human health. Croubels *et al*. (2004) reported that some of these residues may exert genotoxic, immunotoxin, carcinogenic or endocrine effects in human body.

Use of health-hazard materials in livestock and poultry products in the recent years are not been controlled in Bangladesh. This could be due to weak business policy, lack of awareness of producers, excessive dependency on non-technical traders, carelessness of some technical personnel, inadequate policy guidelines and lack of strong monitoring of law enforcing agencies, etc. Antibiotics reducing chick mortality may pose serious health risks to consumers. The nation is claimed under threat as chicken meat and egg are mostly consumed and the cheapest source of animal protein (Hossain and Hasan 2014). Human especially children consuming poultry meat and egg are claimed to become fattened and gained unusual growth that could be due to residual effects of antibiotic and/or growth promoters.

HACCP is a system of extensive evaluation and control over an entire food production process for the sole purpose of reducing potential food-related health risks to consumers. It is a world-wide recognized systematic and preventive approach that addresses biological, chemical and physical hazards through anticipation and prevention, rather than through end-product inspection and testing and thereby reducing the food-borne illness (Gandhi 2009). The hazards associated with poultry products may differ in various regions of the world (Tompkin 1994) because of the differences in environment and practices in different areas. Hoque *et al*. (1997) found a 10%

prevalence of Salmonella in commercial poultry farms. The country has animal food safety regulation those are rather theoretical than practice.

Under these circumstances, poultry feeds and poultry products needed to be analysed. Efforts are given to identify the extent and steps of use of the hazardous materials, chemicals and their derivatives, and microbial contamination in poultry feed and their residual effects in raw and value added poultry products. Critical evaluation is done on HACCP practices on poultry origin food chains. Moreover, nutritive composition of the commercial poultry diets was also worthwhile to be investigated whether the farmers were misguided by the feed manufacturers. Earlier reports indicate that nutritional composition of poultry feed significantly affect growth performances of broilers (Kamran *et al.* 2008, Dairo *et al.* 2010) and layers (Gunawardana *et al.* 2008, Li *et al.* 2013 and Nahashon *et al.* 2007). The present study would help to formulate a guideline to the policy makers and law-enforcing agencies to minimize or stop these unethical practices concerning safe food production as well as building healthy nation.

6.2 Potential risk factors in raw materials and poultry products

Three types of food-borne risk factors for human health can be summarized as follows:

6.2.1 Physical factors

Physical hazards are objects which are not in true sense a part of food, never was meant to be food, but somehow got into the food chain. These may include utensils/equipment for processing in wet market, pieces of glass or utensils, toothpicks, cigarette butts, pebbles, hair, staples, jewellery. These elements may cause injury.

6.2.2 Chemical factors

Chemical products or contaminants can be of different nature e.g. residues of pesticides or other phyto-products applied, veterinary medications, growth promoters and disinfectants in animal production, or disinfectants used in the food-processing industry, environmental contaminants such as heavy metals or dioxins. It is claimed that a few producers used hormones or their derivatives in poultry production causing adverse effects on human health.

6.2.3 Biological factor

Biological hazards are disease-causing bacteria, viruses, parasites, molds, yeasts, and naturally occurring toxins. The transmission of human diseases through food, water and wastewater is a global problem, particularly of developing countries where gastrointestinal diseases are one of the most important causes of morbidity and mortality and food habits adopted by populations may mitigate or increase the hazards (WHO 1976). The first group of risk factors comprises microbiological factors such as *E. coli* and *Salmonella spp*.

7. Sub-project general objective

To identifying the health-hazards materials in poultry industry and their effects on nutritional quality of poultry and poultry products and to find out the ways of producing poultry and poultry products for safe human consumption and mitigating malnutrition.

8. Sub-project specific objectives (component wise)

8.1 Coordination Component: BARC

Under the principal objective of the NATP, the coordination unit of the BARC sub-project had the following specific objectives to achieve the sub-project goal:

- To quantify the caffeine concentration of energy drink and pulp concentration of commercially available fruit juices;
- To determine the contaminants of some selected street foods;
- To quantify the salt concentration of selective processed foods and iodine concentration of iodized salts;
- Co-ordination and monitoring of the component project activities to facilitate smooth implementation.
- Organize disseminating training workshop/seminar and capacity building on food safety and quality.
- Development of communication materials for wide awareness building to the consumers.

8.2 Component – 1: PSTU

i) To identify various hazards in poultry feeds and their effects in egg production performances and hazardous residues in eggs,
ii) To assess the chemical and biological contaminants in raw and value added poultry products at market chain, and
iii) To assess the implementation of HACCP standard in feed and food production from poultry industry.

8.3 Component – 2: HSTU

i) To identify and quantify the effects of various hazards in broiler performances and hazardous residues in broiler meat, and
ii) To assess the implementation of HACCP standard in feed and food production from poultry industry.

9. Implementing location(s)

The coordination unit monitored all activities from the respective unit office at BARC. The sub-project research of PSTU and HSTU components was implemented in the geographical locations of Bangladesh including Uzirpur and Babuganj Upazilas of Barishal, Sadar and Parbatipur of Dinajpur and Savar-Dhamrai of Dhaka. In addition, Rajshahi, Khulna, Gazipur and Chattragram were also included for required baseline data and related information and poultry feed and food samples.

10. Methodologies in Brief (*Combined*)

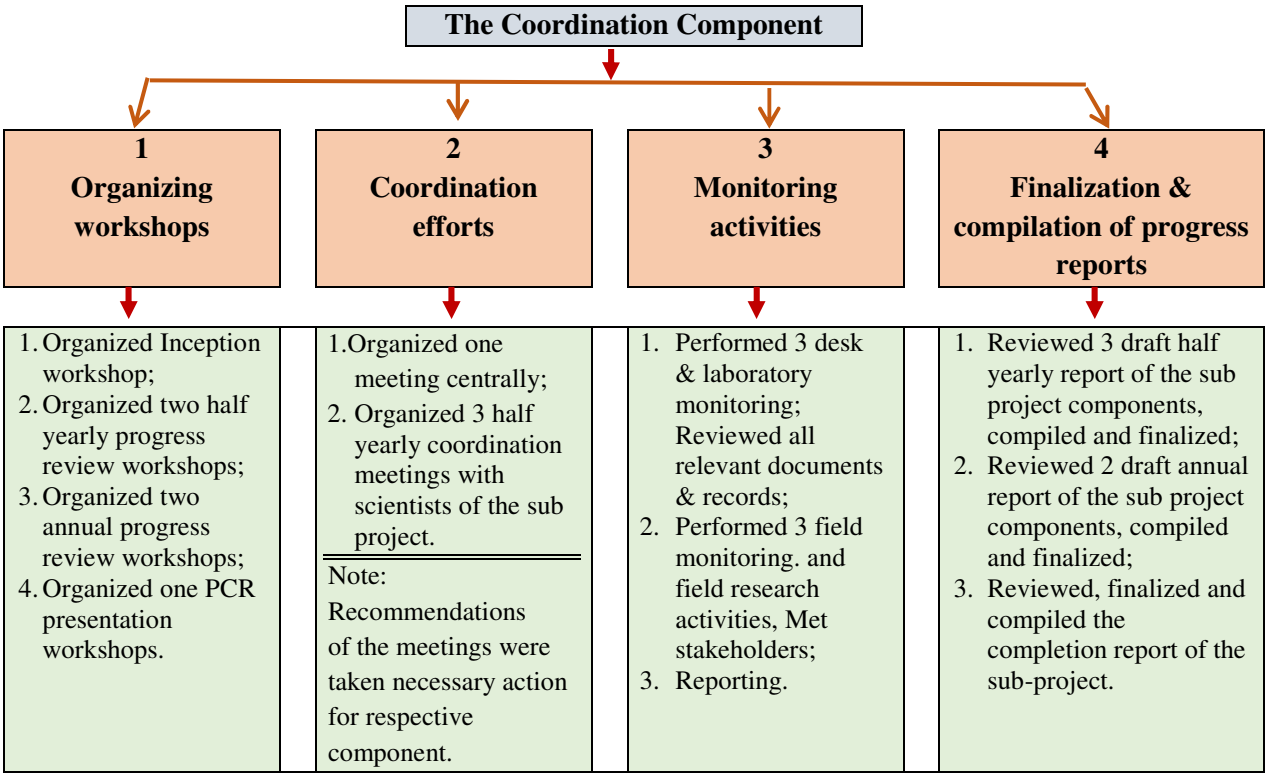
10.1 Approach

10.1.1. Implementation approach for the Coordination Component

The Coordination component as the responsible unit of the sub project to initiate all potential efforts in the process of implementation of each component under the sub project so that the general objectives and goal of the sub project can be achieved through smooth and successful completion of each of the specific objectives as per activity time plan of the project document. To ensure that, the Coordination component, taken into consideration its own activity and objectives and duration of the sub project, thus accordingly designed its own plan of activity (approach) for the proposed period. Followings are the major activities carried out by the Coordination component under the plan:

a. Organizing seminars/workshops.
b. Monitoring the sub-project activities (specifically financial and research activities);
c. Coordination activities within the component of the sub-projects.
d. Review and compilation of half yearly and annual research progress reports.

The implementation approach and activities there under for the Coordination component of the sub-project shown in the following diagram:



Recommendations of the inception, half yearly and annual research progress review workshops and different coordination meetings are furnished hereunder in **Appendices- BARC: A - D.**

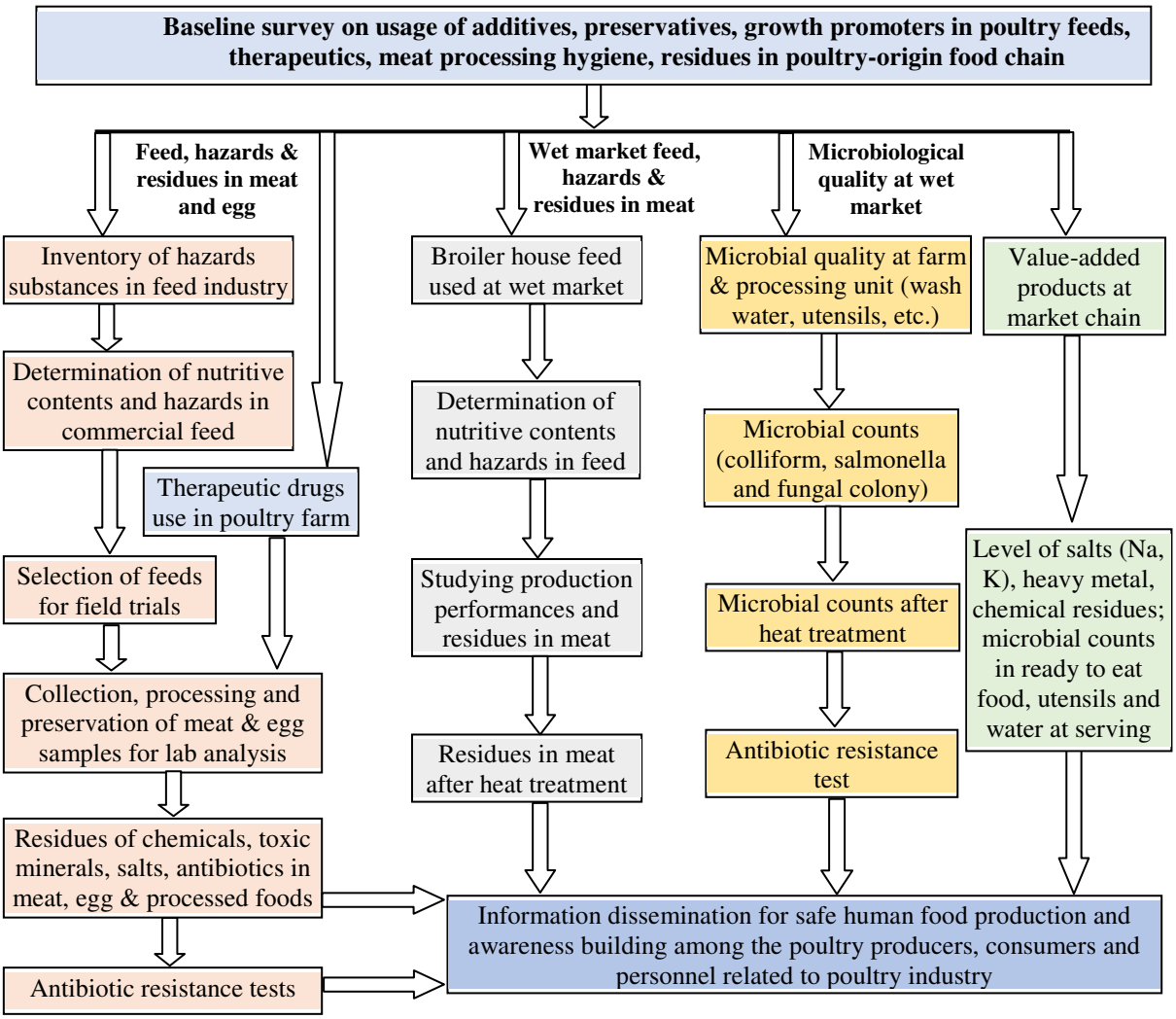
Following table represents the summary statement of achievements performed by the Coordination component of the sub project:

Summary statement of achievements		
Name of activities	**Performance against each activity**	**Remark**
Inception workshop	Organized centrally at BARC in November' 2018	Attended all PI, Co-PI & expert members.
Revision of PP	Done as per recommendations of Inception Workshop	
H-Y-prog. Review Workshop (Date)	Organized centrally at BARC in March' 2019, January'2020.	Attended all PI, Co-PI & expert members
Ann. Prog. review Workshop (Date)	Organized centrally at BARC in July' 2019 & in September' 2020	Attended all PI, Co-PI & expert members.
Coordination meeting (No)	03 07.02.19, 19.10.19 & 25.06.20	One Coordination meeting held centrally.
Monitoring of field and Lab activities	04 (PSTU & HSTU)	Covered all components under sub-project.
Financial achievement (Up to May'21)	100% of released money & 99.7% of total approved budget	Delay in proc. plan approval and Covid-19 incidence hampered desired progress
Reporting performance	Provided project inception reports, SoE, Half yearly and Annual compiled progress reports of all sub project components as per planned time frame.	Major reports are: Inception report (1 no); Compiled half yearly progress report (3 no); Compiled annual progress report (2 no); Monitoring reports (3 no);

Pictorial views of different workshops, coordination meetings and field monitoring activities

10.1.2 Implementation approach for the research components

The sub-project components conducted the research in the respective site based on the approaches presented in the underlined schematic diagram. Farmers were aware about the safe poultry meat and egg production through awareness meetings.



Value addition is the process of increasing the economic value of a product or service through special processing, marketing or manufacturing. Value addition is often driven by the need to offer more convenience to the customer. A number of value-added meat products chicken cutters, burger, lollipop, kabab, roll, fried cut-parts, cutlet, sausage, meatballs, nuggets, strips, treasure, chicken popcorn, etc. as well as egg products viz. pancake, egg patties, egg pizza, egg roll, egg strips, egg cutlet, brined and pickled eggs are now available in Bangladesh market. We already prepared fried and heat-treated chicken for laboratory analysis. Similarly, some commercially available value-added products are also taken into account for determining hazards through laboratory analysis.

10.2 Research Methodology

10.2.1 Coordination component

Because of Covid-19 Pandemic lock down, the research target of the coordination component could not achieve as per activity plan. As all most all the objectives are related with lab based analytical activities, so that, most of the analytical works of the samples in different accredited labs could not completed, because travelling to different corners of the country for collection of research samples, shipment of samples to different accredited labs was totally stopped for about 18 months. However, before non-pandemic situation, the coordination unit completed its research works as per set objectives on Caffeine concentrations of energy drinks, Qualitative analysis of street food, Sodium cyclamate in white sugar and analysis of milk samples for antibiotics, sulphur drugs and heavy metals concentration. Brief description of the methodologies of the research completed so far has been presented as follows:

10.2.1.1 Caffeine concentrations in energy drink samples

A total of 10 (ten) commercially marketed available energy drink sample (including two imported samples) were collected from the retail shops of various locality of Dhaka city (Table 1). Special attention was given for production and expiry date of the products to ascertain desired test result of the samples. The samples were than packed in required amount (250 ml for each sample) in PET bottle with laboratory code of identification. Samples were analysed for their caffeine concentration in triplicate as per SO-IN-MUL-TE-019 by HPLC with the technical assistance of SGS Bangladesh Limited.

Table 1. List of energy drink samples (with code) collected from the local shops

Sl. No	Code name of the energy drink	Sl. No	Code name of the energy drink
1.	ED-1	6.	ED-6
2.	ED-2	7.	ED-7*
3.	ED-3	8.	ED-8
4.	ED-4	9.	ED-9
5.	ED-5*	10.	ED-10

*Imported energy drinks

10.2.1.2 Qualitative analysis of street food

Most commonly used 14 freshly prepared street food samples were collected from 11 different public rush business areas of Dhaka city. Initially 200 gm/ml of samples were preserved in sterilized plastic bags at low temperature (around 0 ^{o}C to inhibit growth) for further analysis. Culture and plate count of the samples for total coliform, E. coli, S. aureus and Salmonella sp. were done following the standard methods in the pathological laboratory of SCG Bangladesh. Types of street food collected and location of collection are shown in Table 2.

Table 2 . Place of collection and list of street foods

Collection Point	Name of Food samples/ items
Jatrabari, Khilgaon, New Market, SadarGhat, Hatirjhil, Karwan bazaar, Dhanmondi, DU, Gabtoli, Mirpur, Mohammadpur	Sugarcane juice
	Jhal muri
	Panipuri
	Vellpuri
	Chotpoti
	Noodles
	Fruit-Drink
	Mixed Fruit-mash (tetul, green banana, jolpai, coriander, spice)
	Fruit-mash (Jambura)
	Fruit-mash(Kodbell)
	Lemon water
	Lemon Water (With Tang)
	Tetul water

10.2.1.3 Determination of Sodium cyclamate in white sugar

Readily available sixteen white sugar samples (branded and non-branded open sold) from different areas of markets/shops of Dhaka city were collected for Sodium cyclamate analysis (Table 7). All the samples were directly collected examining the production and expiry date after the commodity to ascertain their freshness level. An amount of 250 g of sugar sample (for each) after weighing separately preserved in air tight condition in three fresh poly bags and kept them all in room temperature until further analysis. Analysis of samples for detection of Sodium Cyclamate concentration was carried out by the SGS laboratory Singapore following standard analytical procedure.

10.2.1.4 Analysis of pasteurized and unpasteurized milk samples

A total of 16 boiled, pasteurized and unpasteurized milk samples of eight leading milk marketing companies of the country were collected from the local traders and agents. Collected samples were analysed for leftover residue level of antibiotics, sulphur drugs and heavy metals (Table 3). Analyses of all the milk samples were carried out in the accredited laboratories of SGS Bangladesh. Parameters studied under each broad area (antibiotic, sulphur drug and heavy metals) are categorically shown in Table 3.

Table 3. Parameters analysed under different study areas of milk samples

Sl	Study area	Parameters
01.	Antibiotics	Tetracycline, Chlortetracycline, Oxytetracycline,Epitetreacycline, Epichlortetracycline, Epioxytetracycline, Enrofloxacin, Ciprofloxin, Chloramphenicol, Streptomycin
02.	Sulphur drug	Sulfamethazine, Sulfamerazine, Sulfadiazine, Sulfapyridine, Sulfadimethoxine, Sulfathiazole, Sulfamethoxazole, Sulfamethoxazole, Sulfamethiazole, Sulphamethoxypyridazine, Sulphanilamide, Sulphadoxine, Trimethoprim,
03.	Heavy metals	Lead and Chromium

10.2.2. Detailed survey on poultry feed and usages of hazardous materials including antibiotics, additives/growth promoters in poultry industry, and also on value added poultry products at market

Poultry provides the cheapest protein to fulfill the demand of fastest growing population of world. In Bangladesh, consumption of poultry and its product have been increasing for last few decades. Recent report stated that chicken meat accounts for 75 percent of the national demand for meat in Bangladesh (IRIN 2014). Thus poultry production grows rapidly all over the country. As a consequence, different types of hazardous adulterant and contaminant in different stages of production, processing, and marketing chain are being practiced specially in the less developed countries. Chickens are regularly and extensively exposed to various toxic chemicals, antimicrobial and other drugs and various growth promoters/hormone derivatives. This is due to weak business policy, lack of awareness of producers, excessive dependency on non-technical traders, carelessness of some technical personnel, inadequate policy guidelines and lack of strong monitoring of law enforcing agencies, etc. To control over this situation, overall production, marketing and treatment system need to be evaluated. Under these circumstances, the present study was undertaken to: i) investigate present state of poultry feed production in Bangladesh, ii) know the usages of antimicrobial agents, biological and/or chemical growth promoters and health-hazard materials in poultry industry, if any, and iii) make an inventory on available value added poultry products.

Plate 1. Interviewing under survey activities.

A cross-sectional study was conducted among the farmers, feed and chick dealers, medicine seller and practitioners in different areas Bangladesh. A total of 303 farmers and 90 dealers, 85 medicine sellers and 45 practitioners were interviewed to collect data. All participants aged minimum 18 years agreed to participate in the study were included. Pre-tested and structured self-administered interview questionnaires were used in order to collect data by trained interviewers (research staff). First, the questionnaire was prepared in English and then translated to Bengali (local language) and translated back to English again to maintain its consistency. To ensure the quality of data, a pre-test was done and modifications were made accordingly. Sociodemographic characteristics of participants, farm details activities on marketing (feed, chicks), details of antibiotics and other medicines were collected. Some relevant information was

also collected from feed industry personnel, company field officers, and processed food outlets. Some recent data sources were also reviewed. Based on the previous literature and personal interview of the technical personnel of feed industry, we selected some compound feeds and decided to analyze them to quantify certain selected chemicals (antibiotics, anticoccidials, antifungal drugs, etc.) and heavy metals. Feeds contaminated by any of these chemicals were brought into account for further biological experimentations. We were planned to analyze the meat and eggs to observe residual effects of chemicals at the end of experimentation.

10.2.3. Determination of composition and hazardous materials in layer and broiler feeds available in Bangladesh

Poultry sector, in Bangladesh, provides a large portion of dietary protein, income and employment. But, this business faces huge challenges because of unstable market and unplanned marketing systems, fluctuation and scarcity of available raw materials, unethical practices in feed and chick marketing, week control of government regulatory authorities, rumor, etc. However, feed quality of is the main determinants for successful poultry business. Sometimes farmers are cheated by the ill-motives of feed and chick manufacturers. In addition, rumors on poultry feed, meat and eggs are prevailing among the people. Ultimately these factors affected human consumption. In these courses some small farmers become totally robbed. Based on the existing scenario, the present study was designed to evaluate commercial broiler and layer feed at various levels a) to assess the nutritive composition of available layer and broiler feeds in the market, and b) to identify the hazardous materials in feeds.

a. Feed sample collection and their processing

Commercial broiler and layer feeds manufactured by large, medium and small companies were collected from field/farm for laboratory analysis. Firstly, eight commercial broiler, eight layer feeds and three house feed samples were collected from Barishal, Dinajpur and Savar based on the availability. Samples were packed, labelled and subjected to laboratory analysis for toxic elements, nutrient component and antimicrobial drugs following standard procedures as described below from SGS India Ltd.

Table 4. Parameters of broiler and layer feed samples analysed and method of analysis

Parameter	Method of analysis
Lincomycin, Maduramycin, Ciprofloxacin, Tylosin, Enrofloxacin, Neomycin	SO-IN-MUL-TE-006
Oxutetracycline, Clortetracycline	SO-IN-MUL-TE-001
Heavy metals (Cr, Pb, As)	SO-IN-MUL-TE-063A by ICP-MS
Crude protein	AOAC 2001-11
Ca/P	SO-IN-MUL-TE-063 by ICPOES

Analysing laboratory finding, five broiler, five layer and two market/house feeds were selected for biological trials in both locations (Dinajpur and Barishal) as per planned project activities. Another lot of selected five broiler, five layer and two market/house feed samples were sent for laboratory analysis to detect some other selected chemical parameters. These chemical substances were worthwhile to quantify in the feeds.

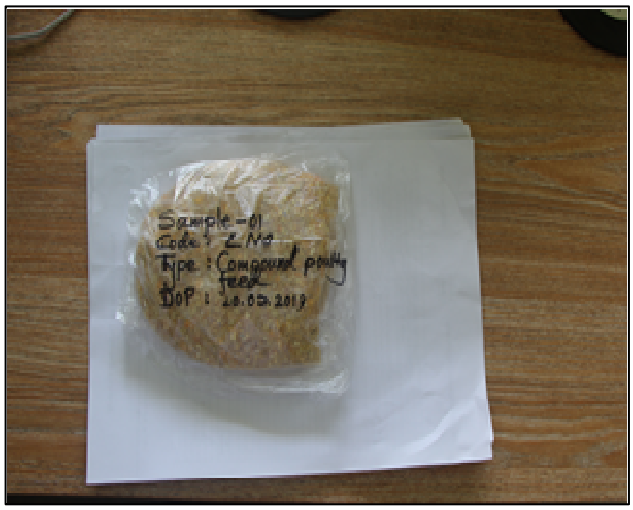



Plate 2. Collection and processing of feed samples.

b. Quantification of elements and data analysis

Analyses were done to assess the nutritional components (CP, Ca and P) and to identify the level of various hazardous chemicals substances having immediate or long-term human health threats. Analysed data were tabulated for interpretation.

10.2.4. Growth and production performances of pullets and egg quality characteristics fed commercial poultry diets

Manipulation of feeding regimes and diets are important considerations in improving egg production. Faulty feed and feeding methods are sometimes responsible for reduced egg production performances of commercial layers. Feed production ethics and quality control assurance is being implemented strictly in developed countries. This is almost absent in Bangladesh although some regulations are in the government paper book. Singh *et al*. (2019) reported that poor quality feeds sometimes results in high mortalities, low productivity, and low product quality which lead to heavy losses. Unlimited feeding in pullets and laying hens lead to deposition of abdominal fat resulting heat stress, and acquire early maturity causing reduced egg size, higher mortality and lowered persistency of production. Therefore, limited feeding in the rearing period often yields benefits in the laying period concerning egg size, more sustained laying ability and lower mortality. Limited feeding also reduces rearing costs. This study was conducted to know the effects of selected commercial layer feeds on growth and egg production performances of pullets, and also on external and internal egg quality.

a. Birds, feed and management

Twenty Hy-line brown pullets of 15-week age were included in a replicate under each dietary treatment. Purposively five commercial feeds were involved in each trial. Weekly birds' weight, daily feed intake, daily egg production, egg weight and mortality for each replicate were recorded. A total of 10 birds from each replicate group were randomly weighed on weekend. External and internal egg quality characteristics were also assessed.

Plate 3. Birds & feed management activities.

Birds were provided with recommended management practices including vaccination and medication. Each trial was conducted in three different seasons of the year (summer, rainy and winter) to assess seasonal variations. The same study was simultaneously conducted at Barisal and Dinajpur sites to validate performances in two geographical locations.

b. Layout and experimental design

Factor 1 : 02 (Barishal and Dinajpur)
Factor 2 : 05 (Commercial feed)
Factor 3 : 03 (Summer, Rainy and Winter)
Replication : 3 (each)
Design : 3-Factor CRD

c. Egg sample collection, data compilation and analysis

Egg samples were collected from different treatment groups for further laboratory analysis. Recorded data on per bird weekly weight gain and feed intake, egg production, survivability, birds' health issues and egg quality characteristics were subjected to analyses for interpretation using GENESTAT statistical package (Lawes Agricultural Trust 1997).

Plate 4. Collection & laboratory analysis of eggs.

10.2.5. Meat yield and its quality of broilers fed compound broiler diets

Over the years, the demand for poultry products in Bangladesh has grown significantly; per capita consumption per year increased to 8.5 kg poultry meat and 5.1 kg (104 pieces) eggs in 2019 (Anonymous 2020b). People are now becoming more involved in this sector and taking it as a business. The sector is now nourishing some big private investors, although it is still very small. Farmers are mostly dependent on commercial feeds and therefore feed production is increasing day by day to meet the growing demand. Based on internal estimates, current demand for poultry feed has been estimated to be 5.08 Million MT/year (based on CAGR 10% growth) (Fuad 2017). Feed industry has registered an around 25% expansion in the last decade, driven by a surge in poultry farming as well as fish and cattle farming. In that year, poultry feed production was 4.45 million tones, fish feed 1.59 million tones and cattle feed was 0.53 million tones (https://www.tbsnews.net/economy/industry/feed-thrives-poultry-grows-204553). Feed quality influences poultry productivity as well as health. Sometimes nutritional qualities of commercial feed are overlooked by some feed manufacturers by taking the advantages of loose monitoring and control in Bangladesh. This ultimately creates huge losses in production. Some feed manufacturers are not so serious about hazardous substances in compound feed. Many of these hazardous substances have proven detrimental to human health. Considering above fact, the study was designed to (i) to measure the effects of feed on the performances of broilers, and (ii) to assess the residual effects of chemical and other hazards on meat.

a. Feed, birds and management

Five commercial broiler feeds selected through screening (based on laboratory analyses) were used for experimentation. Each dietary group contained three replicates having 30 birds each. Day-old chicks were penned in a thoroughly cleaned housed in each season. Supplementary heat was provided up to 15-day age depending on the in-house temperature. Birds were duly vaccinated as per recommendations. Standard management practice was provided throughout the trial period. Medication was done when necessary. Each trial in a season lasted for 28-day in three different seasons of the year (summer, rainy and winter). Sometimes repeated trials were conducted to reach a valid conclusion.

Plate 5. Standard management practice for bird feeding &vaccination.

b. Layout of the experiment

Geographical location: 2 (Dinajpur and Barishal)
Feed : 5 (Commercial feed)
Seasons : 3 (summer, rainy, winter)
Replication : 3 (each)
Design : 3-factor CRD

A trial in each season in an experimental site was replicated in another research site to validate the performances.

10.2.5.1. Meat sample collection, data compilation and analysis

Broiler meat samples were collected from different pens in various seasons. These samples were processed and stored for laboratory analysis. Data on weekly weight, feed consumption, mortality and health issues were recorded. Body weight gain, feed intake, feed conversion and survivability were calculated. Data were analysed using Genstat statistical package (Lawes Agricultural Trust 1997).

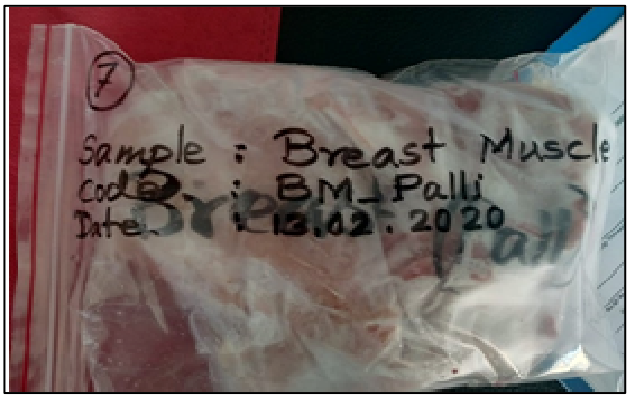


Plate 6. Meat sample analysis.

10.2.5.2. Performances of various hybrids (Sonali) for meat production

As a global effort, poultry sector is an important tool to overcome malnutrition and poverty in developing countries. Poultry often represents a farmer's first investment as a way of increasing income and emerging from the poverty trap. Between the 1960s and the 1980s various agencies made several attempts to improve local poultry through cockerel exchange programmes and the distribution of hatching eggs. However, these initiatives met with limited success. During 19's, several projects including SLDP and the PLDP involved nearly one million women beneficiaries (Dolberg *et al.* 2002) and emphasized the rearing of cross-bred *Sonali* birds and encouraged other small-scale farmers in rural areas to become involved in the poultry sector. Now a day, some large DOC producing farms in Bangladesh are producing slow-growing meat type birds along with layer and broiler chicks. Considering consumers' preferences in rearing meat type *Sonali* birds, companies put tremendous efforts to improve the growth rate of this hybrids. The

present effort was directed towards assessing the performances of two different genotypes of *Sonali* birds under farmer's condition.

a. Bird genotype, diet and management

Two different genotypes were collected from local vendors namely Local Sonali and Kazi Gold. A total of 1000 chicks (600 Local Sonali and 400 Kazi Gold) in two different seasons (summer and rainy) were reared. Two types of feeds (viz. broiler and sonali grower) were tested for growth performances. Up to 21-day age all birds were commonly provided with standard broiler starter feed. Thereafter, each genotype was divided into two groups having three replications each. These two groups of birds were supplied with two different feeds– sonali grower and broiler grower. These two feeds were continued up to weight at marketing (i.e. ≥800g $bitd^{-1}$). All birds were vaccinated for Newcaste disease and Gumboro. Medication was done when necessary.

Plate 7. Feeding management & growth performance of two genotype birds

b. Data recording and analysis

Weekly body weight, feed intake, mortality and the costs of vaccination and medication were recorded. Data were subjected to ANOVA in factorial CRD using Genstat statistical package (Lawes Agricultural Trust 1997).

10.2.6. Effect of house feed on weight change of commercial broilers at different marketing ages

House feed (also called market feed) is a low protein diet used to feed broilers in the wet market. Some feed manufacturers in Bangladesh are producing this sort of feed offering comparatively lower price only for the sustenance of live birds specially broilers before slaughter/or live disposal at wet market. This feed is relatively popular among the retailers in wet market due to lower price. But, instead of using balanced diet it might cause loss of body weight. Growth performance and carcass composition become inferior to those of broiler chicks fed standard high-CP diets when dietary CP content is lowered by more than three to four percentage points (Ferguson *et al*. 1998; Aletor *et al*. 2000). Thus, it is generally not recommended to lower the

dietary CP content by more than about three percentage points (Kornegay and Verstegen 2001, Lewis 2001). In Bangladesh, live bird marketing is a popular practice at wet shop. Sometimes, retailers have to retain live broilers for 2-3 days in the shops with these sorts of house feeds. Some of the retailers use standard broiler diet also. Thus state of weight change of broilers in the wet shops fed different diets is needed to be investigated. The objective of the experiment reported herein was to explore the effects of using house feed on performances of broilers at market age before sacrifice.

a. Diet, bird and management

Two house feed samples were collected form the market and were analysed in an accredited laboratory (SGS India Ltd.). Along with these two, a standard broiler diet was also included to compare the performances. Birds' performances were measured at two harvesting ages i.e. at 28-day and 35-day of age. A total of 270 birds were allocated to three dietary treatment groups having three replications each with 15 birds. The study was replicated at two different geographical locations (Northern and Southern area) and in three different seasons (summer, rainy and winter) of a year. *Ad libitum* feed and clean drinking water was supplied to all the birds throughout the trial period. Standard management was provided for all the birds for a period of 6 days.


Plate 8. Comparing diet performance on birds growth.

a. Data recording and analysis

Daily body weight, feed consumption and mortality were recorded. Weight change, feed consumption and survivability were calculated. All recorded and calculated data were subjected to ANOVA in a 3 (feed)×2(harvesting age)×3(rearing seasons) factorial design in CRD using GENSTAT Package (Lawes Agricultural Trust, 1997).

10.2.7. Residual effects of chemical hazards and heavy metals in broiler meat and eggs

Consumption of poultry meat and processed products has increased for a variety of reasons like, lower price, easy to process, available, acceptable to all communities, etc. Although egg consumption diminished remarkable in the last period of the twentieth century due to its cholesterol content, recent advances in research provides more evidence on the positive health

effects increasing the consumption trades (Reyes-Herrera and Donoghue 2012). Food itself contains chemical and natural compounds with hazardous properties as the main source of nutrients. Among these, the most important ones are the chemical residues including veterinary drugs, pesticides, and dioxins (Ayhan Filazi *et al*. 2017). All food products are at risk of contamination from several resources, and poultry meat and products are no exemptions. Drug residues can be prevented by not using the substance in animal production, where legal monitoring procedures are applied; meanwhile, these contaminants can be difficult to exempt completely due to the background level of pollution in the environment (Di Stefano and Avellone 2014, Andree *et al*. 2010). Now-a-days consumers are raising the question, "Is the agricultural products safe, no chemicals used?" It means that consumers are becoming aware of the quality and safetiness of raw agricultural produces. Although most consumers are mainly worried about the residues of veterinary drugs in their food, there are many more potential contaminants in the environment, which are more likely to contaminate the product from various resources. These include phthalates, persistent organic pollutants, various emerging toxic elements, and pesticides (Sireli *et al*. 2015). Some of these compounds in residual amounts in poultry meat and eggs have important deleterious effects and known to have genotoxic, immunotoxic, carcinogenic, teratogenic, or endocrine disrupting effects (Di Stefano and Avellone 2014). Analysis of relevant chemical substances is a major part of food safety programs to provide consumer safety and agreement with regulatory limits. Modern testing procedures can identify known chemical substances in complex food matrices. Additionally, they can also help to reveal and determine new or unexpected emerging chemical substances (Filazi 2012). The present effort was to quantify potential hazardous threats as residues in poultry meat and eggs.

Samples of breast muscle, thigh muscle and liver from different dietary treatment groups were collected in various seasons. Similarly, egg samples are also collected. Besides dietary groups, a number of known meat and egg samples containing selective therapeutic drugs (known as health hazardous chemicals) were also collected. All these samples are packed and labeled for further laboratory analysis from SGS India Ltd.

10.2.8. Determination of microbial quality of poultry feed, inputs and raw and processed poultry products

The microbiological safety and quality of poultry feed, meat and eggs are equally important to producers, retailers and consumers, and both involve microbial contaminants on the processed product. Most of our poultry farmers are dependent on commercial feed and feed quality is of tremendous importance for profitable farming business. Chowdhuri *et al*. (2011) reported that poultry feeds can be contaminated with food borne pathogenic microorganisms during harvesting, processing, handling and marketing of the bagged feeds. Potential bacterial species like Bacillus, Salmonella, E. coli, Enterococcus, Canpylobacter, Clostridium and Lactobacillus identified in poultry feed have been shown to be of critical importance in tropical region like Bangladesh (Onyeze *et al*. 2013, Hossain *et al*. 2011, Nasrin *et al*. 2007). The consumption of highly nutritious and safe poultry meat has increased worldwide. In chicken, presence of total mesophilic count is an indicator of hygienic level, total coliform count and total faecal coliform count are indicator of faecal and environment contamination whereas total Staphylococci count and S. aureus count are indicator of poor hygienic, handling and temperature control condition

(González-Fandos and Dominguez 2006, Rindhe *et al*. 2008). Presence of food poisoning bacteria in meat is an important public health issue (Mbata 2005). Unfortunately, egg is also an ideal source of nutrients for proliferation of both spoilage and pathogenic contaminating microorganisms. Microorganisms like bacteria and fungi can evade the defense mechanism of eggs and penetrate inside the egg, thus increasing the risk of food-borne illnesses or product spoilage. Salmonella Enteritidis and Salmonella Typhimurium are the most frequent Salmonella serotypes found inside shell eggs that caused food poisoning (Tan *et al*. 2012). Based on the existing scenario prevailed at field level in poultry industry of Bangladesh, the present study was designed to evaluate microbial loads of different sources of poultry farm which negatively affect poultry productivity. The study was thus undertaken to assess the microbial load such as E. coli and Salmonella spp. in selected layer and broiler feeds and other different sources of water.

Some biochemical tests like Catalase test, Methyl Red test, Vogs-Proskauer test, Citrate utilization test, Indole test, Starch Hydrolysis Test and Hydrogen Sulfide (H_2S) Production Test were performed to identify the bacterial isolates strains. These are summarized below.

a. Catalase test

The test was used to differentiate those bacteria that produce the enzyme catalase from catalase producing ones. Aerobes, facultative anaerobes, micro aerophiles can produce while the strict anaerobes are unable to produce the enzyme. The test was done by taking a pure colony by a sterile loop immersing it in 2-3 ml of the hydrogen peroxide in a petri dish. The production of bubbles indicated the positive result.

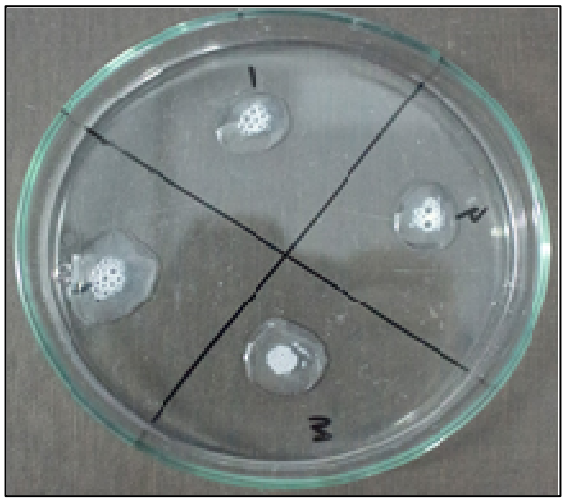


Plate 9. Catalase test: All bacterial samples showed positive results with bubble production

b. Methyl Red test

The test was performed by inoculating a colony of the test organism in 5ml of MR-VP medium. After 48 hour incubation at 37 ^{0}C, 5 drops of methyl red solution was added. The tubes were then shaken and examined. A distinct bright red colour indicated MR positive test and orange colour indicated MR negative.

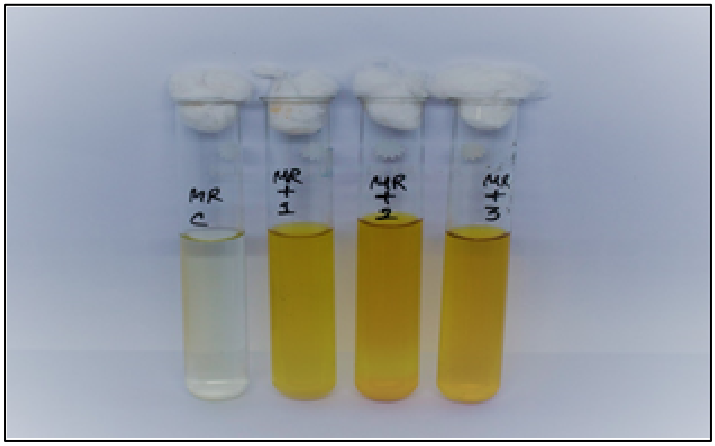


Plate 10. Methyl Red test: **(+)** Positive, (-) Negative, (c) Control,
(1) *E.coli,* (2) *Shigella,* (3) *Salmonella.*

C. Vogs-Proskauer test

The test was performed by inoculating a colony of the test organism in 5ml of MR-VP medium. After 48 hour incubation at 37 ^{0}C, 10 drops of Barritt's reagent A and immediately Barritt's reagent B was added and shacked 3-4 minutes. Development of a deep rose color in the culture 15 minutes following the addition of Barritt's reagent is represents a positive result. The absence of rose coloration is a negative result.

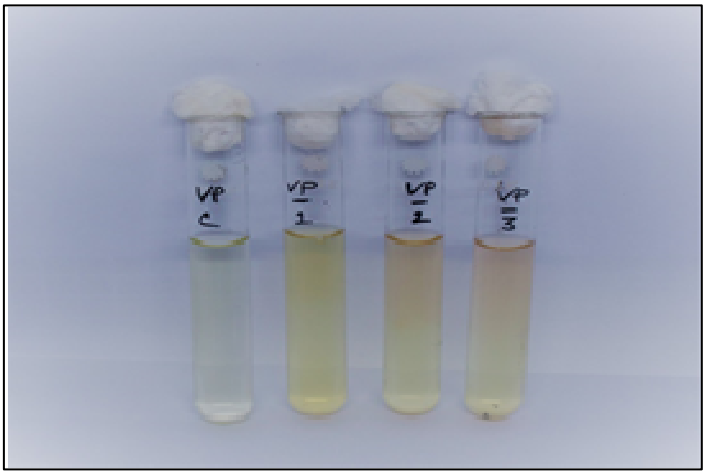


Plate 11. Voges-Proskauer test: (+) Positive, (-) Negative, (c) Control;
(1) *E. coli*, (2) *Shigella*, (3) *Salmonella.*

D. Citrate utilization test

This test was performed to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon. After incubation, citrate-positive cultures were

identified by the presence of growth on the surface of the slant, which was accompanied by blue coloration and citrate-negative cultures showed no growth, and the medium was remain green.

Plate 12. Citrate utilization test. **(-)** tube is negative, showing no growth on slant surface **(+)** tube is positive, showing growth on slant surface; (c) Control, (1) *E. coli*, (2) *Shigella*, (3) *Salmonella*

E. Indole test

This test was done to demonstrate the ability of certain organisms to break the amino acids trptophan to indole which was detected by a colorimetric reaction with kovacsindole reagent. In this test peptone water medium was used. Formation of rose purple colour indicated positive reaction and no colour formation indicated negative reaction.

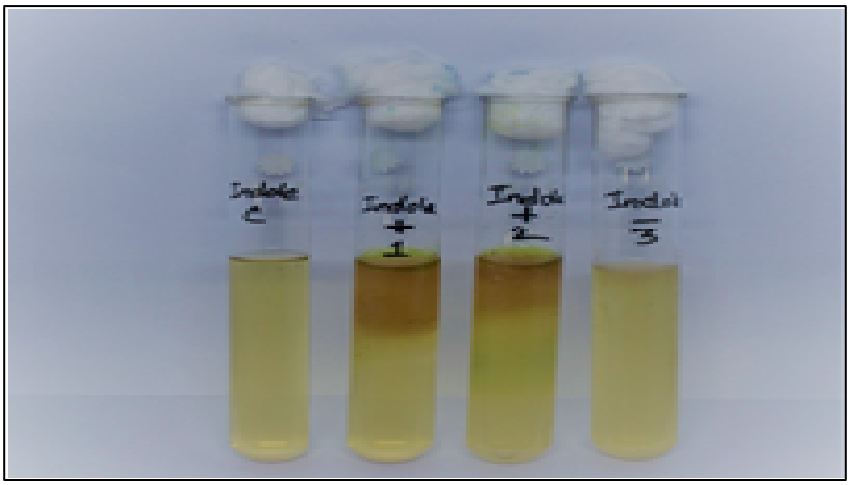


Plate 13. Indole production test: (+) Positive, (-Negative, (c) Control; (1) *E.coli*, (2) *Shigella*, (3) *Salmonella*.

F. Starch Hydrolysis Test

This test was used to determine the organism which was capable of breaking down starch into maltose through the activity of the extra-cellular α-amylase enzyme. It streaked the starch plate in the form of line across the width of the plate. Plates were Incubate at 37 °C for 48 hours and 2-3 drops of iodine solution were added directly onto the edge of colonies. Dark brown color around the bacterial growth indicates positive result.

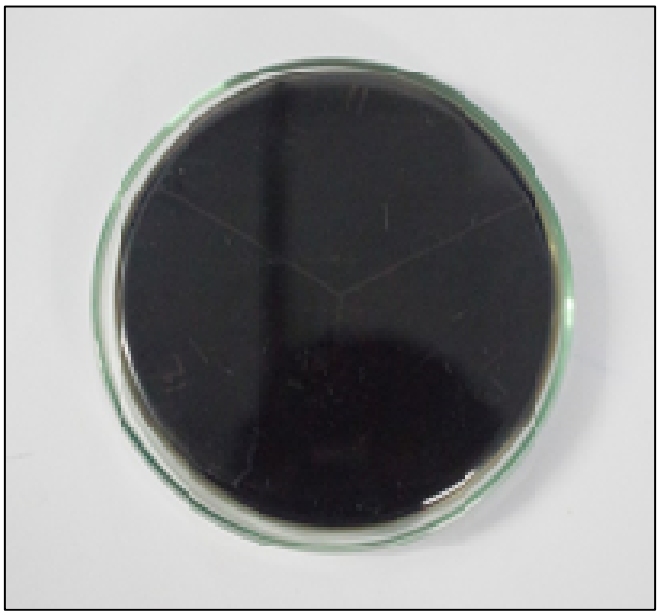


Plate 14. Starch hydrolysis test: A clear zone of hydrolysis was not appeared surrounding the growth of the organism. This is a negative result. All the bacteria showed negative result against starch hydrolysis test.

G. Hydrogen Sulfide (H_2S) production test

This test was used to determine whether the microbe reduces sulfur-containing compounds to sulfides during the process of metabolism. An inoculum from a pure culture was transferred aseptically to a sterile SIM medium. This medium contains ferrous ammonium sulfate and sodium thiosulfate, which together serve as indicators for the production of hydrogen sulfide. The inoculated tubes were incubated at 37 ^{0}C for 48 hours. Ferrous ammonium sulfate in the medium serves as an indicator by formation of an insoluble black ferrous sulfide precipitate that is seen along the line of the stab inoculation and is indicative of H_2S production. Absence of the precipitate is evidence of a negative reaction.

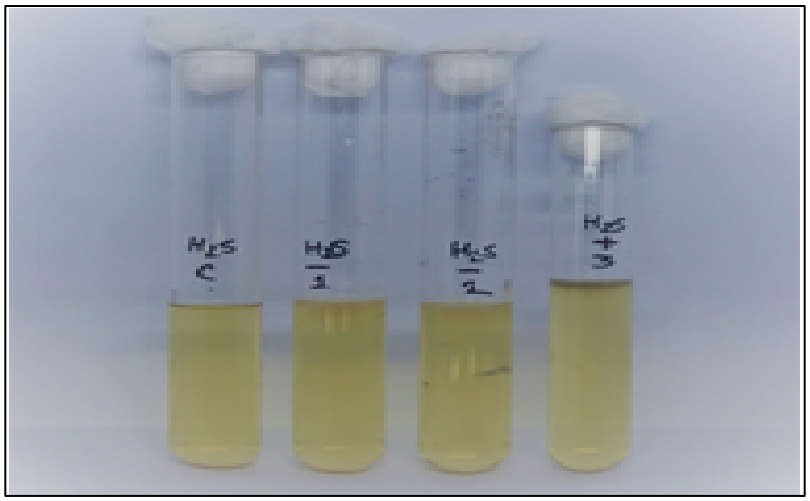


Plate 15. Hydrogen sulfide production test: **(+)** Positive, (-) Negative, (c) Control; (1) *E. coli*, (2) *Shigella*, (3) *Salmonella*

10.2.9. Assessing the implementation of HACCP in poultry feed and poultry origin food production chain

HACCP is a system of extensive evaluation and control over an entire food production process for reducing potential food-related health risks to consumers. An HACCP program maintains safety and wholesomeness of meat, egg as well as poultry because potential hazards that may occur during processing are anticipated, evaluated, controlled and prevented. Rather than monitoring isolated processing steps, an HACCP approach controls the entire production process as an integrated system. Although HACCP provides insurance that poultry is safe, there is no way to completely eliminate all hazards. HACCP plan is based on seven principles. The key to the success of HACCP is employee training, behavior and attitude. However, the study was undertaken to assess the state of implementation of HACCP in poultry industry.

Safety levels were assessed at feed industry, rearing units, processing plant and finished products. Physical observation and microbiological and other laboratory analysis results were the basis of this assessment. Determination of hazards was done in the following arena.

i) Analysis of feed for antimicrobial drugs, heavy metals and microbial colony;
ii) analysis of chick boxes, litter, thin paper, and soil for microbial count;
iii) analysis of drinking water at farm and dressing water at wet shop for microbial loads;
iv) analysis of house feed for antibiotic and heavy metals;
v) analysis of broiler meat for antibiotic, heavy metals and microbial count; and
vi) analysis of eggs for antibiotic and heavy metals.

10.2.10. Organizing awareness meeting at dealers' point

Awareness meeting on technologies of growing commercial chicken for safe meat and eggs production were arranged at dealers' points of Babuganj, Uzirpur, and Gournadi upazils of Barishal district. A total of 20 poultry rearers were present in each gathering and therefore 80 farmers including 12 females were participated. All the gatherings were organized in collaboration with Kazi Farms Ltd. The keys to success in poultry rearing, the common mistakes during rearing, problems faced and their probable solutions, judicial use of antibiotics and nurtaceuticals in growing safe food for human consumption, etc. were thoroughly discussed and presented in each meeting. The recent advancements in poultry production and government's target were also discussed. Farmers were actively participated by raising questions throughout the gathering period.

Plate 16. Awareness meetings at dealers' point in different Upazilas.

11. Results and Discussion (*Combined*)

11.1 Contaminants in selected foods and drinks

11.1.1 Caffeine concentration in energy drinks

Caffeine concentration of eight local energy drink samples showed their presence within the limit of BDS (145/L) ranging from 75.55–116.05mg/Kg (Table 5). However, caffeine concentration was found much higher than the BDS level in case of both the imported fruit juice samples (Code 5 = 285.46 mg/kg and Code 7 = 293.29 mg/kg).

Table 5. Caffeine concentration in different local and imported energy drinks

Brand Name	Results (mg/L)			BDS standard/ Approved level
	BARC Test Result	Range	Company Label	
ED - 1	116.05	75.55 – 293.29	-	Maximum approved level 145mg/L * FDA have no MRL; EU recommended 400mg/L
ED - 2	113.23		-	
ED - 3	97.62		<145	
ED - 9	105.40			
ED - 8	103.86		-	
ED - 6	BLQ (LOQ:10)			
ED - 4	BLQ (LOQ:10)			
ED - 10	75.55		-	
Imported			-	
ED - 5	285.46		320	
ED - 7	293.29		320	

*Two imported energy drinks available in Bangladesh markets

Plate 17. Energy drink samples for laboratory analysis.

11.1.2 Bacterial load in street food samples

Bacterial load analysis for total *Coliform, E. coli, S. aureus* and *Salmonella* sp. of street food samples were found loaded heavily with the pathogens except *Salmonella* sp. (which was not recorded in any of the tested samples). For other three, bacterial load was within the acceptable range with the lowest concentration of *Coliform* and *E. coli* in lemon water (3.6 and 3.6 cell) which is probably due to acidic nature of the sample where survival possibility of bacteria is minimum (Table 6).

Table 6. Level of contamination in street food samples

Qualitative Results of Different Street Foods in Dhaka City					
Collection point	**Type of food samples/ items**	**Total coliform**	***E. coli***	***S. aureus***	***Salmonella* sp**
Jatrabari, Khilgaon, New Market, Sadar Ghat, Hatirjhil, Karwan bazaar, Dhanmondi, Dhaka University, Gabtoli, Mirpur, Mohammadpur	Sugarcane juice	>1100	>1100	< 3	absent
	Jhal muri	>1100	>1100	< 3	absent
	Panipuri	>1100	>1100	< 3	absent
	Vellpuri	>1100	>1100	< 3	absent
	Chotpoti	>1100	>1100	< 3	absent
	Noodles	>1100	>1100	< 3	absent
	Fruit-Drink	>1100	290	< 3	absent
	Mixed Fruit-mash (tetul, green banana, jolpai, coriander, spice	>1100	>1100	< 3	absent
	Fruit-mash (Jambura)	>1100	>1100	< 3	absent
	Fruit-mash(Kodbell)	>1100	>1100	< 3	absent
	Lemon water	3.6	3.6	< 3	absent
	Lemon Water (With Tang)	< 3	< 3	< 3	absent
	Tetul water	< 3	<3	< 3	absent

Plate 18. Street food samples.

11.1.3 Concentration of Sodium cyclamate in sugar samples

Sodium cyclamate is commonly known as Assugrin, Sweet' N Low and Sugar Twin cyclamate. The true brand name of the chemical is "Sucaryl" which is only produced and marketed in the 90 countries of the world by the Merisant Company of Chicago, USA. Sodium cyclamate as a hazardous chemical compound for human being health has been prohibited for human consumption in any form and at any concentration by the USFDA authority. However, its low price, easy availability and as a source of concentrated sweetening agent, its utilization has been continuously increasing particularly by the sugar manufacturing industries of the country with a view to earn maximum without considering health safety of the consumers. The analysis report of the sixteen sugar samples (branded and non-branded open sold) of Bangladesh retail markets revealed that 100% of the sugar samples are contaminated with Sodium cyclamate (<1.0 ppm). Highly alarming concentration of Sodium cyclamate was detected in the nonbranded open sold sugars samples collected from Kachukhet market of Dhaka (Table 7).

Table 7. List of sugar samples with Sodium cyclamate concentration

Sl. No	Name of sugar samples/producer	Collection place/markets	Conc. of Sodium Cyclamate (ppm)
1.	Number One	Motijheel	<1.0
2.	Sugarcane	RayerBazar, Puran Dhaka	<1.0
3	Teer	Dhaka Cantonment	<1.0
4.	Fresh	Gulsan 1	<1.0
5.	ACI	Badda	<1.0
6.	Eglo	Banani	<1.0
7.	SIS sugar cube	SIS On line provider	<1.0
8.	Desi Sugar(BSMC)	Mohakhali Kancha Bazar	<1.0
9.	Swapna	Uttara	<1.0
10.	Saoccha	Tongi	<1.0
11.	Refined sugar (open sold, NB)	Savar Price market	<1.0
12.	Health care	Baipal Kancha Bazar	<1.0
13.	Open sold sugar (NB)	Mirpur Kancha Bazar	<1.0
14.	Open sold sugar (NB)	Kachukhet market	<10.0
15.	Open sold sugar (NB)	Sadarghat	<1.0
16.	Open sold sugar (NB)	KawranBazar	<1.0

NB, Non-branded

11.1.4 Antibiotics, Sulphur drugs and heavy metals in milk samples

Pasteurized and unpasteurized milk samples of eight leading milk marketing companies of the country were collected and analysed for leftover residue level of antibiotics, Sulphur drugs and heavy metals. Analyses of parameters studied under each broad area are categorically

11.1.4.1 Antibiotics

Parameters include Tetracycline, Chlortetracycline, Oxytetracycline, 4-Epitetreacycline, 4-Epichlortetracycline, 4-Epioxytetracycline, Enrofloxacin, Ciprofloxin, Chloramphenicol, and Streptomycin.

Result

Left over/remaining antibiotic residue in pasteurized and unpasteurized milk samples were below detectable level (Table 8).

11.1.4.2 Sulphur drugs

Sulfamethazine, Sulfamerazine, Sulfadiazine, Sulfapyridine, Sulfadimethoxine, Sulfathiazole, Sulfamethoxazole, Sulfamethoxazole, Sulfamethiazole, Sulphamethoxypyridazine, Sulphanilamide, Sulphadoxine, and Trimethoprim were studied.

Result

Left over/remaining sulpher drug residue in pasteurized and unpasteurized milk sample were below detectable level in all cases (Table 9).

11.1.4.3 Heavy metals

Parameters studied were Lead and Chromium.

Result

Left over/remaining heavy metal (Pb, Cr) residue in pasteurized and unpasteurized milk sample shows below MRL in all the cases (Table 10).

Table 8. Left over/remaining antibiotic residue in pasteurized and unpasteurized milk sample

Sl.	Sample name	Tetracycline	Chlortetracycline	Oxytetracycline	4 Epitetracycline	4 Epichlortetracycline	4 Epioxytetracycline	Enrofloxacin	Ciprofloxin	Chloramphenicol	Streptomycin
1	Milk Vita (Pasteurized)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	<(Less)10
2	Milk Vita (Boiled)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
3	Pran (Pasteurized)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
4	Pran (Boiled)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	0.06	BDL (DL:5.0)
5	Arong (Pasteurized)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
6	Arong (Boiled)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
7	Farm Fresh (Pasteurized)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
8	Farm Fresh (Boiled)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
9	RD (Pasteurized)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
10	RD (Boiled)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
11	Igloo (Pasteurized)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
12	Igloo (Boiled)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
13	Savar Dairy (Pasteurized)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
14	Savar Dairy (Boiled)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
15	Unpasteurized Milk (Raw-Savar)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)
16	Unpasteurized (Boiled Milk)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:5)	ND (DL:1.0)	ND (DL:1.0)	ND (DL:0.05)	BDL (DL:5.0)

Table 9. Left over/remaining sulphur drug residue in pasteurized and unpasteurized milk sample

Sl	Sample name	Sulfa methazine	Sulfa merazine	Salfa diazine	Sulfa pyridine	Sulfa dimethoxine	Sulfa thiazole	Sulfa methoxazole	Sulpha chloropyridazine	Sulfa methiazole	Sulphamethoxy pyridazine	Sulpha nilamide	Sulpha doxine	Tri methoprim
1	Milk Vita (Pasteurized)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
2	Milk Vita (Boiled)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
3	Pran (Pasteurized)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
4	Pran (Boiled)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
5	Arong (Pasteurized)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
6	Arong (Boiled)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
7	Farm Fresh (Pasteurized)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
8	Farm Fresh (Boiled)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
9	RD (Pasteurized)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
10	RD (Boiled)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
11	Igloo (Pasteurized)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
12	Igloo (Boiled)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
13	Savar Dairy (Pasteurized) PM-Na-SV-13	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
14	Savar Dairy (Boiled)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
15	Unpasteurized Milk (Raw-Savar)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)
16	Unpasteurized (Boiled Milk)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)	ND (DL:5.0)

Table 10. Left over/remaining heavy metal (Pb, Cr) residue in pasteurized and unpasteurized milk sample

Sl. No.	Sample name and code	Lead (Pb)	Chromium (Cr)
1	Milk Vita (Pasteurized) PM-Na-MK-1	BDL (LOD:0.01)	BDL (LOD:0.05)
2	Milk Vita (Boiled) PM-Nb-MK-2	BDL (LOD:0.01)	BDL (LOD:0.05)
3	Pran (Pasteurized) PM-Na-NP-3	BDL (LOD:0.01)	BDL (LOD:0.05)
4	Pran (Boiled) PM-Nb-NP-4	BDL (LOD:0.01)	BDL (LOD:0.05)
5	Arong (Pasteurized) PM-Na-GA-5	BDL (LOD:0.01)	BDL (LOD:0.05)
6	Arong (Boiled) PM-Nb-GA-6	BDL (LOD:0.01)	BDL (LOD:0.05)
7	Farm Fresh (Pasteurized) PM-Na-FF-7	BDL (LOD:0.01)	BDL (LOD:0.05)
8	Farm Fresh (Boiled) PM-Nb-FF-8	BDL (LOD:0.01)	BDL (LOD:0.05)
9	RD (Pasteurized) PM-Na-DR-9	BDL (LOD:0.01)	BDL (LOD:0.05)
10	RD (Boiled) PM-Nb-DR-10	BDL (LOD:0.01)	BDL (LOD:0.05)
11	Igloo (Pasteurized) PM-Na-IO-11	BDL (LOD:0.01)	BDL (LOD:0.05)
12	Igloo (Boiled) PM-Nb-IO-12	BDL (LOD:0.01)	BDL (LOD:0.05)
13	Savar Dairy (Pasteurized) PM-Na-SV-13	BDL (LOD:0.01)	BDL (LOD:0.05)
14	Savar Dairy (Boiled) PM-Nb-SV-14	BDL (LOD:0.01)	BDL (LOD:0.05)
15	Unpasteurized Milk (Raw-Rajsan, Savar) PM-Na-ON-15	BDL (LOD:0.01)	BDL (LOD:0.05)
16	Unpasteurized (Boiled Milk) PM-Nb-ON-16	BDL (LOD:0.01)	BDL (LOD:0.05)

11.2. Status of poultry feed production and usages of hazardous materials including antibiotics, additives/growth promoters, and preservatives in poultry industry

a. Poultry and feed industry in Bangladesh

Commercial poultry, cattle and fish farms seem to be the most promising enterprises over the last two decades. Personal interviewing data indicate that about 60 lac people are directly involved with the industry for their livelihood. But Covid-19 affects these enterprises a lot, specially poultry and dairy enterprises. Rumours drastically lowed consumers' demand of meat and eggs and therefore a huge pressure is now on the chick and feed manufacturing companies. As a result, number of parents, DOCs, feed production was squeezed. Raw materials price in international market was also affected the production process specially feed production. This influences the retail price of feed at farmers' level. Primary interview data of DOC production is presented in Table 11.

Table 11. Commercial DOC production in Bangladesh

Type of chick (DOC)	2019 (figures are in crores)	2020 (figures are in crores)
Broiler	70.0	51.0
Layer	4.74	4.16
Sonali	35.0	31.20

Commercial feed production experienced almost 25% growth in last one decade (https://databd.co/profiles/industries/profile-animal-feed) due to the augmented protein demand, which results the launch of many commercial feed mills over the past couple of years. According to the Bangladesh Poultry Industries Central Council (BPICC), the number of registered feed mills in Bangladesh is 261 and 135-140 companies renewed licences from the Department of Livestock Services. Besides, more than 200 feed mills are unregistered (Islam and Ali 2021). In 2020, these feed companies produced 6.57 MMT commercial feed, of which poultry feed accounts for 4.45 MMT (38-40% broiler, 6-6.5% layer and 22-23% sonali), fish feed 1.59 MMT and cattle feed 0.53 MMT. This achievement in commercial feed production has hit a roadblock because of the Covid-19 pandemic. After Covid-19 outbreak, feed mills specially small ones compelled to squeeze or stop their production process. It is reported that only 70-80 feed mills are now in operation. It is estimated that about 4.2 lac MT/week (5.04 MMT/year) of feed are now producing in Bangladesh. A major portion of the unregistered feed mills are acting as toll manufacturers. In most cases, unregistered feed mills are producing low-quality feed and the reputation of the leading companies is suffering (Islam and Ali 2021). It was also found that top 10 feed mills produces about 80% of total commercial (branded) feed production in Bangladesh. Nearly 71% market share by top 15 feed mills was also noticed by Mitali Saha (2020). She also mentioned that market share of poultry feed was approximately 63% followed by fish feed (23%) and cattle feed (14%).

Interviewing some of the feed industry personnel revealed that feed industry people added certain chemicals viz. antibiotics (popularly said food grade antibiotics) to suppress organism growth as well as accelerate growth and anticoccidial agents during feed formulation.

a) Antibiotics (lincomycin, tylosin, oxytetracycline, cholotetracycline, neomycin, colistin etc.)
b) Anticoccidial drug (maduromycin, diclazuril, etc.)

b. Field survey

Age, education and income source of respondents

On an average all category of farmers were just over 35 years of age (Table 12). Maximum participants belonged to 31-40 years age group. Majority (34.7%) of farmers had above secondary level education, more than one-half (52.2%) of dealers passed secondary level and, 42.2% of drug sellers had above secondary education. As the interviewing was purposive, all practitioners had technical graduation. Majority of farmers (73.6%) and dealers (74.4%) stated that this business was the main income source, but 62.4% of drug sellers reported others source(s) as main income hub.

Table 12. Age, education and income source of participants

Parameter	Farmers (N=303)	Dealer (N=90)	Drug seller (N=85)	Practitioner (N-45)
Age of respondent				
Below or equal to 30	86 (28.4)	16 (17.8)	15 (17.6)	10 (22.2)
31-40	127 (41.9)	46 (51.1)	39 (45.9)	24 (53.3)
Above 40	90 (29.7)	28 (31.1)	31 (36.5)	11 (24.4)
Mean (STD)	**37.49 (9.70)**	**38.48 (7.54)**	**38.85 (9.01)**	**37.1 (10.0)**
Education				
Literate	15 (5)	10 (11.1)	4 (4.7)	
Primary	78 (25.7)	15 (16.7)	14 (16.5)	
Secondary	99 (32.7)	47 (52.2)	31 (36.6)	
Above secondary	105 (34.7)	18 (20)	36 (42.2)	45 (100)
Source of income				
Poultry or drug	223 (73.6)	67 (74.4)	32 (37.6)	
Others	78 (26.4)	23 (25.6)		

c. Profile of farmers and farms

More than one-half (54.5%) farmers reared layer, 40.0% broiler and only 3.6% reared sonali birds (Table 13). Over two-third (66.3%) farms were located in the rural areas. More than 37% farmers belonged to joint family. About one-half (51.2%) of the respondents run their business only for maximum six months at a time. This is true for broilers, sonali and other meat-type birds. Nearly one third (32) of respondents had a firm size of 500-1000 bird and 27.1% reared 2000 birds or more. Majority (58.7%) reared birds on floor, followed by cage (29.7%) and slat (11.9%). This is because layer birds are mostly reared in cages at 15-week onwards. Nearly one third (29%) farmers got the training on poultry farming and that is why they become looser as a result of poor technical knowhow.

Table 13. Profile of farms of farmers

Parameter	Frequency (%)
Types of farm	
Broilers	121 (39.9)
Layer	165 (54.5)
Cockerel	6 (2.0)
Others	11 (3.6)
Location of farm	
Urban	17 (5.6)
Peri-urban	85 (28.1)
Rural	201 (66.3)
Family size	
Single (less or 4)	98 (32.3)
Joint (above 4)	205 (37.7)
Duration of business	
6 months and below	155 (51.2)
One year	110 (36.3)
More than one year	38 (12.5)
Size of farm	
500	33 (10.9)
501-1000	97 (32.0)
1001-2000	91 (30.0)
Over 2000	82 (27.1)
Rearing method	
Case	90 (29.7)
Slat	38 (11.9)
Floor	177 (58.7)
Training received	
Yes	88 (29.0)
No	215 (71)

d. Business details of respondents

A large numbers (73.6%) of participants financially depend only on poultry business (Table 14). A greater majority (94.7%) of them collected chicks from dealer with average BDT 32.52 for broiler and BDT 48.58 for layer. This phenomenon is prevailing due to the policy of chick and feed manufacturing companies, i.e. all chicks and feeds must have to be sold through their authorized dealers. About 55% farmers bought chicks on credit and nearly one-half (46.3%) collected feed on credit. Procuring chicks and feed from respective dealers on cash-basis was about 28%. Therefore, farmers have to pay more BDT 150-200 per 50-kg bag. Almost all (97.4%) apply vaccines in their flocks following the dealer's instruction. Nearly one-half (45.5%) of farmers dispose their products (live birds and eggs) through dealer and only 27% sold directly to wholesale market.

Table 14. Business details of farmers

Parameter	Frequency (%)
Main income source	
Poultry business	223 (73.6)
Others	80 (26.4)
Chicks collection points	
Dealer	287 (94.7)
Others	16 (5.3)
Prize of chick (Mean+-STD)	
Broiler	32.52 (5.98)
Layer	48.58 (30.32)
Cockerel	17 (8.27)
Payment mode for chicks	
Cash	85 (28.1)
Lending	167 (55.1)
Mixed	51 (16.8)
Payment mode for feed	
Cash	35 (28.9)
Lending	56 (46.3)
Mixed	30 (24.8)
Use of vaccine	
Yes	295 (97.4)
No	8 (2.6)
Selling of birds	
Through dealer	138 (45.5)
To wholesale market	82 (27.1)
Mixed mode (others)	83 (27.4)

e. Medicines used and persons giving advice for medicine and other problems

Almost all the famers (92%) used antibiotics to combat variety of problems faced in their flock (Table 15). Poor biosecurity measure is the single major cause. Anticoccidials drugs were used by 78.1% farmers which are also related to improper biosecurity measures. Enzymes, probiotics, other growth promoters, herbal extracts, etc. were used by the farmers and the average percentage was one-half. Dealers are the sole decision makers for advising various drugs and other neutraceuticals products as they are the investors in case of credit-business. Dealers may ask for advice from a registered veterinarian form respective company, if necessary. Sometimes they also consult representatives of medicine companies. In most of the cases, a dealer decide himself in managing the disease and nutrition related problems prevailing in a flock. The present findings also support in favour of the statement; only about 45% problems were solved following the advice given by a technical person. Indiscriminant and unauthorised usage of drugs stand as a big issue leading drug resistance and threatening human health.

Table 15. Medicines usages and relevant advice

Use of medicine	Frequency (%)
Antibiotic	279 (92)
Anticoccidial	237 (78.1)
Enzyme	212 (69.9)
Probiotic	132 (43.4)
Growth promoter	151 (49.8)
Herbal medicine	128 (42.1)
Who solve the problem, when occurred?	
Dealer himself	80 (26.4)
Technical person	135 (44.6)
Own or others famers	88 (29)

f. Business details of dealers

According to dealers' statement, a majority (82.2%) of them sold their products (chick, feed, vaccine and medicine) on credit-basis (Table 16). Findings revealed that about 84% of them provide unethical technical (veterinary) service to the farmers. Dealers explained that famers often violate the terms and conditions in product disposing and credit turn over. In about 45% cases, farmers sell the product (live bird or egg) without concerning the dealers. Fluctuating market and unexpected low prices was another major factor (about 36%) hindering the business. Regarding antibiotics, ciprofloxacin (28.9%) was most sold item followed by amoxicillin (23.3%). Others antibiotics sold by dealers were oxytetracycline (26.6%), tylosin (13.3%), tilmicosin (15.5%), doxycycline (11.1%), and neomycine (6.66%).

Table 16. Details of business activities of dealers

Parameter	Frequency (%)
Mode of payment	
Case	3 (3.3)
Credit	74 (82.2)
Mixed	13 (14.4)
Type of support	
Vet service	76 (84.4)
Product disposal	7 (7.8)
No service	7 (7.8)
Problems of business	
Delayed payments by retailers	24 (26.6)
Violating terms and conditions by farmers	40 (44.4)
Low price of product	33 (36.6)
Name of antibiotic	
Amoxicillin	21 (23.3)
Doxycycline	10 (11.1)
Neomycin	6 (6.66)
Oxytetracycline	24 (26.6)
Ciprofloxacin	26 (28.9)
Tylosin	12 (13.3)
Tilmicosin	15 (15.5)

g. Drugs type, prescribers details reported by drug seller

Major drugs and pharmaceutical products sold were growth promoting products (98.8) and antibiotics (96.5%). Antococcidial drug (57.6%) was moderate sold items followed by enzyme (27.1%), herbal medicines (22.3%) and probiotics (18.8%). Registered vets' prescription presented in about 60% cases of collecting drugs. In about 32% cases, farmers choose some drugs/pharmaceutical products by intermingling the vets' advice. Only9.4% farmers bought the medicines solely by their own choice. Surprisingly, a majority (75.3%) of drug sellers sold medicine without prescription of vet doctors (Table 17).

Table 17. Drugs type, prescribers details reported by drug seller

Parameter	Frequency (%)
Name of medicine	
Antibiotic	82 (96.5)
Anticoccidial	49 (57.6)
Enzyme	23 (27.1)
Probiotic	16 (18.8)
Growth promoters	84 (98.8)
Herbal	19 (22.3)
Who advised medicine	
Registered physician	50 (58.8)
Own + physician	27 (31.8)
Others	8 (9.4)
Sale without prescription	
Yes	64 (75.3)
No	21 (24.7)

h. Practitioners' experience and areas of advice

More than one-half (51.1%) of practitioners had less than or equal to 5 years of experience in veterinary field (Table 18). Only 22.2% had the vast experience (over 10 years) in this field. About55% of them gave advice on brooding, feeding and diseases management, and 51.1% advised regarding farm biosecurity.

Table 18. Practitioners' experience and areas of advice

Parameter	Frequency (%)
Experience as practitioners	
Up to 5 years	23 (51.1)
6-10 years	12 (26.7)
11 years and above	10 (22.2)
Advice for production	
Brooding, feeding, management	25(55.5)
Diseases management	25 (55.5)
Vaccination	28 (62.2)
Biosecurity	23 (51.1)

i. Advised medicines and additives in the farms

Findings presented in Table 19 indicate that major feed additives/enzymes suggested were acmezyme (28.8%) and multienzyme (26.6%), renazyme (15.5%), phytase (13.3%), and ginger (11.1%) as minor used enzymes. Regarding probiotics, about one-half (51.1) was advised for mixed agents. A variety of growth promoting substances was used; the available agents reported were emmolyte (20%), vit. mix (22.2%) and livatone (8.8%). Main antibiotics were ciprofloxacin (40%) and enrofloxcin (35.5%). Other antibiotics were amoxicillin (22.2%), doxycycline (11.1), oxytetracycline (8.8%), colistin (6.6%), tylosin (6.66%) and tilmicosin (6.66%). Toltazuril (37.7%) was the main drug of choice as anticoccidial agents followed by the salphaclozine sodium (35.5%). There were some herbal product like broncho vet (22.2%) and hepatovet (20%) also prescribed by the practitioners.

Table 19. Advised medicines and additives in the farms

Parameter	Frequency (%)
Feed enzyme	
Acmezyme	13 (28.8)
Multienzyme	12 (26.6)
Phytase	6 (13.3)
Renazyme CS	7 (15.5)
Ginger	5 (11.1)
Feed probiotic	
Mixed probiotic agents	23 (51.1)
Gutpro	7 (15.5)
Black cumins	6 (13.3)
Curds	8 (17.7)
Growth promoter	
Emmolyte	9 (20.0)
Acid Vit mix	10 (22.2)
Livatone	4 (8.8)
Antibiotic	
Amoxicillin	10 (22.2)
Doxicycline	5 (11.1)
Enrofloxacin	16 (35.5)
Oxytetracycline	4 (8.8)
Ciprofloxacin	18 (40)
Colistin	3 (6.6)
Tylosin	3 (6.6)
Tilmicosin	3 (6.6)
Anticoccidial	
Toltazuril	17 (37.7)
Salphaclozine sodium	16 (35.5)
Amprolium hydrochloride	8 (17.8)
Salphadiamithoxine+salphadimidine	9 (20)
Metranidazole	4 (8.9)
Prescribed herbal medicine	
Broncho vet	10 (22.2)
Heapato vet	9 (20.0)
Restoliv	3 (6.7)
Other herbal extract	7 (15.5)

j. Value added processed poultry products

In Bangladesh, demand for frozen or processed food including poultry products is growing day by day at a rapid pace. With rapid urbanisation and an expanding middle class, the market for processed frozen foods has crossed around BDT 800 crore (Shifat 2020). The city dwellers and households are progressively adapting to frozen foods (not only protein- like fish and meats but also vegetables as well). The sector generated USD 371 million in revenue in the fiscal year 2018 and is expanding at over 20 percent a year (Azad 209). Many well-established companies, as well as young entrepreneurs, are focusing on this frozen food sector these days and the competition is getting bigger between them. All of them are showing the ability to beat the competitive market.

k. Market segmentation

The frozen food market of Bangladesh is segmented broadly into four namely product category, product type, freezing techniques and distribution channel as shown in Table 20.

Table 20. Market segmentation of frozen products in Bangladesh

Product category	Product type	Freezing technique	Distribution channel
Ready-to-eat	Frozen Fruits & Vegetables	Individual quick freezing	Supermarkets/Hypermarkets
Ready-to-cook	Frozen meat and fish	Blast freezing	Convenience Stores
Ready-to-Drink	Frozen-cooked ready meals	Belt freezing	Specialty Stores
Others	Frozen desserts	Others	Online Stores
-	Frozen Snacks	-	Others
-	Others	-	-

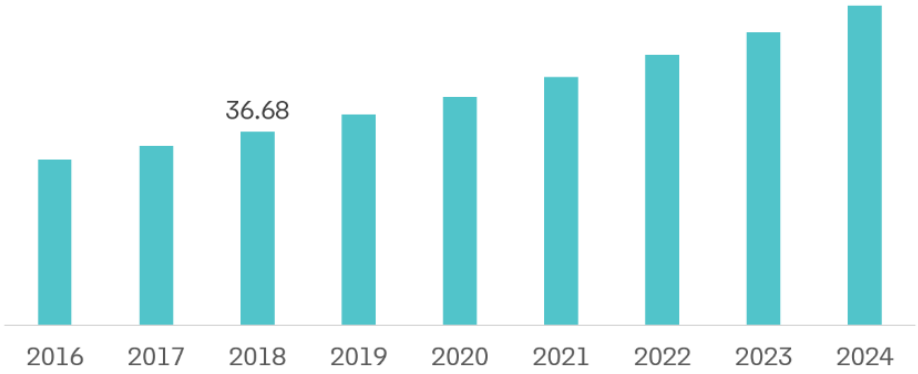
Source: https://www.mordorintelligence.com/industry-reports/bangladesh-frozen-food-market

l. Competitive landscape

The frozen food market is consolidated, owing to few local and international players. The raw material suppliers are either farmers or middlemen, who transfer farm supplies to the frozen food manufacturers. About 15 frozen food companies are mostly controlling the market. The prominent players in the country include Golden Harvest, Pran RFL Group, AG Foods Ltd, Frozen Foods Ltd, Kazi Farms Group, and McCain Foods, among others.

m. Market growth

Online retail frozen food sale represents a small segment of the market, but is expected to be among the fastest-growing segments. The urban population in big cities and old district towns has slowly started preferring online channels, as it is hassle-free, convenient, less time-consuming, and consumers get a larger variety of goods to choose from (https://www.mordorintelligence.com/industry-reports/bangladesh-frozen-food-market). Some e-commerce companies, like Chaldal, Parmeeda, Priyoshop, Shwapno, Othoba, Daraz, etc., are the major players in the Bangladeshi market. Many online channels provide an option for same-day delivery, which increases their brand value, thereby increasing the market penetration. A market forecast trend of frozen food in Bangladesh through growing buyers interest has ben presented in Fig 1 that reflects an increasing trend with the passing of time.



Source: https://www.mordorintelligence.com/industry-reports/bangladesh-frozen-food-market

Fig 1. Frozen food market growth: Revenue in USD million, Bangladesh, 2016-24.

n. Quality control issues

Ingredients and preservatives used, hygienic standards maintained during production and preservation, presence of health hazardous materials, authorised regulatory body, etc. are big questions for these items as safe human food. Producers declare to have their own mechanism to ensure quality of the food items. But no standard is yet set for the country. Both Bangladesh Standards and Testing Institution (BSTI) and Bangladesh Food Safety Authority say it is not within their jurisdictions to certify the standard of processed frozen food items (Azad 2019). Consumers have to solely rely on the assurance given by the food producers.

In concluding remark, it may be said that a company has to adopt key factors like taste, availability of products; visibility of products at the point of purchase; customer satisfaction; customer loyalty; continuous innovation, etc. are crucial and needed to achieve leadership. Customers focus on more taste for choosing any brand.

11.3. Nutrient content and hazardous materials in layer and broiler feeds

a. Chemical hazards

Among eight commercial broiler feeds under investigation, feed 3, 5 and 8 were free from hazardous chemicals (Table 21). Feed sample 4 and 6 contained lincomycine, but the levels were far below the permissible limit (500mg/kg feed) (Survey 2019-20). Actually, there are no recommended limits for the inclusion of various chemical substances like antibiotics, acticoccidial agents, etc. in commercial poultry feed. We cited the maximum limit for each chemical that is practically used in the feed industry of Bangladesh. Chlortracycline and epichlortracycline content in broiler feeds 1 and 2 was below practical level (500mg/kg feed). Similarly, oxytetracycline and its derivative levels were lower in feed 7 against MRL of 500mg/kg feed (Survey 2019-20). None of the feed samples contains ciprofloxacin, enrofloxacin or neomycin. Tylosin content in feed 1 was surprisingly higher (level is also below the practical level), although other feed contained no such chemical. Feeds 1 and 7 contained moderate levels of maduramicin which are much lower than the recommended level of 300mg/kg (Survey 2019-20). It was also invented that maduramicin, salinomycin-Na, diclazuril, etc. are alternatively used in broiler and layer grower feeds as aniticoccidial drug.

None of the market feed/house feeds under trial contained any hazardous chemical substances except maduracycin (Table 21) which is much lower than the maximum recommended level (Survey 2019-20).

Chlortracycline and its derivate were detected in commercial layer feed samples 1 and 6 (Table 22), which were below the level in practice. Oxytetracycline and its derivative found in sample 4, but it was far below the permissible limit. None of other chemical hazards was reported in any of the feed sample. Besides these facts, all samples are free from chemical hazards.

Table 21. Chemical analysis of commercial broiler feed

Chemicals	Feed-1 (BFCP)	Feed 2 (BFNH)	Feed 3 (BFA1G)	Feed 4 (BFA1F)	Feed 5 (BFPR)	Feed 6 (BFRR)	Feed 7 (SFPL)	Feed 8 (BFMA)
A. Antibiotics (µg/kg)								
Lincomycine	ND	ND	ND	10.8	ND	12.1	ND	ND
Chlortracycline	15.4	5066.9	ND	ND	ND	ND	ND	ND
4-Epichlortracycline	12.9	2608.7	ND	ND	ND	ND	ND	ND
Oxytetracycline	ND	ND	ND	ND	ND	ND	3860.6	ND
4-Epioxytetracycline	ND	ND	ND	ND	ND	ND	11536.8	ND
Ciprofloxacin	ND	ND	-	-	ND	ND	ND	-
Enrofloxacin	ND	ND	-	-	ND	ND	ND	-
Tylosin	3465.67	ND	-	-	ND	ND	ND	-
Neomycin	ND	ND	-	-	ND	ND	ND	-
Maduramicin	87.25	ND	-	-	ND	ND	107.08	-
B. Heavy metals mg/kg								
Pb	0.42	0.14	1.09	0.59	0.09	0.88	Nil	0.16
Cr	1.46	0.40	1.90	1.80	1.15	2.51	0.89	1.17
As	19.63	0.08	0.15	0.16	0.15	11.83	0.13	0.22
C. Nutrient components (%)								
CP	21.9	20.1	18.9	18.6	20.9	20.0	21.7	18.5
Ca	0.64	0.76	0.99	0.89	0.70	0.83	0.99	0.66
P	0.78	0.86	1.06	0.70	0.61	0.59	0.50	0.63

ND, Not detected

b. Heavy metals

Heavy metal contaminations in poultry products obviously come from feed or water and these heavy metals mostly transfer from the soil to plant, animal and consequently man through progressive food chain and bioaccumulation. Considering this consequence, no lead in any commercial poultry feed is somewhat surprising. However, all broiler feeds under investigation contained various levels of lead (Table 22). Lead concentrations were below the permissible limit of 1mg/kg in the United Kingdom (Nicholson et al. 1999); however it is also much lower than the maximum permitted limit of 5mg/kg suggested by FAO/WHO (2005). Chromium levels in all the feed samples were found to be above the maximum acceptable limit of 0.3mg/kg (Act No. 21, NRC 994). Arsenic levels in broiler feed 1 and 6 were unusually higher. All other feed samples also contained higher concentration of arsenic. Suggested recommended amount of As in chicken feed is more than 0.012 mg/kg yet less than 0.050 mg/kg feed (Nielsen 1998). According to DeSesso *et al*. (1998), nutritional recommendations for As are 0.025-0.050 mg/kg feed.

Table 22. Chemical analysis of broiler house feed/market feed

Chemicals	Feed 1 (HFPA)	Feed 2 (HFNH)	Feed 3 (HFAT)
A. Antibiotics (µg/kg)			
Lincomycine	ND	ND	ND
Chlortracycline	ND	ND	ND
4 Epichlortracycline	ND	ND	ND
Oxytetracycline	ND	ND	ND
4 Epioxytetracycline	ND	ND	ND
Ciprofloxacin	ND	-	ND
Enrofloxacin	ND	-	ND
Tylosin	ND	-	ND
Neomycin	ND	-	ND
Maduramycin	ND	-	54.23
B. Heavy metals mg/kg			
Pb	3.32	0.14	11.76
Cr	2.54	1.14	32.71
As	0.12	0.06	0.30
C. Nutrient components (%)			
CP	10.54	12.22	12.22
Ca	1.35	1.23	2.51
P	0.51	0.60	1.06

ND, Not detected

Table 22 indicates that lead concentrations in house feeds were amazingly uncommon and were remarkably higher than any of the permissible limit except sample 2. Similarly, chromium levels in all three house feed samples were abnormally greater compared to maximum recommended level.

Lead contents in layer feed samples 2, 4 and 5 exceed the limit of UK, but were below the maximum recommended level suggested by FAO/WHO 2003 (Table 23). Other samples contained a little amount. Chromium concentrations in all feed samples were far beyond the acceptable limit, among then sample 6 was the lowest. Arsenic levels also exceeded the permissible limits. Arsenic contents followed the similar trend as found in case of chromium.

The levels of heavy metals in poultry feed are the result of the levels of those elements in the different feedstuffs used. The best approach is to be critical and selective in the feedstuffs used, rather than trying to correct things after the feed is made. Minerals and animal protein including bones are often a source of heavy metals. So individual feedstuffs should be checked and selection is done with low concentrations of heavy metals (Kan 2019).

Table 23. Chemical analysis of commercial layer feed

Chemicals	Feed 1 (LFNH)	Feed 2 (LFIN)	Feed 3 (LFNO)	Feed 4 (LFPA)	Feed 5 (LFA1)	Feed 6 (LFAG)	Feed 7 (LFAT)	Feed 8 (LFNA)
A. Antibiotics (µg/kg)								
Lincomycine	ND	ND	ND	ND	ND	ND	ND	ND
Chlortracycline	319.5	ND	ND	ND	ND	17.19	ND	ND
4 Epichlortracycline	156.8	ND	ND	ND	ND	5.18	ND	ND
Oxytetracycline	ND	ND	ND	9.12	ND	ND	ND	ND
4 Epioxytetracycline	ND	ND	ND	12.40	ND	ND	ND	ND
Ciprofloxacin	ND	ND	ND	ND	ND	-	-	-
Enrofloxacin	ND	ND	ND	ND	ND	-	-	-

Chemicals	Feed 1 (LFNH)	Feed 2 (LFIN)	Feed 3 (LFNO)	Feed 4 (LFPA)	Feed 5 (LFA1)	Feed 6 (LFAG)	Feed 7 (LFAT)	Feed 8 (LFNA)
Tylosin	ND	ND	ND	ND	ND	-	-	-
Neomycin	ND	ND	ND	ND	ND	-	-	-
Maduromycin	ND	ND	ND	ND	ND	-	-	-
B. Heavy metals mg/kg								
Pb	0.17	3.31	Nil	3.62	3.15	0.12	0.12	0.04
Cr	0.78	1.80	0.83	2.04	2.35	0.56	1.52	2.34
As	0.06	0.11	0.20	0.22	0.16	0.09	0.61	0.19
C. Nutrient components (%)								
CP	16.41	15.98	15.5	15.78	14.26	16.36	16.32	15.57
Ca	2.96	3.25	5.4	2.44	5.14	4.47	4.46	6.59
P	0.69	0.51	0.62	0.52	0.88	0.51	0.69	0.66

ND, Not detected

c. Nutrient composition

Results for nutrient analysis indicate that crude protein per cent in broiler feeds were more or less nearer to standard nutrition recommendations except feed 8, where it was much lower (https://idoc.pub/download/cobb500-broiler-performance-and-nutrition-supplement-3no7gk0q1xld) (Table 23). Only feed 8 contained required amount of calcium and all other starter feed were sub-standard. On the other hand, all feed samples possessed relatively higher amount of phosphorus.

All house feed samples contained much lower amount of crude protein as indicated in the label of bags (Table 22). Calcium levels were higher, but phosphorus levels were nearer to requirements except feed 3. Crude protein levels of commercial layer feeds were nearer to the recommendations other than feed 5 (Table 23). Phosphorus contents were reasonable, although sample 5 possessed higher amount.

11.4. Performances of pullets and egg quality characteristics fed different commercial poultry diets

a. Body weight

Average initial body weight (15-week weight) of each pullet significantly ($p>0.01$) different at two geographical locations (Table 24). It could be due to differences in management (feeding, cleaning, medication and vaccination, etc.) events provided for the birds during rearing period. But, with the advancement of age the birds recovered the difference and body weights were almost similar. Appropriate/controlled feeding and other management practices contributed to achieve the target body weights in both the sites. Feed had no influence on body weight of birds during rearing (Table 25). It indicates that presence of chemical substances in feeds did not affect the growth performances of birds irrespective of geographical locations and seasons. On the contrary, rearing season had profound ($p>0.01$) influence on body weight of birds throughout the study period (Table 27). Initial weight of each bird in rainy season was remarkably higher than that either in summer or winter. This trend was almost continued up to 25-week of age. Thereafter, summer-reared birds gained the highest weight followed by rainy and winter seasons. Differences in initial weights of pullets derived from different flocks in various seasons may be the possible reason. Lower body weight is the resultant factor of less feed consumption which interacted to produce significant variations in GL×CF×SE interaction results (Table 29). Other than rainy season, no birds gained recommended 20-week body weight of 1630-1730g (Hy-line Brown Commercial Layer Management Guide 2015).

Table 24. Performances of pullets reared in different geographical locations

Geographical location	Body weight (g b^{-1})				Feed intake (g b^{-1})				Survivability (%)
	15-week	20-week	25-week	30-week	15-week	20-week	25-week	30-week	
Barishal	1084.30	1574.90	1808.80	1853.10	60.74	88.43	100.92	108.43	99.70
Dinajpur	1194.50	1558.40	1805.60	1877.00	64.12	89.05	109.03	111.67	99.71
SED/LSD	25.340	10.650	14.290	8.900	1.312	1.305	1.868	2.104	0.284
Level of significance	**	NS	NS	NS	**	NS	**	**	NS

NS, Not significant; **, p>0.01

b. Feed intake

Pullets reared at Dinajpur consumed significantly (p>0.01) higher amount of feed compared to that of Barishal except on 20-week (Table 24). This could be due to less control over daily feed supply in controlling body weight after the onset of lay. Similarly, per bird feed intake was affected (p>0.01) by feed type except on 25-week (Table 25). Birds fed Diet-LFIN consumed lowest amount of feed on 25-week and onwards. Findings indicate that birds fed Diet- LFIN required less amount of feed to achieve target body weight. Feed consumption depends on a variety of factor, like physiological condition of an individual, palatability, feed form, smell, ambient temperature, stage of production, etc. One or more of these factors might influence dietary intake in Diet- LFIN. Birds on all other dietary groups feed similar amount of feed. Rearing season influenced (p>0.01) feed intake of birds (Table 28). Lower feed intakes were recorded throughout the trial period except 25-week in winter-reared pullets. On the other hand, birds' feed intake was more from 20-week onwards during summer months. Cold shock and feed toxicity at Dinajpur and feed toxicity and outbreak of micoplasma and salmonella at Barishal might be the contributors in lower feed consumption during winter. Higher feed intake recorded in summer months during laying period is difficult to explain. But, higher amount of feed allocation, wastage of feed, etc. may be the possible reasons. However, higher consumption in summer and lower in winter ultimately interacted to produce significant variations (Table 29).

Table 25. Performances of pullets feed various commercial poultry feeds

Feed	Body weight (g b^{-1})				Feed intake (g b^{-1})				Survivability (%)
	15-week	20-week	25-week	30-week	15-week	20-week	25-week	30-week	
LFNO	1140.40	1587.20	1818.40	1878.70	64.77	89.94	104.52	110.24	99.89
LFA1	1158.10	1554.80	1796.30	1855.20	62.81	90.57	104.40	110.66	99.61
LFNH	1140.60	1568.20	1824.80	1877.40	60.46	86.67	105.73	110.82	99.83
LFPA	1136.10	1553.40	1794.90	1857.00	61.94	88.86	105.83	111.01	99.20
LFIN	1121.70	1569.60	1801.60	1857.00	62.15	87.66	104.39	107.50	100.00
SED/LSD	14.080	16.840	22.590	14.070	2.074	3.170	1.923	3.327	0.777
Level of significance	NS	NS	NS	NS	**	**	NS	**	NS

NS, Not significant; **, p>0.01

c. Health issues and survivability of birds

All birds were vaccinated following the recommendations of Kazi Farms Ltd., the local vendor of Hy-line International. A very few birds were died of and as a result survivability of birds in all groups was not affected (Tables 23-25). But, mortality (due to egg bound and vent picking in cages) was relatively higher at Dinajpur during summer which interacted to produce significant results (Table 29). Micoplasmosis was the most common disease outbreak recorded.

Sometimes salmonellosis, feed toxicity, egg bound, and cannibalism contributed to lower survivability of pullets.

Table 26. Effect of seasonal variations on growth performances of pullets

Rearing season	Body weight (g b^{-1})				Feed intake (g b^{-1})				Survivability (%)
	15-week	20-week	25-week	30-week	15-week	20-week	25-week	30-week	
Summer	1139.40	1551.50	1769.70	1963.70	62.43	100.72	110.80	113.44	99.88
Rainy	1222.10	1623.30	1828.60	1887.30	64.96	81.99	101.08	110.97	99.87
Winter	1056.70	1525.10	1823.30	1744.30	59.90	83.52	103.04	105.73	99.37
SED/LSD	40.06	34.690	46.560	29.960	1.606	2.456	2.287	2.577	0.347
Level of significance	**	**	**	**	**	**	**	**	NS

NS, Not significant; **, p>0.01

d. Egg production

Age at 1st egg differed significantly (p>0.01); pullets reared at Dinajpur site laid their 1st egg earlier than Barishal (Table 26). Similarly, 1st egg came late (p>0.01) during summer (Table 20) compared to other seasons. But, feed had no influence on age at 1st egg (Table 28). According to the breeder, birds should start laying on 126-day age (Hy-line Brown Commercial Layer Management Guide 2015). Pullets laid 50% eggs on about 151-day of age against the recommended age of 140-day. Fifty percent and ≥90% egg production was found on almost similar ages at both the sites (154.67 vs 155.33 and 174.29 vs 178.42 days) (Table 27). Birds fed LFNH diet reached 50% and peak (≥90%) production much earlier than other groups (Table 25). It was also found that summer-reared birds were late producers (Table 29). In practice, we were behind the breeder's recommendations. Thus, we become looser with respect to farm productivity and economic returns. Interaction effects were significant (Table 30).

Table 27. Egg production performances of birds in different geographical locations

Geographical location	Age at 1st egg (d)	Weight of 1st egg (g)	Age at 50% production (d)	Weight at 50% production (g)	Age at ≥90% production (d)	Weight at ≥90% production (g)
Barishal	142.18	48.53	155.33	52.15	178.42	56.72
Dinajpur	140.24	44.58	154.67	48.65	174.29	54.55
SED/LSD	1.930	1.291	0.786	1.259	1.668	1.172
Level of significance	**	**	NS	**	NS	**

NS, Not significant; **, p>0.01

Table 28. Effect of commercial feed on egg production performances of birds

Feed	Age at 1st egg (d)	Weight of 1st egg (g)	Age at 50% production (d)	Weight at 50% production (g)	Age at ≥90% production (d)	Weight at ≥90% production (g)
Nourish	142.67	46.54	159.22	49.88	178.56	55.98
A1	140.28	46.13	154.67	49.89	180.44	55.54
New Hope	140.39	46.43	151.94	50.00	167.67	54.93
Paragon	141.83	47.07	155.22	50.86	179.89	55.61
Index	140.89	46.62	153.94	51.36	175.22	56.21
SED/LSD	1.147	0.767	3.306	0.748	7.016	0.697
Level of significance	NS	NS	**	NS	**	NS

NS, Not significant; **, p>0.01

e. Egg weight

Eggs at Barishal site were heavier (p>0.01) that of Dinajpur throughout the study period (Table 27), although feed had no effect (Table 28). Similarly, birds reared during rainy season laid smaller (p>0.01) eggs and summer the heavier (Table 29). Average egg weight at onset of lay, 50% and ≥90% production stages were 48.8–50.0, 50.2–52.2 and 53.1–55.3g, respectively (Hy-line Brown Commercial Layer Management Guide 2015). Egg size depend on genotype, ambient temperature, lighting, age feed intake, water consumption, body weight and physique, nutrition, disease and egg cooling and storage condition (https://www.livestocking.net/factors-affecting-egg-size). Interaction effects were significant for all ages (Table 30).

Table 29. Seasonal influence on egg production performances of birds

Rearing season	Age at 1st egg (d)	Weight of 1st egg (g)	Age at 50% production (d)	Weight at 50% production (g)	Age at ≥90% production (d)	Weight at ≥90% production (g)
Summer	146.23	47.01	157.87	50.31	188.87	56.90
Rainy	138.00	46.33	155.97	49.54	177.73	54.71
Winter	139.40	46.32	151.17	51.35	162.67	55.30
SED/LSD	2.364	0.594	2.561	1.542	5.435	1.435
Level of significance	**	NS	**	**	**	**

NS, Not significant; **, p>0.01

f. Egg quality characteristics

Results demonstrated in Table 31 indicate that internal egg quality parameters were not affected by dietary treatments except yolk colour score. Yolk colour is scored on a 1 to 15 scale with 1 being very pale yellow and 15 a deep orange. Yolk colour is influenced by many factors including ingredients used, pigment (oxycarotenoid) levels both in ingredients and additives, length of storage, feed intake of birds, stability of fats in the feed, antioxidants, calcium, vitamin A, drug use, mycotoxins, health, hours of daylight.

It is well recognised that yolk colour is important to consumers. Recent surveys in Europe have confirmed that yolk colour is one of the main parameters by which the quality of eggs is judged. It is certainly true that consumers in most parts of the world prefer deeply hued yolks. It is interesting to note that, when presented with samples of eggs with yolk colours corresponding to Colour Fan scores of 8, 10, 12 and 14, most of the consumers questioned in the European surveys expressed a preference for the darkest colours (Beardsworth and Hernandez 2004). In our study, we have found a score of 7-10.

Table 30. Effect of interaction of Geographical Location (GL), Commercial Feed (CF) and Rearing Season (SE) on the performances of pullets

Interaction	Body weight (g b^{-1})		Feed intake (g b^{-1})		Egg production performances				Survivability (%)
	15-week	30-week	15-week	30-week	Age at 1st egg (d)	Weight of 1st egg (g)	Age at ≥90% egg (d)	Weight at ≥90% egg (g)	
$GL_1 \times CF_1 \times SE_1$	1074.60	1933.70	61.97	109.82	151.33	47.43	201.33	57.10	100.00
$GL_1 \times CF_1 \times SE_2$	1173.30	1916.50	66.15	110.24	127.33	47.80	169.33	54.80	100.00
$GL_1 \times CF_1 \times SE_3$	1037.10	1771.10	59.00	105.89	146.67	48.33	173.00	58.67	99.33
$GL_1 \times CF_2 \times SE_1$	1087.00	1894.70	60.97	111.03	148.33	47.80	205.00	57.73	100.00
$GL_1 \times CF_2 \times SE_2$	1193.20	1892.10	63.73	111.00	130.33	47.97	164.33	53.23	99.33
$GL_1 \times CF_2 \times SE_3$	1056.90	1742.00	58.71	106.54	150.33	54.67	178.00	60.00	99.00
$GL_1 \times CF_3 \times SE_1$	1055.30	1868.00	59.54	108.66	150.67	47.37	177.33	57.10	100.00
$GL_1 \times CF_3 \times SE_2$	1183.30	1874.90	61.00	109.50	132.00	47.37	163.67	53.87	100.00
$GL_1 \times CF_3 \times SE_3$	1006.60	1747.90	57.64	107.12	144.00	50.67	167.00	57.67	99.00
$GL_1 \times CF_4 \times SE_1$	1046.90	1913.00	60.17	109.50	149.67	47.97	192.00	57.90	98.85
$GL_1 \times CF_4 \times SE_2$	1180.80	1903.70	62.84	110.03	129.00	48.07	181.31	54.07	100.00
$GL_1 \times CF_4 \times SE_3$	993.30	1772.70	57.78	107.02	148.00	49.33	171.67	57.00	100.00
$GL_1 \times CF_5 \times SE_2$	1035.30	1921.00	60.47	109.39	150.00	48.57	201.33	58.80	100.00
$GL_1 \times CF_5 \times SE_2$	1165.40	1908.70	62.99	109.99	129.00	47.90	163.67	52.53	100.00
$GL_1 \times CF_5 \times SE_3$	974.80	1736.30	58.24	100.69	146.00	46.67	167.33	60.33	100.00
$GL_2 \times CF_1 \times SE_1$	1205.70	2020.70	47.16	117.00	144.00	44.66	183.67	56.09	100.00
$GL_2 \times CF_1 \times SE_2$	1244.10	1883.00	70.82	111.98	153.33	45.40	192.00	56.14	100.00
$GL_2 \times CF_1 \times SE_3$	1107.80	1747.30	63.55	106.53	133.33	45.60	152.00	53.06	100.00
$GL_2 \times CF_2 \times SE_1$	1219.10	2018.30	64.59	116.67	141.33	45.58	184.00	56.41	100.00
$GL_2 \times CF_2 \times SE_2$	1265.50	1864.30	66.97	111.93	139.00	41.17	194.00	52.87	99.33
$GL_2 \times CF_2 \times SE_3$	1127.10	1719.80	61.92	106.79	132.33	38.58	157.33	52.43	100.00
$GL_2 \times CF_3 \times SE_1$	1225.30	2073.30	61.83	117.00	137.00	44.68	166.00	54.20	100.00
$GL_2 \times CF_3 \times SE_2$	1275.10	1930.60	63.06	113.58	147.00	45.77	182.33	57.27	100.00
$GL_2 \times CF_3 \times SE_3$	1098.20	1769.90	59.81	109.07	131.67	42.70	149.67	49.48	100.00
$GL_2 \times CF_4 \times SE_1$	1227.10	1971.70	63.80	117.33	144.33	48.00	186.33	57.35	100.00
$GL_2 \times CF_4 \times SE_2$	1277.70	1844.90	66.05	113.52	148.00	46.76	189.00	55.92	100.00
$GL_2 \times CF_4 \times SE_3$	1090.70	1735.80	61.02	108.65	132.00	42.27	159.00	51.44	96.33
$GL_2 \times CF_5 \times SE_2$	1217.70	2022.30	63.88	118.00	145.67	48.06	189.67	56.28	100.00
$GL_2 \times CF_5 \times SE_2$	1262.70	1854.00	66.02	107.90	145.00	45.08	177.67	56.45	100.00
$GL_2 \times CF_5 \times SE_3$	1074.20	1699.60	61.29	99.04	129.67	43.42	151.67	52.89	100.00
SED/LSD	98.130	94.740	5.080	8.150	7.475	5.001	17.186	4.539	2.922
Significance level	**	**	**	**	**	**	**	**	**

**, p>0.01

Table 31. Internal egg quality characteristics

Feed	Egg weight (g)	Albumen height (mm)	Yolk colour score	Haugh unit
Nourish	55.9	4.73	8.17	65.5
A1	59.3	4.92	7.33	67.8
New Hope	59.6	5.30	9.00	71.5
Paragon	59.3	5.48	8.00	69.7
Index	59.0	4.48	9.33	64.0
SED/LSD	3.520	0.745	0.995	5.24
Level of significance	NS	NS	*	NS

11.5. Effects of commercial feed on meat yield and quality of broilers

a. Weight gain

Day-old weights of broiler chicks in all the cases showed significant (p>0.01) differences (Tables 32-35). Tullett and Burton (2008) reported that over 97% of the variation in chick weight at hatch can be explained by two factors, fresh egg weight and weight loss during incubation. These two factors still account for a high (87%) proportion of the variation in chick weight at 21-day after setting when chicks would be removed from the hatcher.

Variations in day-old chick weight may be due to the initial egg weight, water loss during incubation, weight of shell and other residues at hatch (Tullett and Burton 1982), post-hatch water loss from chicks (Wilson 1991), season and sex of chicks (Khan *et al*. 1975, Wilson 1991), maternal age (Lotte 2006). Seasons (summer, rainy, autumn and winter) had influence on egg weight (Pandey *et al*. 1989, Adams and Bell 1998, Chen-DengFei *et al*. 2001), chick weight at hatch (Uddin *et al*. 1994) and hatching weight is positively correlated with final fattening weight (Tandron *et al*. 1987). A seasonal effect on the efficiency of egg utilization has been found (Galpin 1983), and was attributed to changes in maternal mechanism, which reduced the dependence of chick weight on egg weight in summer (Wilson 1991). On the contrary, Saleh *et al*. (1992) did not find any significant effect of season on egg weight, but birds hatched in summer were of better quality than winter

Table 32. Performances of broilers in different geographical locations

Geographical location	Body weight (g b^{-1})		Feed intake (g b^{-1})	Feed conversion	Survivability (%)
	Initial	28-day			
Barishal	42.11	1689.80	2196.30	1.31	96.50
Dinajpur	45.43	1501.00	2077.40	1.39	96.91
SED/LSD	0.269	33.870	37.360	0.020	0.830
Level of significance	**	**	**	**	NS

NS, Not significant; **, p>0.01

Similar to day-old weight, all factors also had profound influence on weight gain at 28-day age (Tables 32-35). Although chick weight was higher, slaughter weight was significantly lower at Dinajpur. Birds fed BFPR and BFNH appreciably heavier than others (Table 24). Again, winter-reared birds gained highest weight followed by summer and rainy seasons (Table 34). Growth depression at Dinajpur as well as during summer may be explained by the reduced feed intake which is regulated by elevated environmental temperature and relative humidity (Scott *et al*. 1992, Sundararasu *et al*. 1989). The mentioned two commercial feed were probably more balanced with respect to various nutrients to produce heavier broilers. It

implies that presence of hazardous substances found in the feeds under trial did not influenced much in achieving higher growth of broilers.

Table 33. Performances of broilers feed various commercial poultry feeds

Feed	Body weight (g b^{-1})		Feed intake (g b^{-1})	Feed conversion	Survivability (%)
	Initial	28-day			
Provita	43.56	1618.10	2150.50	1.33	96.71
Palli	43.38	1550.70	2127.70	1.38	96.64
CP	44.00	1588.10	2150.50	1.36	96.90
RRP	43.88	1582.10	2140.80	1.36	95.85
New Hope	44.02	1613.00	2114.70	1.31	97.40
SED/LSD	0.425	53.550	22.210	0.031	1.312
Level of significance	**	**	NS	**	NS

NS, Not significant; **, p>0.01

b. Feed consumption and feed conversion

Chicks at Dinajpur were relatively poor feed converter (Table 32). Results presented in Table 33 indicate that birds fed BFNH were the best regarding feed consumption and feed conversion. Winter-reared birds consumed more feed and convert it less efficiently (Table 34). Lowered digestibility, nutrient retention, energy expenditure in struggling (wing fluffing, crouching of body, frequent drinking, lying down, diffing litter, panting, etc.) to disseminate heat, etc. may be the reasons for poor feed utilization in a high temperature-high humidity environment.

Table 34. Effect of seasonal variations on performances of broilers

Rearing season	Body weight (g b^{-1})		Feed intake (g b^{-1})	Feed conversion	Survivability (%)
	Initial	28-day			
Summer	43.58	1581.90	2115.60	1.33	96.83
Rainy	43.07	1542.50	2047.30	1.33	97.84
Winter	44.65	1646.80	2247.60	1.37	95.44
SED/LSD	0.329	41.480	45.760	0.024	1.016
Level of significance	**	**	**	**	NS

NS, Not significant; **, p>0.01

c. Health issues and survivability of birds

Birds' survivability in all the study was almost similar and did not influenced by location, feed, rearing season or by the interactions (Tables 32 – 35). Better management practices (feeding, cleaning and disinfection, immunization, appropriate medication, etc.) helped to reduce mortality. However, most common health related problems faced were as follows:

a) electricity failure and interrupted power during early-stage cause chilling and flock tends to huddle together resulting suffocation, immune suppression, omphalitis, etc.,
b) litter dampness on later stage resulting in harmful gas accumulation, specially in rainy season,
c) ammonia accumulation during winter months as a result of improper ventilation,
d) sometimes vaccination failure,
e) mycoplasmosis specially during winter months,
f) coccidiosis in flock etc.

Table 35. Effect of interaction of Geographical Location (GL), commercial feed (CF) and Rearing Season (SE) on feed intake of broilers

Interaction	Body weight (g b^{-1})		Feed intake (g b^{-1})	Feed conversion	Survivability (%)
	Initial	28-day			
$GL_1 \times CF_1 \times SE_1$	40.79	1621.80	2115.60	1.30	97.44
$GL_1 \times CF_1 \times SE_2$	40.91	1642.90	2144.00	1.30	97.44
$GL_1 \times CF_1 \times SE_3$	44.64	1875.90	2430.80	1.30	94.77
$GL_1 \times CF_2 \times SE_1$	40.86	1595.20	2115.10	1.33	95.24
$GL_1 \times CF_2 \times SE_2$	40.77	1562.80	2165.80	1.32	95.24
$GL_1 \times CF_2 \times SE_3$	43.65	1812.90	2413.60	1.33	96.05
$GL_1 \times CF_3 \times SE_1$	41.13	1579.40	2077.90	1.32	95.05
$GL_1 \times CF_3 \times SE_2$	41.35	1600.30	2087.10	1.30	95.05
$GL_1 \times CF_3 \times SE_3$	43.87	1828.60	2395.30	1.31	98.72
$GL_1 \times CF_4 \times SE_1$	40.84	1640.40	2123.30	1.30	100.00
$GL_1 \times CF_4 \times SE_2$	40.87	1640.40	2146.20	1.30	100.00
$GL_1 \times CF_4 \times SE_3$	44.77	1719.40	2268.20	1.32	89.21
$GL_1 \times CF_5 \times SE_2$	40.98	1628.20	2049.50	1.26	100.00
$GL_1 \times CF_5 \times SE_2$	40.90	1628.20	2113.10	1.30	100.00
$GL_1 \times CF_5 \times SE_3$	45.25	1820.40	2398.80	1.32	93.27
$GL_2 \times CF_1 \times SE_1$	45.00	1619.40	2173.00	1.35	97.22
$GL_2 \times CF_1 \times SE_2$	44.67	1449.80	1936.00	1.34	97.33
$GL_2 \times CF_1 \times SE_3$	45.33	1498.70	2103.70	1.40	96.08
$GL_2 \times CF_2 \times SE_1$	45.00	1451.50	1995.00	1.37	97.22
$GL_2 \times CF_2 \times SE_2$	44.33	1411.70	1982.90	1.40	100.00
$GL_2 \times CF_2 \times SE_3$	45.67	1470.00	2194.00	1.55	96.08
$GL_2 \times CF_3 \times SE_1$	48.00	1557.90	2186.20	1.41	97.22
$GL_2 \times CF_3 \times SE_2$	46.00	1483.30	2015.30	1.36	97.33
$GL_2 \times CF_3 \times SE_3$	43.67	1479.00	2141.30	1.45	98.04
$GL_2 \times CF_4 \times SE_1$	46.15	1539.00	2165.30	1.41	94.44
$GL_2 \times CF_4 \times SE_2$	45.67	1505.00	1999.00	1.33	97.33
$GL_2 \times CF_4 \times SE_3$	45.00	1448.00	2142.70	1.48	94.12
$GL_2 \times CF_5 \times SE_2$	47.00	1586.00	2155.60	1.36	94.44
$GL_2 \times CF_5 \times SE_2$	45.33	1501.00	1983.90	1.32	98.67
$GL_2 \times CF_5 \times SE_3$	44.67	1514.30	1987.30	1.31	98.04
SED/LSD	1.041	131.180	144.710	0.076	1.855
Significance level	**	**	**	**	NS

**, p>0.01; NS, Not significant

11.6. Performances of various hybrids (Sonali) under farmers' condition performances up to 21-day

a. Initial body weight of Kazi Gold genotype was significantly higher than the local one (Table 36) and therefore growth rate and feed consumption were remarkably higher than those of Local Sonali. This is due to genetic make-up of these birds. Higher body weight along with increased feed intake ultimately contributed in gaining heavier weight in case of Kazi Gold. Despite variations in day-old weight, birds in both the seasons achieved similar body weight on 21-day irrespective of genotypes. But, birds in rainy season are better feed converter (Table 37). Interaction effects were significant in all the three weeks (Table 38). Results indicate that Kazi Gold during rainy season gained the highest weight and also consumed more feed up to 21-day age.

Table 36. Performances of various genotypes (sonali) up to 21-day

Genotype	Body weight (g b^{-1})				Feed intake (g b^{-1})		
	Initial	7-day	14-day	21-day	7-day	14-day	21-day
Kazi Gold	42.54	99.95	199.50	327.60	83.82	153.70	213.40
Local Sonali	30.65	73.63	131.60	209.20	74.06	125.00	150.40
LSD	0.561	4.563	5.760	12.590	2.531	7.850	12.410
Level of significance	*	*	*	*	*	*	*

*, p>0.05

Table 37. Seasonal variations in performances of sonali genotypes up to 21-day

Season	Body weight (g b^{-1})				Feed intake (g b^{-1})		
	Initial	7-day	14-day	21-day	7-day	14-day	21-day
Summer	36.21	83.37	170.70	267.30	85.19	155.40	189.00
Rainy	36.98	90.22	160.40	269.50	72.70	123.30	174.80
SED/LSD	0.561	4.563	5.760	3.860	2.531	7.850	12.410
Level of significance	*	*	*	NS	*	*	*

*, p>0.05; NS, Not significant

Table 38. Effect of interaction of Genotype (GE), and Rearing Season (SE) on the performances of sonali up to 21-day

Interaction	Body weight (g b^{-1})				Feed intake (g b^{-1})		
	Initial	7-day	14-day	21-day	7-day	14-day	21-day
$GE_1 \times SE_1$	42.09	96.61	203.70	324.70	90.33	178.10	211.60
$GE_1 \times SE_2$	42.99	103.30	195.20	330.50	77.31	129.20	215.30
$GE_2 \times SE_1$	30.33	70.13	137.70	209.90	80.05	132.70	166.40
$GE_2 \times SE_2$	30.97	77.13	125.70	208.50	68.08	117.40	134.30
LSD	0.794	6.453	8.140	17.800	3.579	11.100	17.550
Level of significance	*	*	*	*	*	*	*

*, p>0.05

b. Performances up to weight at marketing

Results indicate that Kazi Gold chicks crossed the target market weight of 800g b^{-1} on 45-day (Table 39). But, Local Sonali achieves this weight on 60-day age. Feed intake was decreased due to outbreak of coccodiosis on 42-day onwards. Therefore, weight gain was slightly slower down. Results in Table 40 reveals that birds fed broiler grower feed finally gained marketable weight at 45-day and this weight was achieved on 60-day by the same birds consumed sonali grower feed. Therefore, higher amount of sonali grower feed were required to attain marketable weight. Birds irrespective of genotype and feed type reached the target body weight on 45-day during summer and on 60-day in rainy season (Table 41). Higher body weight in Kazi Gold demanded higher feed and thus the differences were marked ($p \geq 0.05$). It is noted that birds consumed more sonali grower feed to meet their nutrient requirements. Interaction effects (Table 42) indicate that Kazi Gold offered broiler grower in both the seasons gained about 829g body weight which is slightly higher than that of sonali grower. Similarly, local sonali fed broiler grower gained slightly higher weight compared to sonali grower and differences were not large.

c. Bird health and survivability

Survivability was almost similar in all the cases (Tables 39-42). It was experienced that omphalitis due to improper brooding, power failure during early ages lowed the immunity level of birds. Besides, coccidisis outbreak is almost general phenomenon. As a result, increased chick mortality, medication cost is the most common.

Table 39. Performances of various genotypes (Sonali) from 21-day onwards

Genotype	Body weight (g b^{-1})							Feed intake (g b^{-1})							Survivability (%)
	28-day	35-day	42-day	45-day	49-day	56-day	60-day	28-day	35-day	42-day	45-day	49-day	56-day	60-day	
Kazi Gold	460.40	611.40	762.60	822.70	-	-	-	253.90	277.10	378.60	223.80	-	-	-	99.25
Local Sonali	298.30	413.70	505.10	-	652.20	768.00	828.30	189.70	187.80	261.00	-	241.30	265.20	197.50	98.25
SED/LSD	17.050	31.100	29.200	-	-	-	-	7.750	9.450	10.320	-	-	-	-	0.00
Level of significance	*	*	*	-	-	-	-	*	*	*	-	-	-	-	NS

**, p>0.01; NS, Not significant

Table 40. Effect of feed on the performances of Sonali genotypes from 21-day onwards

Feed type	Body weight (g b^{-1})							Feed intake (g b^{-1})							Survivability (%)
	28-day	35-day	42-day	45-day	49-day	56-day	60-day	28-day	35-day	42-day	45-day	49-day	56-day	60-day	
Broiler grower	380.20	512.20	650.60	828.50	-	-	-	220.70	224.80	313.40	212.10	-	-	-	98.75
Sonali grower	378.50	512.90	617.00	-	667.00	760.60	805.10	222.90	240.10	326.30	-	264.60	250.30	216.20	98.75
SED/LSD	11.370	14.200	29.200	-	-	-	-	5.170	9.450	10.320	-	-	-	-	0.00
Level of significance	NS	NS	*	-	-	-	-	NS	*	*	-	-	-	-	NS

Table 41. Seasonal variations on the performances of Sonali genotypes

Season	Body weight (g b^{-1})							Feed intake (g b^{-1})							Survivability (%)
	28-day	35-day	42-day	45-day	49-day	56-day	60-day	28-day	35-day	42-day	45-day	49-day	56-day	60-day	
Summer	388.50	518.90	673.80	822.70	-	-	-	310.50	201.10	358.50	223.80	-	-	-	98.50
Rainy season	370.20	506.20	583.90	-	615.90	760.00	822.80	133.10	263.90	281.10	-	145.70	195.10	210.10	99.00
SED/LSD	17.050	31.100	29.200	-	-	-	-	7.750	9.450	10.320	-	-	-	-	0.00
Level of significance	*	*	*	-	-	-	-	*	*	*	-	-	-	-	NS

Table 42. Effect of interaction of Genotype (GE), Feed (FE) and Rearing Season (SE) on the performances of Sonali from 21-day onwards

Interaction	Body weight (g b^{-1})							Feed intake (g b^{-1})							Survivability (%)
	28-day	35-day	42-day	45-day	49-day	56-day	60-day	28-day	35-day	42-day	45-day	49-day	56-day	60-day	
$GE_1 \times FE_1 \times SE_1$	474.70	603.00	817.70	829.30	-	-	-	344.40	246.30	416.60	210.60	-	-	-	100.00
$GE_1 \times FE_1 \times SE_2$	451.80	614.20	743.20	829.60	-	-	-	154.40	293.60	320.10	209.90	-	-	-	99.00
$GE_1 \times FE_2 \times SE_1$	467.50	621.20	790.80	816.00	-	-	-	348.40	233.40	420.70	236.90	-	-	-	98.00
$GE_1 \times FE_2 \times SE_2$	447.70	607.30	698.80	815.70	-	-	-	168.40	335.20	357.10	237.60	-	-	-	100.00
$GE_2 \times FE_1 \times SE_1$	302.60	426.00	554.80	-	667.90	790.00	865.90	272.20	159.70	287.70	-	297.20	374.20	146.70	98.00
$GE_2 \times FE_1 \times SE_2$	291.70	405.80	487.00	-	606.80	761.30	838.50	112.00	199.70	229.00	-	138.10	186.80	210.50	98.00
$GE_2 \times FE_2 \times SE_1$	309.30	425.40	531.90	-	710.20	762.40	802.00	277.20	164.80	308.80	-	379.10	300.10	222.90	98.00
$GE_2 \times FE_2 \times SE_2$	289.70	397.70	464.50	-	624.10	758.40	806.90	97.60	227.10	218.40	-	150.80	199.80	210.00	99.00
SED/LSD	34.100	60.21	58.400	33.940	50.870	38.320	36.530	10.960	18.910	20.640	14.310	18.010	18.350	13.020	0.00
Level of significance	*	*	*	NS	*	NS	NS	*	*	*	*	*	*	*	NS

11.7. Effect of house feed on weight change of broilers harvested at different marketing ages

a. Composition of house feed

Table 43 demonstrated that CP% was much lower (10.54 and 12.22) than standard broiler diet (17.5-18.0). Bu, house feed contained higher Ca% (1.35 and 2.51) than standard finisher diet (0.76). It was also found that nutrient content specially CP% was also lower than the value labeled on the bag. Comparing analytical results with those of labeled composition it can be concluded that manufacturers are providing exaggerated misleading information. So, retailers become looser. Moreover, excessive mineral and lower CP contents indicate no or very weak control or monitoring of regulatory authority over feed manufacturing process.

Table 43. Chemical analysis of commercial broiler house feed

Nutrient components	Feed 1 (HFPA)	Feed 2 (HFAT)	Feed 3 (Standard diet)
CP %	10.54 (minimum 15±1)	12.22 (minimum 13±1)	17.50-18.0
Ca%	1.35 (minimum 1.20)	2.51 (minimum 1.00)	0.76
P%	0.51 (minimum 0.45)	1.06 (minimum 0.60)	0.67*

Figures in the parentheses indicate labeled composition on the bags; *, available P

b. Body weight and weight gain

Body weight and weight gain irrespective of harvesting age and rearing season were significantly different among the treatment groups except initial weight of birds (Tables 44). Although protein contents were different (10.54 and 12.22%), birds fed house feeds gained almost similar weight through the study period. Total gain after 6 days on two house feeds were 127 and 194g, respectively which were much lower ($p>0.01$) than that of standard broiler diet (499g). Nutritionally balanced standard broiler diet attributed to the large variations in weight gain among the treatment groups. Harvesting age also influenced ($p>0.01$) daily weight and weight gain; birds harvested on 35-day was naturally much heavier than those on 28-day (2843.00g vs 1849g) (Table 45). Thus, total gain was also much higher in birds harvested on 35-day. Birds reared during summer were lighter ($p>0.01$) than rainy season ones (Table 46) which could be to relatively higher ambient temperature, panting, etc. Higher initial weight contributed to the differences in daily weight gain. Although seasonal variations in day-to-day body weight were prominent, total gain was almost nearer to each other (297 and 250g). Increased feed consumption (Table 49) yielded relatively higher weight during summer (Table 46). Interaction effects on initial and final body (6^{th} day weight) were significant.

Table 44. Effect of house feed on weight gain and survivability of broilers at wet market

House feed	Daily body weight change (g b^{-1})							Weight gain (g)	Surviva-bility (%)
	Initial	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6		
HFAT	2105.00	2165.00	2188.00	2209.00	2213.00	2231.00	2232.00	127.00	100.00
HFPA	2043.00	2082.00	2132.00	2182.00	2199.00	2225.00	2238.00	194.00	98.81
Standard broiler diet	2069.00	2192.00	2272.00	2342.00	2385.00	2494.00	2569.00	499.00	96.37
SED/LSD	34.500	108.600	114.100	130.900	140.000	143.600	159.600	99.300	1.907
Level of significance	NS	**	**	**	**	**	**	**	NS

NS, Not significant; **, $p>0.01$

Although performances of broilers are the resultant factors of the genetic constituent, management, and feed formulation, environmental concerns associated with the concentrated production of broilers remain a priority issue today in which they are reared (College of Agriculture and Natural Resources Bulletin 2006). Nutritionally balanced broiler diet attributed to these differences in weight gain among the treatment groups. Previous study found an increased protein level associated with increased weight gain of broilers (Jackson *et al*. 1982). Higher initial weight contributed to heavier weight in birds harvested on 35-day. This result was supported by Coban *et al*. (2014) who found increased body weight up to 49 days and thereafter weight gain became decreased compared to feed intake. Broiler's weight is increased up to certain periods and after that profitability decreases with extended growth periods (Baéza *et al*., 2012). Ferket and Gernat (2006) reported that environmental stresses had the most profound effects on flock-to-flock variation of feed intake that influenced both the body weight gain and feed conversion in meat-type poultry. Dietary, harvesting age and seasonal variations in weight of birds interacted to yield significant differences.

Table 45. Variations in weight gain and survivability harvested on two different ages

Harvesting age	Daily body weight change (g b^{-1})							Weight gain (g)	Survivability (%)
	Initial	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6		
28-day	1665.00	1699.00	1728.00	1773.00	1786.00	1827.00	1849.00	184.00	99.17
35-day	2480.00	2593.00	2666.00	2715.00	2745.00	2806.00	2843.00	363.00	97.62
SED/LSD	79.800	88.700	32.900	106.900	114.300	117.200	130.300	81.100	1.557
Level of significance	**	**	**	**	**	**	**	**	NS

NS, Not significant; **, p>0.01

c. Health issues and survivability

Vaccinated birds and applying standard management practices helped to exhibit no major issues affecting bird health. Thus, survivability was almost similar (Tables 44-45 & 50). Survivability irrespective of feed, harvesting age and rearing season ranged from 96.37-100 percent.

Table 46. Weight gain and survivability of broilers harvested on two different seasons

Rearing season	Daily body weight change (g b^{-1})							Weight gain (g)	Survivability (%)
	Initial	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6		
Summer	1979.00	2057.00	2105.00	2153.00	2193.00	2242.00	2276.00	297.00	98.41
Rainy	2166.00	2236.00	2289.00	2336.00	2338.00	2391.00	2416.00	250.00	98.37
SED/LSD	79.800	88.70	93.200	106.900	114.300	117.200	130.300	28.600	1.557
Level of significance	**	**	**	**	**	**	**	NS	NS

NS, Not significant; **, p>0.01

d. Feed consumption

Unlike weight, daily feed intake was affected by feed type (Table 38). With the advancement of age, feed consumption slightly decreased except HFAT. The lowest consumption was reported in birds fed Paragon house feed and this pattern was persisted up to end of trial. Per bird feed consumption also gradually decreased from second day onward. Feed consumption trend found in HFAT is the usual phenomenon. However, boilers on HFAT feed consumed more also. Progressively lower feed consumption trends were found in all the broilers

harvested on 28 and 35-day age in different seasons from second day onward, though it was slightly recovered at the end of the trials. Summer-reared birds ate slightly higher amount of feed, and seasonal effects produced variable results.

Table 47. Effect of house feed/market feed on feed intake of broilers

House feed	Daily feed intake (g b^{-1})					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
HFAT	158.10	163.10	157.10	152.20	165.80	162.60
HFPA	148.80	158.00	145.30	147.90	134.50	133.00
Standard broiler diet	155.70	171.50	154.20	147.10	150.20	150.70
SED/LSD	5.130	8.390	9.480	10.320	28.510	28.300
Level of significance	NS	NS	NS	NS	*	*

NS, Not significant; *, p>0.05

Table 48. Feed consumption of broiler harvested on two different ages

Harvesting age	Daily feed intake (g b^{-1})					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
28-day	120.50	132.70	122.60	125.60	126.60	120.80
35-day	188.00	195.70	181.80	172.60	173.80	176.80
LSD	11.850	19.390	21.910	23.850	31.600	31.460
Level of significance	**	**	**	**	**	**

NS: Not significant; **, p>0.01

This progressively lowered consumption pattern is difficult to explain. Birds' physiological state, gut environment, orientation of new feeds might be possible reasons. Heavier birds harvested on 35-day ate significantly higher amount of feed at the end. Lowered feed intake during rainy season can be explained only by body physiological conditions of birds. Variable results (p>0.01) in body weight, feed consumption and survivability were interacted to yield significant results (Table 50). We found birds reared during summer were lighter (p>0.01) than rainy season ones which was in line of result of Sarma *et al*. (2020). Broilers exhibits optimal feed intake and weight gain when reared within the comfortable zone. Ferket and Gernat (2006) reported that environmental stresses had the most profound effects on flock-to-flock variation in feed intake that influenced both the body weight gain and feed conversion in meat-type poultry.

Table 49. Variations in feed intake of broilers harvested on two different seasons

Rearing season	Daily feed intake (g b^{-1})					
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6
Summer	150.30	165.90	154.70	162.30	161.50	150.50
Rainy	158.10	162.50	149.70	135.90	138.90	147.00
SED/LSD	4.180	6.850	7.740	23.850	23.280	11.110
Level of significance	NS	NS	NS	**	*	NS

NS, Not significant; *, p>0.05; **, p>0.01

Table 50. Effect of interaction of House Feed (HF), Harvesting Age (HA) and Rearing Season (SE) on the performances of broilers

Interaction	Body weight (g b^{-1})		Feed intake (g b^{-1})		Survivability (%)
	Initial	6-day	Day-1	Day-6	
$HF_1 \times HA_1 \times SE_1$	1717.00	1785.00	129.50	161.50	100.00
$HF_1 \times HA_1 \times SE_2$	1703.00	1845.00	132.50	128.50	100.00
$HF_1 \times HA_2 \times SE_1$	2359.00	2467.00	188.00	171.90	100.00
$HF_1 \times HA_2 \times SE_2$	2643.00	2831.00	182.30	188.70	100.00
$HF_2 \times HA_1 \times SE_1$	1575.00	1758.00	107.40	103.60	100.00
$HF_2 \times HA_1 \times SE_2$	1695.00	1785.00	126.70	117.10	100.00
$HF_2 \times HA_2 \times SE_1$	2293.00	2582.00	189.30	154.10	95.24
$HF_2 \times HA_2 \times SE_2$	2610.00	2826.00	171.80	157.10	100.00
$HF_3 \times HA_1 \times SE_1$	1598.00	2004.00	80.80	101.30	100.00
$HF_3 \times HA_1 \times SE_2$	1705.00	1920.00	145.90	112.50	95.00
$HF_3 \times HA_2 \times SE_1$	2333.00	3062.00	207.00	210.60	95.24
$HF_3 \times HA_2 \times SE_2$	2640.00	3288.00	189.30	178.20	95.24
SED/LSD	195.600	319.300	29.020	77.060	3.814
Level of significance	**	**	**	**	NS

HF_3, Standard broiler diet; **, p>0.01

11.8. Residual effects of dietary chemicals and heavy metals in broiler meat and eggs

a. Chemical residues in muscle and liver of broilers

Despite some of the feed samples contained certain chemical hazards (like lincomycine, chlortracycline, epichlortracycline, oxytetracycline, epioxytetracycline, tylosin or maduramicin) in varying levels, but none of these, as residues, were reported in liver and muscle of broilers except maduramycin and lincomycin (Tables 51-56) either in summer or winter. Lincomycin residue found in the liver sample (Feed 6) during winter was much lower than the cited MRL as mentioned in Table 52 (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252 Fcodex%252FStandards%252FCXM%2B2%252FMRL2e.pdf). Similarly, maduramycin residues in both liver and muscles either in summer or winter season were also lower than the MRL values (https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/maximum-residue-limits-mrls/list-maximum-residue-limits-veterinary-drugs-foods.html).

Table 51. Residues of chemicals and heavy metals in liver of broilers reared in summer

Chemicals/heavy metals	Feed 1 (BFPC)	Feed 2 (BFNH)	Feed 5 (BFPR)	Feed 6 (BFRR)	Feed 7 (BFPL)	MRL
Lincomycine (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Ciprofloxacin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Tylosin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Oxytetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Chlortetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Maduromycin (⌠g/kg)	BLQ	BLQ	BLQ	ND	BLQ	-
Pb (mg/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Cr (mg/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
As (mg/kg)	1.34	BLQ	BLQ	1.28	BLQ	2.0 mg/kg

ND, Not detected; BQL, Below limit of quantification

Table 52. Residues of chemicals and heavy metals in liver of broilers in winter

Chemicals/heavy metals	Feed 1 (BFPC)	Feed 2 (BFNH)	Feed 5 (BFPR)	Feed 6 (BFRR)	Feed 7 (BFPL)	MRL
Lincomycine (⌠g/kg)	BLQ	BLQ	BLQ	15.52	ND	0.5 mg/kg
Ciprofloxacin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	ND	-
Tylosin (⌠g/kg)	ND	BLQ	BLQ	BLQ	ND	-
Oxytetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Chlortetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Maduromycin (⌠g/kg)	32.98	BLQ	BLQ	ND	ND	0.5 mg/kg
Pb (mg/kg)	BLQ	BLQ	BLQ	BLQ	ND	-
Cr (mg/kg)	BLQ	BLQ	BLQ	BLQ	ND	-
As (mg/kg)	BLQ	BLQ	BLQ	1.40	ND	2.0 mg/kg

ND, Not detected; BQL, Below limit of quantification

Table 53. Chemicals and heavy metal residues in breast muscle of broilers during summer

Chemicals/heavy metals	Feed 1 (BFPC)	Feed 2 (BFNH)	Feed 5 (BFPR)	Feed 6 (BFRR)	Feed 7 (BFPL)	MRL
Lincomycine (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Ciprofloxacin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Tylosin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Oxytetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Chlortetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Maduromycin (⌠g/kg)	15.14	BLQ	BLQ	BLQ	BLQ	0.1 mg/kg
Pb (mg/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Cr (mg/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
As (mg/kg)	0.08	BLQ	0.06	0.10	BLQ	0.5 mg/kg

ND, Not detected; BQL, Below limit of quantification

b. Heavy metal residues in muscle and liver

Arsenic residues in varying levels in both muscle and liver samples during summer and winter seasons were common, but the levels were below the permissible limit (https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Maximum%20Levels%20of%20Contaminants%20in%20Foods%20_Beijing_China%20-%20Peoples% 20Republic%20of_12-11-2014.pdf) ANZFA, 2001) (Tables 51-56). A thigh muscle contained chromium residue during summer which was also far below the MRL value (FAO/WHO 2003). All other samples were free from chromium contamination. All liver samples were free from chromium and lead contamination (Tables 51 & 52). Results indicate that lead residue only in thigh muscle was found during summer (Table 45) and the level was within MRL level (Australia New Zealand Food Standards Code - Standard 1.4.1 - Contaminants and Natural Toxicants. https://www.legislation.gov.au/Details/F2011C00542.

Table 54. Chemicals and heavy metal residues in thigh muscle of broilers reared in summer

Chemicals/heavy metals	Feed 1 (BFPC)	Feed 2 (BFNH)	Feed 5 (BFPR)	Feed 6 (BFRR)	Feed 7 (BFPL)	MRL
Lincomycine (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Ciprofloxacin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Tylosin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Oxytetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Chlortetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Maduromycin (⌠g/kg)	20.96	BLQ	BLQ	17.81	BLQ	0.1 mg/kg
Pb (mg/kg)	BLQ	BLQ	BLQ	0.05	0.04	0.1 mg/kg
Cr (mg/kg)	BLQ	BLQ	0.09	BLQ	0.06	0.1 mg/kg
As (mg/kg)	0.10	BLQ	BLQ	0.14	BLQ	0.5 mg/kg

ND, Not detected; BQL, Below limit of quantification

Table 55. Chemicals and heavy metal residues in breast muscle of broilers in winter

Chemicals/heavy metals	Feed 1 (BFPC)	Feed 2 (BFNH)	Feed 5 (BFPR)	Feed 6 (BFRR)	Feed 7 (BFPL)	MRL
Lincomycine (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Ciprofloxacin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Tylosin (⌠g/kg)	ND	BLQ	BLQ	BLQ	BLQ	-
Oxytetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Chlortetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Maduromycin (⌠g/kg)	ND	BLQ	BLQ	BLQ	ND	-
4 Epioxytetracycline	ND	ND	ND	ND	ND	-
Pb (mg/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Cr (mg/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
As (mg/kg)	BLQ	BLQ	ND	0.09	BLQ	0.5 mg/kg

ND, Not detected; BQL, Below limit of quantification

Table 56. Chemicals and heavy metal residues in thigh muscle of broilers in winter season

Chemicals/heavy metals	Feed 1 (BFPC)	Feed 2 (BFNH)	Feed 5 (BFPR)	Feed 6 (BFRR)	Feed 7 (BFPL)	MRL
Lincomycine (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Ciprofloxacin (⌠g/kg)	BLQ	BLQ	BLQ	BLQ	BLQ	-
Tylosin (⌠g/kg)	ND	BLQ	BLQ	BLQ	BLQ	-
4 Epioxytetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Oxytetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Chlortetracycline (⌠g/kg)	ND	ND	ND	ND	ND	-
Maduromycin (⌠g/kg)	15.71	ND	BLQ	ND	ND	0.1 mg/kg
Pb (mg/kg)	BLQ	BLQ	BLQ	ND	ND	-
Cr (mg/kg)	BLQ	BLQ	BLQ	0.07	ND	-
As (mg/kg)	BLQ	BLQ	BLQ	0.09	BLQ	0.5 mg/kg

ND, Not detected; BQL, Below limit of quantification

c. Chemical and heavy metal residues in eggs

Results indicate that none of the chemicals and heavy metals had residual effect except chromium content in raw eggs (Table 57). The value found in Feed 2 was far below the MRL level (https://apps.fas.usda.gov/gainfiles/200608/146208660.doc).

Table 57. Residues of chemicals and heavy metals in egg during winter

Chemicals/heavy metals	Feed 1 (LFNH)	Feed 2 (LFIN)	Feed 3 (LFNO)	Feed 4 (LFPR)	Feed 5 (LFA1)	MRL
Lincomycine (∫g/kg)	-	-	-	-	-	-
Ciprofloxacin (∫g/kg)	-	-	-	-	-	-
Tylosin (∫g/kg) (**known sample**)	-	-	ND	-	-	-
4 Epioxytetracycline (∫g/kg)	ND	-	-	ND	-	-
Oxytetracycline (∫g/kg)	-	-	-	ND	-	-
Chlortetracycline (∫g/kg)	ND	-	-	-	-	-
Maduromycin (∫g/kg)	-	-	-	-	-	-
Pb (mg/kg)	-	BLQ	-	BLQ	BLQ	-
Cr (mg/kg)	BLQ	0.06	BLQ	BLQ	BLQ	1 mg/kg
As (mg/kg)	-	-	-	-	-	

ND, Not detected; BQL, Below limit of quantification

11.9. Quantification of drug and heavy metal residues in meat and egg samples of chicken

During the period of rearing, birds were treated, as and when necessary, with different therapeutic doses of antibiotics and some other drugs. Samples of such treated raw and roasted meat and eggs were collected and analyzed to quantify the residues of therapeutic drugs (Tables 58). The study reveals higher concentrations of residues compared to raw samples in most of the roasted breast meat and liver samples. In study also focused that most of the breasted muscle are with higher concentration of residues after heat treatment (roasting). It might be due to the water loss through evaporation during roasting/cooking that has resulted higher concentration of residues per unit of sample and this finding is not in agree with the findings of Gowtham *et al*. (2020). But, the current study findings made similar observations of Lolo *et al*. (2006) where the author reported increased residue concentration of drugs in muscles treated with heat (roasting).

Unlike breast muscles, thigh muscle demonstrated variable results. However, the common scenario found only in case of 4-Epioxytetracyclin and ciprofloxacin, were these two components are degraded through heat treatment. It is usually expected that concentration of residues became lower after heat treatment of meat. But, specific drug has definite heat tolerance capability; some degraded in relatively low and some on higher temperature. Besides, individual tolerance ability may also be a factor. Results also indicate that only tylosin had no residual effect even after providing therapeutic doses both on meat and eggs.

Table 58. Remaining drugs residues in raw and roasted breast and thigh meat of broilers

Active ingredients in commonly used therapeutics	Breast meat (µg/kg)		Thigh meat (µg/kg)		MRL (µg/kg)
	Raw	Roasted	Raw	Roasted	
Sulfadiazine	124.18	174.77	60.73	206.96	100 *
Trimethoprim	≤50.00	≤50.00	≤50.00	≤50.00	--
Erythromycin	BDL	BDL	BDL	12.77	--
Oxytetracycline	5.30	337.49	885.04	215.22	100-2000**
4 Epioxytetracyclin	24.19	ND	1443.14	ND	--
Tylosin	BDL	BDL	BDL	BDL	--
Doxycycline	854. 57	1452.58	762.94	6379.03	100***
Ciprofloxacin	19.22	ND	34.81	ND	100***
Tilmicosin	254.31	430.43	272.74	212.97	--

*Council Regulation (EEC), Codex Alimentarius Commission, FAO/WHO and Code of Federal Regulations
**European Union, Australia; Health Canada Veterinary Drugs Directorate; US Food and Drug Administration
***European Union

Table 59. Remaining drugs residues in raw and roasted liver samples of broilers

Active ingredient in commonly used therapeutics	Residues of drugs (µg/kg)		MRL (µg/kg)
	Raw	Roasted	
Sulfadiazine	90.35	94.77	100 *
Trimethoprim	≤50.00	69.59	--
Erythromycin	66.38	87. 36	--
Oxytetracycline	8.35	226.82	100-2000**
4 Epioxytetracyclin	28.15	ND	--
Tylosin	BDL	BDL	--
Doxycycline	6445.25	3567.71	100**
Ciprofloxacin	530.06	ND	100**
Tilmicosin	5455.68	20909.38	--

*Health Canada Veterinary Drugs Directorate, EU, USDA
**EU

Table 60. Remaining drugs residues in fresh egg of layer

Active ingredient in commonly used therapeutics	Residues (µg/kg)	MRL)
Sulfadiazine	1072.25	100 µg/kg*
Trimethoprim	ND	-
Erythromycin	2582.82	50 ppb**
Oxytetracycline	385.07	400 ppb**
4 Epioxytetracyclin	ND	400 ppb**
Tylosin	BLQ	300 ppb**
Doxycycline	5811.36	-
Ciprofloxacin	ND	-
Tilmicosin	1489.85	-

* Codex Alimentarius Commission
** https://www.sfa.gov.sg/docs/default-source/default-document-library/veterinary-drug-residues.pdf

It was stated earlier that some chemicals/drugs and heavy metal residues were reported in a few meat samples fed different commercial poultry diets. Those meat and liver samples were also analyzed and results presented in Table 61. Results indicate that none of these samples had residues after roasting.

Table 61. Residues of drugs and heavy metals in thigh meat and liver of broilers

Drug/heavy metal	Thigh meat (Feed-BFCP)		Liver (Feed-BFCP)		Liver (Feed-BFRR)		MRL (mg/kg)
	Raw	Roasted	Raw	Roasted	Raw	Roasted	
Lincomycine (µg/kg)	-	-	-	-	15.52	ND	0.50
Maduromycin (µg/kg)	15.71	BDL	32.98	ND	-	-	0.10 in muscle 0.50 in liver
As (mg/kg)	-	-	-	-	1.40	ND	2.00

Notably, present findings are based on the analytical results of small portion of samples. Covid-19 epidemic as well as lack of fund hampered the research activities. Therefore, to know the exact scenario further study should be needed considering representative samples.

11.10. Determination of microbial quality of poultry feed, raw and process meat and egg

Tube well water at farmhouse found free from *E. coli* and *Salmonella* (Table 62). But, tap water fond contaminated with salmonella. Litter materials (rice husk) found contained both *E. coli* and *Salmonella* even after sun-drying. Commercial broiler and layer feed possessed varying amount of *E. coli* and *Salmonella* which is probably due to age of feed, plant hygiene, source of ingredient, storage condition, etc. Both *E. coli* and *Salmonella* were found in raw meat. Some of the biochemical results are shown in Table 63.

Table 62. Identification of microbial load from different sources

Sources	E. coli (cfu/ml or cfu/g)	MRL	Salmonella spp. (cfu/ml or cfu/g)	MRL
Tube well water (Dinajpur)	-	<1cfu/100ml[1]	-	-
Tap water	-		2	
Soil	13×10^{6}	-	41×10^{6}	-
Rice husk (as a litter)	15×10^{5}	-	57×10^{5}	-
Chick box	29×10^{4}	-	2×10^{4}	-
Newspaper (as a thin paper)	580×10^{3}	-	246×10^{4}	-
Dressing broiler water	3	-	2	-
Broiler meat	9×10^{10}	500 CFU/g as the upper limit	7×10^{10}	-
Broiler droppings	530×10^{10}		285×10^{10}	
Layer feed (NOUL)	10×10^{9}		25×10^{9}	3.15×104 (Wray et al., 1996)
Layer feed (NEHL)	18×10^{10}		2×10^{10}	
Layer feed (AA1L)	18×10^{10}		25×10^{10}	
Layer feed (PARL)	9×10^{10}		6×10^{10}	
Layer feed (INDL)	21×10^{10}		8×10^{10}	
Broiler feed (RRPB)	7×10^{9}		3×10^{9}	
Broiler feed (NEHB)	8×10^{9}		5×10^{9}	
Broiler feed (CPCB)	3×10^{9}		-	
Broiler feed (PALB)	38×10^{9}		9×10^{9}	
Broiler feed (PROB)	28×10^{9}		15×10^{9}	

[1]Source: https://www.unicef.org/bangladesh/sites/unicef.org.bangladesh/files/2018-10/Drinking%20Water%20 Quality % 20 in%20Bangladesh.pdf [2]Established by the PTS 201.054 https://www.redalyc.org/journal /3613/ 361364361013 /html/)

Table 63. Biochemical test results

Organisms	Catalase activity	MR	VP	Citrate use	Indole Production	Starch Hydrolysis	Hydrogen Sulfide
E.coli	+	+	-	-	+	-	-
Shigella	+	+	-	-	+	-	-
Salmonella	+	+	-	+	-	-	+

12. Research highlights

Title of sub-project: ***Value addition and standardization of nutritional level in selected food items from animal and plant origin***

Background

Food contamination is a global food safety issue. With the increase in production, different types of hazardous adulterant and contaminant in different stages of production, processing, and marketing chains of feed and food are practiced in Bangladesh. Chickens often exposed to various toxic chemicals, antimicrobial and some other drugs and some growth promoters/hormone derivatives due to weak government policy, lack of awareness of producers, excessive dependency on non-technical traders, carelessness of some technical personnel, inadequate policy guidelines and lack of strong monitoring of law enforcing agencies, etc. There are four primary categories of food safety hazards to consider: biological, chemical, physical, and allergenic (https://blog.smartsense.co/food-safety-education-month-hazards-prevention). Understanding the risks associated with each can dramatically reduce the potential of a foodborne illness. Attitudes in society towards food safety and contamination are often rooted in tradition and habit (Lesa A. et. al. 2019). Food and feed safety laws are essential, with monitoring of feed, food and water contamination, as well as enacting measures to reduce and eliminate hazardous substances, adulterants and pollutants.

Objectives

Along with the general objective of the sub-project there are a set of specific objectives under each components to achieve the project goal. However, for convenience of presentation, all these specific objectives has been combined together and categorized into five as per nature of activities are as follows:

1. Quantification of hazards and contaminants in selected drinks and some other food items;
2. Inventory on poultry industry and identifying various hazards in poultry feeds;
3. Determining the effects of feeds on egg production and growth performances of birds;
4. Determining the residues in poultry-originated food;
5. Assessing the implementation of HACCP standard in feed and food production.

Methodology followed

Hazards, adulterants, contaminants and their residues in poultry feed, milk, meat, egg, drinks and some other food items were identified through survey. Quantification were done from an accredited laboratory, SGS Bangladesh Ltd. Specific methodology was followed for each of

the studies designed as described in methodology section of this report. Data were analyzed in appropriate design of the experiments using computer based statistical package.

Key findings : Findings are summarized according to the objectives stated in this section.

Objective 1: Quantification of hazards, and contaminants in selected drinks and some other food items

- Caffeine concentration of local energy drink was within the limit of BDS (145/L), but it was much higher in case of both the imported fruit juice samples.
- Street food samples were heavily loaded with total *Coliform, E. coli,* and *S. aureus* except *Salmonella* sp. But lemon and *tetul* water contained low bacterial colony.
- All of 16 sugar samples (branded and non-branded) in Bangladesh markets were contaminated with Sodium cyclamate (<1.0 ppm). Highly alarming concentration was detected in the non-branded sugars samples collected from Kachukhet Market of Dhaka.
- Antibiotics, Sulphur drugs and heavy metals residue levels in pasteurized and unpasteurized milk samples were within permissible limits.

Objective 2: Inventory on poultry industry and identifying various hazards in poultry feeds

- Broiler, layer and sonali DOC in 2020 were 51.0, 4.16 and 31.20 crores, respectively against 70.0, 4.74 and 35.0 crores in 2019. During Covid-19 outbreak commercial feed (including poultry) production lowered down to 5.04 MMT/year against 6.57 MMT in 2019-2020.
- Ciprofloxacin (28.9%) found as the most common antibiotic. About 75.3% drug sellers sold medicine without prescription and main antibiotics prescribed by the veterinarians were ciprofloxacin (40%) followed by enrofloxcin, amoxicillin, doxycycline, oxytetracycline, colistin, tylosin and tilmicosin. Toltazuril (37.7%) was the main anticoccidial drug followed by salphaclozine sodium.
- None of the commercial broiler feeds under investigation contains ciprofloxacin, enrofloxacin or neomycin. Feeds 3, 5 and 8 were free from all selected chemical hazards. Feeds 4 and 6 contained lincomycine and feeds 1 and 7 contained moderate levels of maduramicin, but the levels were far below the permissible limit. Chlortracycline and epichlortracycline content in Feed 1 was also below recommended level, but remarkably higher in Feed 2. Oxytetracycline and its derivative levels were exceptionally high only in Feed 7 against MRL value. Tylosin content only in Feed 1 was exceptionally higher, although other feed contained no such chemical.
- None of three market/house feeds contained hazardous substances except maduracycin.
- All broiler, layer and house feeds contained varying levels of heavy metals all house feed samples contained much lower amount of crude protein than the mount shown in the bags label. Calcium levels were higher, but phosphorus levels were variable

Objective 3: Determining the effects of feeds on egg production and growth performances of birds

- Weights of pullets were influenced geographical locations, not by feed and season. Pullets came to lay earlier at Dinajpur and during summer. But, feed had no influence. Pullets achieved delayed 50% and peak eggs production.

- Other than rainy season, no birds gained target weight of 1630-1730g at 20-week age.
- Survivability was almost similar. However, micoplasmosis, salmonellosis, feed toxicity, egg bound, and cannibalism were the enlisted diseases/disorders.
- Though birds' survivability's recorded similar, but most common health related problems were suffocation, immune suppression, omphalitis, gaseous accumulation, mycoplasmosis, coccidiosis, etc.
- Initial body weight of Kazi Gold genotype was much higher than the local one and hence growth rate and feed consumption were remarkably higher in Kazi Gold..
- Birds reared on broiler grower feed gained marketable weight at 45-day and this weight was achieved on 60-day by the same birds consumed Sonali grower feed.
- Birds irrespective of genotype and feed type reached the target body weight on 45-day during summer and on 60-day in rainy season. Birds received more Sonali grower feed.
- Though survivability was similar. Omphalitis, mycoplasma and coccidisis outbreak is common. Survivability was almost similar irrespective of feed, harvesting age and rearing season.

Objective 4: Determining the residues in poultry-originated food

Feeds under trial contained either of lincomycine, chlortracycline, epichlortracycline, oxytetracycline, epioxytetracycline, tylosin or maduramicin in varying levels. But none of these were retained in liver and muscle of broilers except maduramycin and lincomycin.

- Lincomycin residue found in the liver sample (Feed 6) during winter was much lower than the cited MRL. Similarly, maduramycin residues in both liver and muscles both in summer and winter seasons were also lower than the MRL values.
- Arsenic residues were reported during summer and winter seasons in varying levels in both muscle and liver, but it was below the permissible limit.
- Muscle samples were free from chromium contamination except a thigh muscle that contained negligible amount chromium residue during summer. Liver samples were free from chromium and lead contamination.
- None of the chemicals and heavy metals had residual effect except chromium content in raw eggs that was far below the MRL level.
- An egg sample containing therapeutic dose of tylosin had no residual effect.

Objective 5: Assessing the implementation of HACCP standard in feed and food production

- Commercial feeds were found to be contaminated either with antibiotics, heavy metals and microorganisms in varying levels and needed to be addressed during feed manufacturing.
- Chick boxes, thin paper, soil, drinking water, wash water and litter at farm level were contaminated with microorganisms and attention should be paid to reduce microbial load.
- Microbial loads were reported in raw broiler meat and thus care should be taken in using water for washing the carcass at wet market.
- Poultry meat and eggs were found to be health-friendly although varying levels of chemicals and heavy metals were detected in poultry feed, raw meat and eggs.

Keywords: Hazards, **C**ontaminants, Poultry feed, Animal and plant originated food.

B. Implementation Status

1. Procurement (component wise)

i. Coordination component

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical No.	Financial (Tk)	Physical No.	Financial (Tk)	
Office equipment					
Desktop computer	1	435000/	1	435000/-	Target achieved as per plan
Laptop	1		1		
Laser Printer	1		1		
Color Printer	1		1		
UPS	1		1		
Scanner	1		1		
Digital Camera	1		1		
(b) Lab &field equipment	Not applicable				
(c) Other capital items	Not applicable				

ii.Component-1: PSTU

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical No.	Financial (Tk)	Physical No.	Financial (Tk)	
a) Office equipment					Target achieved 100%
Furniture					
Executive Table	1	98500	1	98500	
Executive Chair	1		1		
Visitor/front Chair	4		4		
Steel Almira	1		1		
Computer Table	1		1		
Computer Chair	1		1		
Steel Rack	1		1		
Computer and accessories					
Desktop	2	385000	2	382000	
Laptop	1		1		
Scanner	1		1		
UPS	2		2		
Photocopier	1		1		
Digital camera	1		1		
b) Lab and field equipment					
Refrigerator	1	1189323	1	1189323	
Oven	1		1		
Whole egg analyser	1		1		
Deep fridge (-30)	1		1		
Balance	1		1		
Thermoscan	1		1		
c) Others capital items					
Motorcycle	1	446400	1	446400	
Software	1		1		

iii. Componene-2: HSTU

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical No.	Financial (Tk)	Physical No.	Financial (Tk)	
a) Office equipment					
Multipurpose Book Self	01		01		
Executive Chair	01		01		
Visitor /Front chair	04	100000	04	99950	
Office/Steel Almira	01		01		Target achieved 100%
Computer table	01		01		
Computer chair	01		01		
b) Lab and field equipment					
Refrigerator	01		01		
Deep freeze (-20^{0}C)	01		01		
Oven	01	275000	01	274600	
Ceiling fans	05		05		
Thermoscan	01		01		
Balance analytical	02		02		
c) Other capital items					
Digital camera	01		01		
Desktop computer	02	235000	02	234800	
Laptop computer	01		01		
Scanner	01		01		
UPS	02		02		

2. Establishment/renovation facilities

i. Coordination component: *Not applicable*.

ii. Component-1: PSTU

Description of the facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
Poultry shed management	01	100	--	--	Target achieved 100%

iii. Component-2: HSTU: *Not applicable*

3. Training/Study tour/Seminar/Workshops/Conference organized

i. Coordination component

Description	Number of participants			Duration (Days)	Remarks
	Male	Female	Total		
Coordination component					
Inception Workshop (1)	56	7	63	1 day	All workshops held at the Conference room of BARC as per schedule of activity of the Coordination component
Half yearly Research Prog. Review Workshop (2 no.)	65+62	9+8	144	1+1 = 2 days	
Annual Research Prog. Review Workshop (2 no.)	60+63	7+8	138	1+2 =3 days	

Project Completion Report Review Workshop (1 no)	45	6	52	1 day	
Component-1: PSTU					
Others Awareness meeting – 04 No.	68	12	80	01 day	
Component-2: HSTU					
					Not applicable

C. Financial and Physical Progress (Combined and Component wise)

i. Financial progress *(Combined)*

Items of expenditure/ activities	Total approved budget (Tk.)	Fund received	Actual expenditure	Balance unspent	Physical progress (%)	Reasons for deviation
A. Contractual Staff Salary	7673074	7644162	7641201	2961	99.58	
B. Field Research/ Lab expenses and supplies	15335796	14576436	14484018	92418	94.45	
C. Operating Expenses	1307510	1297640	1272713	24927	97.34	
D. Vehicle Hire and Fuel, Oil & Maintenance	1083025	1074394	1038542	35852	95.89	
E. Training/ Workshop / Seminar etc.	228600	228600	228600	0	100.00	
F. Publications and printing	429900	412516	412400	116	95.93	
G. Miscellaneous	383167	360873	359080	1793	93.71	
H. Capital Expenses	3197800	3166797	3165450	1347	98.99	
Grand Total	**7673074**	**7644162**	**7641201**	**2961**	**99.58**	

ii. Coordination component (BARC)

Items of expenditure/ activities	Total approved budget (Tk.)	Fund received	Actual expenditure	Balance unspent	Physical progress (%)	Reasons for deviation
A. Contractual Staff Salary	3941545	3941545	3941545	0	100.00	
B. Field Research/ Lab expenses and supplies	680441	680441	680441	0	100.00	
C. Operating Expenses	256854	255826	255826	0	99.60	
D. Vehicle Hire and Fuel, Oil & Maintenance	186338	185856	185856	0	99.74	
E. Training/ Workshop / Seminar etc.	228600	228600	228600	0	100.00	
F. Publications and printing	349900	332700	332700	0	95.08	
G. Miscellaneous	121322	121155	121155	0	99.86	
H. Capital Expenses	435000	435000	435000	0	100.00	
Grand Total	**6200000**	**6181123**	**6181123**	**0**	**99.70**	

iii. Component-1: PSTU

Items of expenditure/ activities	Total approved budget (Tk.)	Fund received	Actual expenditure	Balance unspent	Physical progress (%)	Reasons for deviation
A. Contractual Staff Salary	1976123	1978234	1975273	2961	99.96	
B. Field Research/ Lab expenses and supplies	10898981	10442033	10352641	89392	94.99	
C. Operating Expenses	643158	634316	633781	535	98.54	
D. Vehicle Hire and Fuel, Oil & Maintenance	661687	653538	617686	35852	93.35	
E. Training/ Workshop / Seminar etc.	0	0	0	0	0	
F. Publications and printing	50000	49816	49700	116	99.40	
G. Miscellaneous	159883	137756	136425	1331	85.33	
H. Capital Expenses	2153400	2122397	2121100	1297	98.50	
Grand Total	**16543232**	**16018090**	**15886606**	**131484**	**96.03**	

iv. Component 2: HSTU

Items of expenditure/ activities	Total approved budged (Tk.)	Fund Received	Actual expenditure	Balance unspent	Physical progress (%)	Reasons for deviation
A. Contractual Staff Salary	1755406	1724383	1724383	00	98.23	
B. Field Research/ Lab expenses and supplies	3756374	3453962	3450936	3026	91.87	
C. Operating Expenses	407498	407498	383106	24392	94.01	
D. Vehicle Hire and Fuel, Oil & Maintenance	235000	235000	235000	00	100.00	
E. Training/ Workshop / Seminar etc.	00	00	00		00	
F. Publications and printing	30000	30000	30000	00	100.00	
G. Miscellaneous	101962	101962	101500	462	99.55	
H. Capital Expenses	609400	609400	609350	50	99.99	
Grand Total	**6895640**	**6562205**	**6534275**	**27930**	**94.76**	

D. Achievement of Sub-project by objectives (Tangible from): Technology generated/ developed

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Outputs	Outcome (short term effect of the research)
To identify various hazards in poultry feeds and their effects in egg production performances and hazardous residues in eggs	1. Detailed survey to explore the: i. present state of poultry industry ii. hazardous materials in poultry industry, and iii. value added poultry products	1. A number of antimicrobial (known as feed-grade antibiotics) and anticoccidial drugs are used in feed. 2. About 85% dealers provide veterinary services to farmers, when necessary. 3. Antimicrobial drugs viz. ciprofloxacin, enrofloxcin, amoxicillin, doxycycline, oxytetracycline, colistin, tylosin	Extent of antimicrobial drug and supplemental products are invested and the scope of unethical use of these in poultry business will be reduced.

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Outputs	Outcome (short term effect of the research)
		and tilmicosin, toltazuril, salphaclozine sodium and additives, enzymes, probiotics and growth promoters were suggested by veterinarians. 4. Value added frozen poultry products include ready-to-eat, ready-to-cook, ready to fry and snakes, etc.	
	Determination of nutrient composition and hazardous materials in layer and broiler feeds	1. Lincomycin, tylosin, oxytetracycline, cholotetracycline, neomycin, colistin etc. are at times used in feed industry. 2. Broiler feeds were contaminated either of chlortracycline, oxytetracycline, tylosin, lincomycin or maduromycin. 3. House feeds were free from antibiotics. 4. Layer feeds were contaminated either with chlortracycline, oxytetracycline or maduromycin. 5. Both broiler and layer feed contained varying levels of heavy metals. 6. House feed contained much lower CP.	Hazard free poultry feed will be manufactured and health-friendly meat and eggs will beproduced.
	Growth and production performances and egg quality characteristics of pullets	1. Weight of pullets was not often affected by feeds and geographical locations. But, seasonal variations were significant. 2. Feed consumption patterns of birds were variable. 3. Although birds at Dinajpur attained maturity earlier, but they reached peak production at similar age. 4. Birds at Barishal laid larger eggs that that of Dinajpur. 5. Survivability was almost similar. 6. Dietary treatments did not affect internal eggs quality.	Good management practices will enhance better productivity and profitability.
To identify and quantify the effects of various hazards in broiler performances and hazardous residues in broiler meat	Meat yield and its quality in broilers fed selected commercial feed	1. Weight gain and feed intake of bird was significantly affected by feed, location and season. 2. Survivability was almost similar. 3. The common health problems were suffocation, immune suppression, omphalitis, gaseous accumulation, mycoplasmosis, coccidiosis, etc	Better production and profitability will be achieved.
	Performances of various chicken hybrids (Sonali)	1. Kazi gold genotype showed production excellence over local sonali and achieved earlier market weight. 2. Survivability was almost similar. 3. Omphalitis, mycoplasma and coccidiosis outbreak are common.	Genetic excellence will boost Kazi gold genotype for better profitability within relatively shorter period.
	Effect of house feed on weight change of broilers at different marketing ages	1. Inferior growth due to lower CP content of house feeds compared to standard broiler diet. 2. Progressively lowered feed consumption pattern was reported up to the end.	Standard broiler feed will be used to achieve better weight at wet marker.

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Outputs	Outcome (short term effect of the research)
To assess the chemical and biological contaminants in raw and value added poultry products at market chain	Residual effects of chemicals and heavy metals in poultry meat and eggs	1. No chemicals residues were found in liver and muscle of broilers except maduramycin and lincomycin. 2. Arsenic residues were found in muscle and liver, but all liver and muscle samples were free from chromium and lead contamination except a thigh muscle. 3. Diet had no influence in egg content. 4. No residual effect of therapeutic tylosin was found in raw egg.	Safe eggs will be produced for healthy nation.
	Determination of microbial quality of poultry feed, raw and processed poultry products	1. Tubewell water fair, but tapwater contained salmonella. 2. Litter material was infected with E. coli and Salmonella. 3. Commercial broiler and layer feed possessed varying amount of E. coli and Salmonella. 4. Both E. coli and Salmonella were found in raw meat.	
To assess the implementation of HACCP standard in feed and food production from poultry industry.	Awareness building among the stakeholders to produce safe food from poultry	Four awareness meetings were arranged in three upazilas of Barishal	Hazard free poultry products will be avaulable.
	i. Feed analysis ii. Microbial count in tube well and tape water, soil, litter, thin brooding paper and chick boxes for microbial loads iii. Analysis of broiler meat and eggs for antibiotic and heavy metals.	1. Broiler, layer or house feeds were contaminated either with antibiotics, heavy metals or microorganisms. 2. Chick boxes, soil, drinking water, washing water and litter were also contaminated with microorganisms. 3. Microbial loads were found in raw broiler meat. 4. Residues of some of the chemicals and heavy metals were detected in raw meat and eggs. 5. No effect of therapeutic tylosin in eggs.	Frequent monitoring of feed mills, dealers points, etc. to reduce unjustified and unauthorized use of hazardous chemicals and also implementation of HACCP standard in the industry.

E. Information/Knowledge generated/Policy generated

General/specific objectives of the sub projects	Major technological activities perform in respect of the set objectives	Output	Outcome (Short term effect of the research)
Objective 1 Quantify the caffeine concentration of energy drinks and the concentration;	• Collection of local produced and imported energy drink samples from the city markets; • Analysis of energy drink samples for determining the caffeine concentration of the samples in accredited laboratories	• Caffeine concentration of eight local energy drink samples showed their presence within the limit of BDS (145/L) ranging from 75.55 – 116.05mg/Kg. However, caffeine concentration was found much higher than the BDS level in case of both the imported fruit juice samples	Consumers will become aware in using imported energy drinks
Objective 2 Determine the type and level of	• Collection of commonly used street foods of Dhaka city, marketed sugar	• Street foods were found highly Contaminated/loaded with germs • Sixteen sugar samples (branded	Consumers will become aware about the consumption of street food with highly

General/specific objectives of the sub projects	Major technological activities perform in respect of the set objectives	Output	Outcome (Short term effect of the research)
contaminants of some selected street foods of city areas; and to evaluate the type and level of antibiotics, Sulphur drugs and heavy metal contaminants in market milks	samples and milk samples; • Analysis of street foods for bacterial load determination; • Analysis of sugar samples for sodium cyclamate determination; and • Analysis of milk samples for antibiotic, Sulphur drugs and heavy metal determination	and non-branded open sold) of Bangladesh markets revealed that 100% of the sugar samples are contaminated with Sodium Cyclamate (<1.0 ppm). Highly alarming concentration of Sodium Cyclamate was detected in the non-branded open sold sugars samples collected from Kachukhet Market of Dhaka; **Milk samples:** a. Left over/remaining antibiotic residue in pasteurized and unpasteurized milk samples were below detectable level b. Left over/remaining Sulpher drug residue in pasteurized and unpasteurized milk sample were below detectable level in all cases. c. Left over/remaining heavy metal (Pb, Cr) residue in pasteurized and unpasteurized milk sample shows below MRL in all the cases.	contaminated germs and toxic level of marketed sugars
Objective 3 To identify various hazards in poultry feeds and their effects in egg production performances and hazardous residues in eggs	• Detailed survey to explore the**:** status of DOCs and poultry feed production, • hazardous materials in poultry feed, additives/growth promoters, preservatives, usages of antibiotics/ therapeutics in poultry industry, and value added poultry products	1. Commercial broiler, layer and sonali DOC in 2020 were 51.0, 4.16 and 31.20 crores, respectively against 70.0, 4.74 and 35.0 crores in 2019. 2. Among 261 registered feed mills, 140 renewed license. More than 200 feed mills are unregistered. 3. Feed companies produced 6.57 MMT commercial feed, of which poultry feed accounts for 4.45 MMT (38-40% broiler, 6-6.5% layer and 22-23% sonali), fish feed 1.59 MMT and cattle feed 0.53 MMT. Now it squeezes to 5.04 MMT/year. 4. Antibiotics (known as food-grade antibiotics) to suppress organism growth as well as accelerate growth and anticoccidial agents are used in feed. 5. About 92% farmers used antibiotics, anticoccidial drugs, enzymes and growth promoters of different trade names in every flock. 6. More than 84% dealers provide veterinary services to their farmers. Among the antibiotics used, ciprofloxacin ranked first followed by oxytetracycline, amoxicillin, tylosin, tilmicosin, doxycycline, and neomycine. 7. About 75.3% dealers/drug sellers sold medicine without physician's prescription. 8. Veterinarians' suggestions on antibiotics by rank were namely	1. Scope of unethical and unauthorized practices in feed and poultry business will be reduced through strict monitoring and surveillance. 2. Better long-term future planning for increased production and ensuring safe quality feed, food and value-added poultry.

General/specific objectives of the sub projects	Major technological activities perform in respect of the set objectives	Output	Outcome (Short term effect of the research)
		ciprofloxacin, enrofloxcin, amoxicillin, doxycycline, oxytetracycline, colistin, tylosin and tilmicosin. Toltazuril was the main drug of choice as anticoccidial drug followed by the salphaclozine sodium. There were additives, enzymes, probiotics and growth promoters also. 9. Value added frozen foods include ready-to-eat, ready-to-cook, ready to fry and snakes, etc. mostly produced by about 15 prominent companies.	
	Determination of nutrient composition and hazardous materials in layer broiler feeds and selection of feeds.	1. Some of the feed manufacturers used lincomycine, chlortetracycline, oxytetracycline and its derivative, tylosin and maduramicin in varying levels in broiler feeds. But, no one added ciprofloxacin, enrofloxacin or neomycin. 2. Broiler feeds contained various levels of lead, chromium and arsenic levels. 3. Similarly, some of the hazardous chemicals like chlortracycline and its derivate, oxytetracycline and its derivative were reported in some layer feeds. Some feed companies declare that grower feeds contain maduromycin. 4. All layer feeds were also contaminated with heavy metals in different levels. 5. House feeds were free from antibiotics. But, they were contaminated with heavy metals also. 6. Broiler feeds contained standard CP. But Ca and P contents were variable. 7. Nutrient content of layer feeds were nearer to standard levels. 8. House feed contained much lower CP and higher Ca levels. 9. Based on the findings, five boiler, five layer and two house feeds containing various levels of chemical hazards, heavy metals and nutritional components for further field experimentation.	1. Nutritionally balanced feed formulation will be increased to reduce feed cost and increase productivity. 2. Increased possibility of manufacturing chemical hazard free poultry feed production. 3. Opportunity to choose feed ingredients for producing feeds with minimum levels of heavy metals.
	Growth and production performances and egg quality characteristics of pullets	1. Weight gain of pullets from 15-week onwards was almost similar provided with different feeds and geographical locations. But, seasonal variations in body weight were large. 2. After onset of lay, pullets at	1. Proper management including feeding and disease management helped to attain target weight and sexual maturity and thus increased productivity and profitability.

General/specific objectives of the sub projects	Major technological activities perform in respect of the set objectives	Output	Outcome (Short term effect of the research)
		Dinajpur consumed more feed. However, feed consumption patterns were variable. 3. Pullets at Dinajpur gained maturity earlier than Barishal. But, they reach peak production at similar age. 4. Birds at Barishal laid larger eggs that that of Dinajpur. 5. Survivability was almost similar. Micoplasmosis, salmonellosis, feed toxicity, egg bound, and cannibalism were the most common problems. 6. Dietary treatments did not affect internal eggs quality.	
Objective 4 To identify and quantify the effects of various hazards in broiler performances and hazardous residues in broiler meat	Meat yield and its quality in broilers fed selected commercial feed	1. Slaughter weights of birds were significantly lighter at Dinajpur, heavier on feed Provita and New Hope and larger in winter. 2. With respect to feed conversion, birds at Dinajpur were inferior, the best fed New Hope and less efficiently during winter. 3. None of location, feed, rearing season or their interactions influenced birds' survivability. 4. The common health problems were suffocation, immune suppression, omphalitis, gaseous accumulation, mycoplasmosis, coccidiosis, etc	1. Balanced feed, optimum management, cleanliness and health management reduce disease incidence, decrease mortality and increase performances and profitability.
	Performances of various chicken hybrids (Sonali)	1. Genotypic excellence boosts rapid growth and attains early market weight of Kazi Gold compared to local Sonali offered broiler grower or sonali grower feed. Birds irrespective of genotype and feed gained target weight of 800g b-1 on 45-day, whereas local Sonali attain this on 60-day. 2. Survivability was almost similar. 3. Improper brooding during early ages causes omphalitis, mycoplasma outbreak in the flock. Coccidisis outbreak is almost general phenomenon.	1. Genetic excellence along with appropriate brooding and other management events achieved higher productivity of meat birds of public preference at least 15 days earlier. 2. Reduced total cost and thus farmers benefitted.
	Effect of house feed on weight change of broilers at different marketing ages	1. Due to lower CP content of house feeds, they produced much inferior gain compared to standard broiler diet at the end of a 6-day trial. 2. Birds, irrespective of feed and harvesting age, gained almost similar weight at the end. 3. Progressively lowered feed consumption pattern was reported up to the end.	1. Wet marker retailers may use house feed for only 1-2 days retention. Besides, broiler feed seems better for weight gain.
Objective 5 To assess the chemical and biological contaminants in raw	Residual effects of chemicals and heavy metals in poultry meat and eggs	1. None of the chemicals reported to found in commercial broiler feeds was retained in liver and muscle of broilers except	1. Egg, broiler meat and other edible parts produced from commercial feeds are

General/specific objectives of the sub projects	Major technological activities perform in respect of the set objectives	Output	Outcome (Short term effect of the research)
and value added poultry products at market chain		maduramycin and lincomycin. Levels of these two were within the MRL value. 2. Arsenic residues were found in both muscle and liver, but it was below the permissible limit. 3. All liver and muscle samples were free from chromium and lead contamination except a thigh muscle. 4. Only a muscle sample contained lead residue which was below MRL. 5. Dietary content of chemicals had no influence in egg content. 6. Chromium content in eggs derived from dietary source was below MRL. 7. No residual effect of therapeutic tylosin was found in raw egg.	safe for human consumption. 2. Safe eggs are produced even after therapeutic doses of tylosin.
	Determination of microbial quality of poultry feed, raw and processed poultry products	1. Tube well water is free from E. coli and Salmonella. 2. Tap water contained salmonella. 3. Litter material was infected with E. coli and Salmonella even after sun-drying. 4. Commercial broiler and layer feed possessed varying amount of E. coli and Salmonella. 5. Both E. coli and Salmonella were found in raw meat.	
Objective 6 To assess the implementation of HACCP standard in feed and food production from poultry industry.	Awareness building among the stakeholders to produce safe food from poultry 1. Analysis of feed for antibiotics, heavy metals and microbial loads 2. Analysis of chick boxes for microbial count 3. Analysis of tube well and tape water, soil, litter and thin brooding paper for microbial loads 4. Analysis of house feed for antibiotic and heavy metals. 5. Dressing hot waters of processing shops for icroorganisms. 6. Analysis of broiler meat for antibiotic, heavy metals and microbial count. 7. Analysis of eggs for antibiotic and heavy metals.	Four awareness meetings were arranged in three upazilas of Barishal 1. Most of the commercial broiler and layer feeds were found to be contaminated with antibiotics, heavy metals and microorganisms in varying levels. 2. Chick boxes, soil, drinking water, washing water and litter material at farm were also contaminated with microorganisms. 3. Microbial loads were found in raw broiler meat and hot dressing water of processing plant. 4. House feed was adulterated with heavy metals. 5. Residues of some of the chemicals and heavy metals were detected in raw meat and eggs, though the levels were below MRL. 6. No effect of therapeutic tylosin in eggs.	1. Ample opportunities are there to reduce the use of chemicals in different feed production steps and broiler processing at wet market. 2. Judicial application of the knowledge of feed ingredient selection and procurement for father use in feed formulation to reduce heavy metals load in compound feed. 3. Frequent monitoring of feed mills, dealers points, etc. to reduce unjustified and unauthorized use of hazardous chemicals.

F. Materials Development/Publication made under the sub-project

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ ***booklet/leaflet***/flyer etc.	-	01 (One)	স্ট্রীট ফুড কতটা নিরাপদ ও স্বাস্থ্যকর. **ড. মোঃ মনিরুল ইসলাম.** বাংলাদেশ কৃষি গবেষণা কাউন্সিল, ফার্মগেট, ঢাকা-১২১৫
	02	-	1. ব্রয়লার মুরগির খ্যাদ্য ও মাংসে ৰতিকর উপাদান এবং মানুষের স্বাস্থ্য ঝুকি; Dept. of poultry Scs, PSTU & Deptt. Dairy & Poultry Scs. HSTU. Publisher: Graphics Mart, Kalibari Road, Barishal,Publishing year: 2021 2. বানিজ্যিক লেয়ার মুরগীর ডিম কি মানুষের স্বাস্থ্যের জন্য ৰতিকর Dept. of poultry Scs, PSTU & Deptt. Dairy & Poultry Scs. HSTU. Publisher: Graphics Mart, Kalibari Road, Barishal, Publishing year: 2021
Journal publication	02	01	Effect of house feed on weight change of commercial broilers harvested at different marketing ages in Bangladesh. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 14, Issue 7 Ser. II (July 2021), PP 01-04. www.iosrjournals.org
Video clip/TV program	01	03	E coli and coliform in Street Food ***Ekushe TV;*** ***Channel 24*** ****https://youtu.be/6BVv9YwkrwM**
Newspaper/Popular article		04	Harmful bacteria E coli, coliform found in Fuchka, Chotpoti***;*** ***"The Busuness Standard" News-3.*** **Prothom Alo.** **Amader Samoy.** **The Daily Star.** ****https://fb.watch/7f7WW79r_c/**
Other publications, if any	MS Thesis 02	-	I. Fattening performances and meat quality of broilers fed commercial feeds containing hazardous substances I. Growth, egg production of pullets and egg quality as affected by different layer feeds containing hazardous substances in different season

G. Description of generated Technology/Knowledge/Policy

i.a. Technology fact sheet: *The present research is not involved with any technology generation related activities.*

i.b. Description of generated knowledge

1. Health risk facts in commercial feed and chicken meat

Introduction

Food is a source of joy for people around the world, but those limited by their resources. Although the basic stapes are essential for biological energy production, these do not satisfy protein requirements. The availability of affordable and accessible protein through the poultry sector, as a result, has been a considerable development. Chicken is the most popular single meat source in Bangladesh, and it is the second most consumed meat in the world, behind pork. This universal popularity can be explained by the fact that this meat is not a subject of culturally or religiously set limitations, and it appears as nutritionally valuable foods with low fat content, in which there are more desirable unsaturated fatty acids than in other types of meat (Barroeta 2007, Cavani et al. 2009). Kris Gunnars (2020) calculated the daily intake protein based on Dietary Reference Intake of 0.8 g per kg body weight. This amounts to i) 56 g per day for an average sedentary man and 46 g for an average sedentary woman. A recent report indicates that per capita annual meat (broiler and *sonali*) consumption is 6.3 kg which shares about 40% of the total meat intake in Bangladesh (https://wpsa-bb.com/poultry-at-a-glance/) against 37% share in 2016 (Hamid et al. 2017). The prominent rating in poultry meat consumption is influenced by many factors, viz. short fattening period, excellent space utilization, high reproductive ability, excellent feed conversion, satisfactory nutritional value and relatively low sales prices (Gordana Kralik et al. 2017). Quick growth and fattening of meat type chicken, achieved in combination with superior chicken genotype and balanced diets, are nowadays done in an intensive way where balanced feed with increased content of microalgae and vegetable and fish oils is used to enrich poultry products with desirable fatty acids. But there is a common belief persists among a large portion of population of Bangladesh that feed manufacturers are using hazardous chemicals and ingredients in commercial poultry feeds which ultimately is transmitting to chicken meat causing cancer, liver damage, obesity, etc. in human body. Considering the facts, the present efforts are directed towards searching the hazardous materials in commercial feeds and their effects in productivity and meat quality of broilers.

Description

a. Commercial broiler and house feeds and their quality

Poultry, especially chickens are very sensitive to feed quality and forms. Success in the farming business mostly depends on feeding quality feed and feed formulation system. Broilers have different feed requirements in terms of various nutrients during different stages of growth. Young broilers have a high protein requirement for the development of muscles, feathers, etc. As the broilers grow, their energy requirements for the deposit of fat increase and their protein requirements decrease. They require high protein content in their starter rations than in the grower and finisher rations. Nutritionists should look at several critical parameters whilst formulating broiler feed like nutritive value of raw materials, amino acid digestibility, amino acid and calorie ratio, selection of suitable fat source, calcium requirement of birds, electrolyte balancing, immuno-modulation, seasonal management, micronutrient optimisation, etc.

Some commercial broiler and house feeds of large, medium and small companies were subjected to laboratory analysis for toxic elements, nutrient component and antimicrobial drugs following standard procedures. Study findings indicate that about one-half of the broilers and all house feeds were free from chemical hazards. It was also found that some feed manufacturers at times add one or more antibiotics. Heavy metals (Pb, Cr and As) contents varied from feed to feed and these are derived from raw feed ingredients, not from exogenous sources. Grain and grain by-products uptake these heavy metals from contaminated soil and water.

b. Commercial feed and residues in meat

It was earlier mentioned that some manufacturers at times mix antibiotics in powder form in feeds. Besides, therapeutics is another important source. But in most of the cases as detected under the study, there found no residual effects of these in broiler meat. The reported residual effects of these chemicals substances in raw meat was within safe levels i.e. these chemicals substances were at all not harmful for human health. Similarly, different heavy metals in feed had no health hazard effect in broiler meat. It is concluded that dietary chemical hazardous substances and heavy metals have a nominal residual effect and broiler meat is safe for human consumption.

c. Nutritive value of poultry meat

Chicken meat is a good source of good-quality protein with a low fat content. It is very important to select a good genotype and balanced nutrition and to have good production conditions during chicken fattening. Supplementary functional ingredients in diet improve the nutritional value of chicken meat. It becomes a functional food through the increase in the content of bioactive substances (n-3 PUFA, carnosine, selenium, etc.) that have beneficial effects on human health. However, chicken meat is appropriate for quick and simple preparation. When compared to other meat, it is proved that chicken breast meat contains more protein and less fat than red meat. The nutritive content of different types of meat (per 100 g) presented in appendix A of component 2.

Benefits of the knowledge

People would be encouraged consuming poultry meat raised in intensive way providing commercial feed and thereby protein malnutrition will be reduced and industry will be benefitted.

2. Table eggs from commercial flock -safe enough for human consumption

Introduction

Would we think without eggs in our daily life? It becomes a dietary mainstay, not only for breakfast but to feed finicky kids, stand in for a quick lunch or supper, blend raw into holiday nogs, and as an ingredient in all kinds of sweet and savory dishes (Kathleen M. Zelman, 2004). The inexpensive sources of protein offer tremendous nutrition and can play a role in a healthy diet. Eggs are wonderfully versatile, and can be eaten on their own or used in baked goods, sauces, puddings, ice cream and casseroles. In spite of that egg had unwholesome misconceptions for few decades due to its high cholesterol content, rumours about hazardous substances in poultry feed and residues in egg, etc. It is fact that laying hens

may often expose to various hazards from the environment they belong. They can ingest contaminants via feed, water, and veterinary drugs, and those can be transferred to the eggs. Eggs contain a high percentage of fat and have the potential to accumulate polychlorinated biphenyls and doxins. Such chemicals substances may enter the egg production chain and potentially affect human health, although fewer people will be affected by chemical hazards in eggs compared with microbiological hazards (USDA 1997). Chemical hazards can be internationally introduced during the egg production process, or unintentionally enter the egg production chain. Beran and Baum (1997) reported that major source of chemical contamination of eggs is through exposure of laying hens to chemicals at the layer farm. Inappropriate management, e.g. lack of good agricultural and veterinary practices, and inadequate hygiene by personnel and equipment, has the potential to result in introducing chemical hazards into a laying flock. Higher stocking density of cage flocks might result in increased infection rates of pathogens resulting in a higher need for medication (EFSA 2005). Heavy metals can potentially contaminate the egg production chain through feed and water, or through contamination during processing. Heavy metals in feed tend to accumulate in the liver and kidney of birds, but not much specific information is available for transfer of these compounds to eggs. Some previous finding also indicates that relative contribution of eggs and egg products to dietary exposure to heavy metals is low.

Description

a. Hazardous compound in layer feeds

A general assumption is that residues might be lower in more extensive production system, as less or no protective or curative substances are used. A general assumption is that residues might be lower in more extensive production system, as less or no protective or curative substances are used.

Contamination at layer farm in the egg production chain may be result of contaminated feed, use of additives, cleaning and sanitizing agents, insecticide, pesticide and antimicrobial spray and use of veterinary drugs to treat hens. In hot-humid countries like Bangladesh, different feed-grade chemical agents like antibiotics, anticoccidial drugs, etc. are often reported to add in varying levels during feed manufacturing process. Heavy metal accumulations in feed are usually derived from environmental sources. These can be transfer from the soil to plant, animal and consequently man through progressive food chain and bioaccumulation. Richard Alexis Ukpe and Augustine A Chokor (2018) reported that poultry feed also has the potential to contaminant several components of the environment. Most often, poultry feed produced from contaminated plant materials will also be contaminated.

However, findings of laboratory analysis indicate that commercial feeds were almost free from chemical contaminations except a few instances. There are very low levels of some of the antibiotics were found in the compound feed. It was also found that Cr and As accumulation in most of the feed was much higher than the recommendations, but Pb contents amount minimal.

b. Hazardous residues in table eggs

Heavy metal contamination of the food chain is well-said issue in Bangladesh. A general assumption is that residues might be lower in more extensive production system, as less or no protective or curative substances are used. Antimicrobials fed to poultry either from feed or

therapeutic sources are an important public health issue because of possible pathogenic microbial resistance. However, research findings indicate that chemicals and heavy metal residues in raw table eggs were almost nil. Therefore, we may say that table eggs from commercial flock contain a very negligible amount of antibiotic and heavy metal residues and it is safe enough for human consumption

c. Egg Nutrients

How much nutrients in an egg? This table has the answers to the question. Nutritional value is expressed as per 100g of egg. The nutrients information of different birds eggs are shown in appendix B of component 2.

The American Heart Association previously recommended limiting cholesterol to 300 milligrams per day, but the guidelines changed as research shows that dietary cholesterol is not associated with an increased risk of heart disease. Eggs are a significant source of dietary cholesterol, but several studies have shown that eating them is not associated with an elevated risk of heart disease. In fact, research suggests that eating eggs could actually help lower the risk for heart disease in some people (Shin et.al 2019).

Finally, know that the taste of an egg is influenced by the hen's diet. Some people think they can taste a difference between white- and brown-shelled eggs, but the shell color does not influence flavors. Some cooks prefer brown eggs simply because brown shell bits are easier to see to remove from a cracked egg in a bowl or from a hard-boiled egg.

Benefits of the knowledge

People would be encouraged consuming table eggs from commercially raised poultry species with low price. The inexpensive sources of protein offer tremendous nutrition and can play a role in a healthy diet applicable for almost all age groups.

ii. Effectiveness in policy support (if applicable)

Findings and information generated under the present study could be effectively utilized along with the existing respective policy/regulations of the sector for safe poultry meat and egg production activities. The indicative supportive findings have been shown as follows:

- Greater extent unauthorized and indiscriminate application of antibiotics and other drugs in different steps of poultry industry at field level is a serious threat to the sector. Upgrading of present regulatory and monitoring functions is a prime requirement for the development of the sector;
- Certain feed-grade antibiotics and others drugs are used in commercial poultry feed although their residues effects in meat and egg are far below the permissible limits. Amendment of regulatory guidelines to control use of any sort of drug is importantly necessary.
- Microbial loads in various arenas of poultry units and products which is the outcomes of prevailing hot-humid environment and partly unhygienic conditions. To control contamination in production centers, there should be particular regulation functional in field on maintaining of prescribed temperature and humidity control range;
- Chemical and biological hazards in poultry industry and adulteration/contamination sources/steps in poultry products were identified that indicates non-implementation of

existing respective regulation in the sector. Emphasize should be given on the present regulatory practices on priority basis for the interest of the sector and consumers, as well.

H. Technology/ Knowledge generation/Policy support (as applied)

i. Immediate impact on generated technology (commodity and non-commodity)

The set objectives and activities thereunder of the present research sub-project are not related with the development of any technology. However, the knowledge and information generated through the research can contribute significantly to the sector by establishing safe production of poultry meat and eggs by mitigating the identified issues of the sector. Thus, the impacting areas can be narrated as follows:

a. Knowledge about qualitative aspects of feed, meet and eggs production process of the farmers will increase on the basis of the research findings.
b. Management of hygiene practices throughout the total production system of poultry feed, meat and egg will increase significantly and food safety in the sector will be established.
c. Consumer's interest towards poultry meat, egg and other products intake will be increased.

ii. Generation of new knowledge that help in developing more technology in future

Considerable information has been invented on the quality of commercial broiler and layer feed of various manufacturers and also on the yield and productivity of chicken. Moreover, levels of residuals effects in poultry products also determined. As the process requires long way to go and huge monetary involvement, relevant government agencies should take the responsibilities in determination of hazardous residues in commercial feed and poultry products periodically to ensure safe food production process which should be a continuous process. Routine check-up to ensure quality standards and safety of these products should be done by highly professional institutions of Bangladesh.

iii. Technology transferred that help increased agricultural productivity and farmers' income

Not applicable at this stage

iv. Policy support

1. One of the country's national policies is to increase the production of poultry meat and egg to meet the national demand. This research assessed feeds intake, weight gain and feed conversion of broilers and, growth, production performances and egg quality of layers. Application of these management facts in commercial production levels may increase the production of meat and egg. In addition, it will help in mitigating malnutrition and creating new employment in poultry industry.
2. Indiscriminate and unauthorized use of antibiotics and other drugs has become our national concern in recent time. Bangladesh government has been trying to reduce the abuse and unauthorized use of drugs/antibiotic to reduce the drug resistance. This project explored that a large numbers farmers used drug advised by unauthorized persons or by their own choice. This result may trigger the policy makers to increase awareness among the farmers and enforce the laws to combat the problems.
3. Food safety is another concern of our policy makers. Residual effect of drugs and heavy metals in poultry feed, meat and egg is a big concern for human health. This

investigation isolated antibiotics and others drugs used in feed as well as their residues in meat. The results will help policy makers to take proper steps to reduce adverse effects on human health and also to stop rumors against the poultry industry.

4. Implementation of HACCP is another concern for both food production and health policy. HACCP is world-wide recognized logical approach that systematically evaluates and control the entire food production process for reducing potential food-related health risks to consumers. This research discovered the chemical/ biological hazards and the steps of their adulteration/contamination of poultry products. This output will help to control the steps of contamination for production of safe poultry.

I. Information regarding desk and field monitoring

i. Desk monitoring (description and output of consultation meeting, monitoring workshops/seminar etc.)

Monitoring team	Date (s) of visit	Total visit	Output
Component 1: PSTU			
1. Technical Division/Unit, BARC	08.09.2019	01	Recording and maintenance of project information, preparation of documents
2. PIU-BARC, NATP-2	11.07.2020 06.10.2020	02	Maintenance of accounts and procurement documents, identification of equipment and furniture
Component 2: HSTU			
1. Technical Division/Unit, BARC	11.02.2019	01	Reporting of financial, cash book, register etc. and project information documents, total number of analyzing feed sample and base line survey
2. Project Coordinators Unit	6.12.2020	02	Visit the laboratory and discuss the progress of field experiment
3. PIU-BARC, NATP-2	18.03.2021	01	Suggestion about procurement- related documents and HACCP activities of different field of poultry industry, monitoring of furniture and other field equipment

ii. Field monitoring (date and no. of visit, name and addresses of team visit and output)

Monitoring team	Date (s) of visit	Total visit	Output
Component 1: PSTU			
1. Technical Division/Unit, BARC	08.09.2019	01	Received suggestions in conducting field experimentation and data management
2. PIU-BARC, NATP-2	11.07.2020 06.10.2020	02	Detailed discussion on the background and the status of on-going field experiments
3. Others visitors Kazi Farms Ltd. HSTU Team	 26.12.2019 06.04.2020 12.10.2020 02.02.2021 18.02.2021 19.02.2021 25.04.2020 26.04.2020	 04 02	Management and therapeutic (when necessary) suggestions followed by field observations
Component 2: HSTU			
1. Technical Division/ Unit, BARC	11.02.2019	01	Suggested to mention the specific names of chemicals/antibiotics or heavy metals in the experiment
2. Project Coordinators Unit	7.12.2020	01	Visit the experimental shed of broiler and layer and discuss the research activities.

3. PIU-BARC, NATP-2	19.03.2021	01	Monitoring the research activities and suggestion the improvement of research activities.
5. Other visitors PSTU Team	03.06.2021 04.06.2021 26.12.2020 27.12.2020 26.09.2019 27.09.2019	06	Visit the experimental trial and discuss about research activities and suggestions followed by field observations.

iii. Weather data, flood/salinity/drought level (if applicable) and natural calamities:

Three years (2018 – 2020) average weather data of Patuakhali region

Parameters	Seasons						Remarks
	Pre-Monson (January – April)		Monson (May – August)		Post Monson (Sept – December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	127	6	379	234	270	9	
Av. Temperature (^{O}C)	28.7	19.3	29.0	27.5	27.4	20.3	
Av. Humidity (%)	73	63	88	80	87	72	
Flood (year & category)	Strong upstream water pressure and heavy rainfall in September 2020, water level of the rivers have increased and lowland areas, river banks and many parts of shoals have been marooned. Many poultry farms were under floodwater. Heavy rainfall due to deep depression also raised river water and immerge some of the roads and crop field in October 2020.						
Av. Salinity (ppt)	0.9	1.4	0.5	1.0	0.8	1.2	
Natural calamity (Frequency & category)	Tides due to Cyclone "Yaas" inundated low-lying areas of Banuganj and Uzirpur upazila and damaged crop in the end of May 2021						

**Regional Office, Dept. of Metrology; Basisal*

Three years (2018 – 2020) average weather data of Dinajpur region

Parameters	Seasons						Remarks
	Pre-Monson (January – April)		Monson (May – August)		Post Monson (Sept – December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	30	3.6	234.9	138.5	250.1	3.7	
Av. Temperature (^{O}C)	38	16	37	33	26	17	
Av. Humidity (%)	52	34	80	57	78	50	
Flood (year & category)	None						
Av. Salinity (ppt)	0.5	0.6	0.4	0.6	0.8	0.9	
Natural calamity (Frequency & category)	-	-	-	-	-	-	

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J. Sub-project auditing (covers all types of audits performed)

i. Coordination component: BARC

Types of audits	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial & Performance Audit by FAPAD on 29.10.19 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	2157869.00	Financial management of the component found running smoothly till the end of the project. No query or objection raised at any stage of operation by the audit teams.	Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 09.12.20 for the year 2019-2020.	No objection raised, found all relevant documents updated as per guideline	1460104.00		Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 11.10.21 for the year 2020-2021.	No objection raised, found all relevant documents updated as per guideline	1627805.00		Financial management & project performance found satisfactory

ii. Component 1 (PSTU)

Types of audits	Major observations/issues/ objections raised; if any	Amount of audit (Tk.)	Status in the sub project end	Remarks
Financial & performance audit by the FAPAD Audit Team (2018-2019)	No objection raised by the audit team	4038931.00	Sub-project financial management was found satisfactory throughout the whole period	Satisfactory
Financial & performance audit by the FAPAD Audit Team (2019-2020)	No objection raised by the audit team	6365654.00		Satisfactory
Financial & performance audit by the FAPAD Audit Team (2020-2021)	No objection raised by the audit team	5517371.00		Satisfactory

iii. Component 2 (HSTU)

Types of audits	Major observations/issues/ objections raised; if any	Amount of audit (Tk.)	Status in the sub project end	Remarks
Financial & performance audit by the FAPAD Audit Team (2018-2019)	No objection raised by the audit team	790227.00	Sub-project financial management was found satisfactory throughout the whole period	Satisfactory
Financial & performance audit by the FAPAD Audit Team (2019-2020)	No objection raised by the audit team	3186093.00		Satisfactory
Financial & performance audit by the FAPAD Audit Team (2020-2021)	No objection raised by the audit team	2558300.00		Satisfactory

K. Lessons Learned

1. The findings of the research reflected that only one-third of the farmers of the study area received training on different aspects of poultry farming. While majority of the farmers (92%) by applying their own knowledge used antibiotics against infectious organisms during farming and only 45% farmers solved the problems of their farms consulting technical personnel. Generally, they followed dealers' suggestions along with their own experiences. In majority cases (84%), dealers provide unauthorized veterinary services to the farmers which can invite new damages and threat.
2. Qualitative information on poultry feed focuses that majority of the commercial broiler and layer feeds are adulterated/contaminated with hazardous antibiotics, heavy metals and microorganisms.
3. Broiler growth parameters were influenced by geographical location, commercial feed and rearing season without any influence on survival rate.
4. Geographical location, feed, and rearing season also influenced growth and egg production performances. Egg quality characteristics were not affected by feed.
5. Microorganisms were isolated from different steps of poultry production and processing indicates poor hygiene practices in the existing culture and production system.
6. Residual antibiotics and heavy metals found in broiler meat. Residual heavy metal also detected in egg contents. But these residues were within permissible limits.

L. Challenges (if any)

1. Participating farmers had strong faith on their knowledge base, so it was difficult task to take them out of their belief in managing the farm.
2. Application of drugs based on dealers' advice along with own experience for treating the birds in the farm without taken into consideration the advice of technical person is a great challenge towards mitigating safe poultry meat and egg production issues.
3. Heavy rainfall during rainy season sometimes interrupted the management events in the shed. In addition, Covid-19 outbreak also interrupted feed procurement from different sources those cumulatively became a challenging issue for the farmers to survive their business and to continue the research interruptedly, as well.

M. Suggestions for future planning (if any)

1. Farmer's knowledge gap particularly on farm managements, health and hygiene management, selection of drugs and application protocol, residue effect and other hazards are the weaknesses those acts negatively against safe poultry meat and egg production. Adequate hands-on management training of the farmers and operators as a continuous process can play positive role towards meeting the gap.
2. Strengthening of regulatory and enforcement agencies to protect production, importing marketing and unethical application of unauthorized drugs and chemicals and feed ingredients in poultry farming should be established.
3. Commercial feed mills should be taken under regular monitoring and surveillance for controlling of adulteration/contamination practices of unwanted chemicals antibiotics and microorganisms.

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Signature of the Coordinator	**Counter signature of the Head of the organization/authorized representative**
	
(Dr. Md. Monirul Islam)	***(Dr. Shaikh Mohammad Bokhtiar)***
Date: 25.11.21	Date: 25.11.21
Member Director (Fisheries)	***Executive Chairman***
Bangladesh Agricultural Research Council	***Bangladesh Agricultural Research Council***

APPENDICES

1. Coordination component (BARC)

Appendix A. Recommendation of the Inception Workshop and status of action taken

Recommendations	Action Taken
Component 1 (PSTU)	
• Suggested simplification of the flow diagram shown under Approach and Methodology;	Has been simplified by reducing some steps
• As per objective of the research, works on value addition need to be focused more clearly under methodology;	It has enumerated in the Methodology section.
• Outputs and outcomes of the project are not stated properly. Expected outputs and outcomes of the project are to be mentioned under the line of demarcation (like, Outputs will present the result/production of the work while the outcome will be the ultimate broader aspect of impact of the result/production);	It was re-written.
Component 2 (HSTU)	
• Flow diagram shown under Approach and Methodology seems complex. Simplification of the same suggested;	It has been done.
• Commercial feed samples from whole sale market should be given preference for quantification of hazardous materials and its residue;	Feed samples were collected from dealers point from where farmers procure.
• HACCP standards to be implemented should be specify in the methodology;	It has been detailed out in the Methodology section.
• Outputs and outcomes of the project are not stated properly. Expected outputs and outcomes of the project are to be mentioned under the line of demarcation (like, outputs will present the result/production of the work while the outcome will be the ultimate broader aspect of impact of the result/production);	It was re-written.

Appendix B. Recommendation of the Half-yearly Workshops

Workshop recommendations	Actions taken
Component -1 (PSTU)	
• Suggested to indicate the location and project sites under the base line survey and various other field experiments by area mapping instead of simple description;	Not possible to imply
• If possible , the study of prevailing Cr character/nature (hexavalent or teytravalent) to understand the type and degree impact to be occurred;	Completed up to a limited level;
• Name of four feed diet/rations not clearly indicated;	Done accordingly
• Lingering of growth time (slower growth) through appropriate feeding management and arrangement of clear drinking water for bird as an mitigation measure for reducing the residue level and effect of antibiotics and heavy metals suggested;	Necessary steps taken
Component -2 (HSTU)	
• No specific suggestion made;	

Appendix C. Recommendation of the Annual Workshop

Recommendations of the First Annual Workshop	Action taken
Component 1 (PSTU)	
• Study and identification of the form of heavy metal "Cr" (Hexavalent or Trivalent) emphasized to understand the degree of health hazards;	Hexavalent Cr is more carcinogenic. But, the laboratories can analyze total chromium only.
Component 2(HSTU)	
• List of different house feed applied to different animals/birds, their body weight, state of development, rate of feed application and frequency of application are to be shown clearly in the report;	It has been included in the report.
Recommendations of the Second Annual Workshop	**Action taken**
Component 1 (PSTU)	
• Baseline survey data should clearly represent the proportion of respondents (by %) involve with use of therapeutic drugs, antibiotics, anti-coccidial drugs, enzymes, growth promoters and vaccines etc ;	Presented in details in the report.
• Biological trials of selected commercial feeds for determining the hazardous substances in poultry products within the remaining working period of the project urged;	Carried out as per research plan
• Effects of boiling time on chemical residues in broiler meat, determination of salt level and chemical residues (if any) in value added products are need to completed within shortest time. In this matter, as per plan, communication with the relative agencies may be re-instead and strengthening initiative may be taken by the respective PI.	Actually Covid-19 pandemic affected the sending process of frozen samples to Indian for Laboratory analysis from SGS India Ltd.
• Immediate completion of laboratory trials for microbial safety analysis of available poultry feed and evaluation of implementation level of HACCP standards emphasized ;	It is already done.
Component 2(HSTU)	
• Cross interactive tests of selected feed for heavy metals suggested to follow for better result;	The whole analyses were done from an accredited Laboratory, SGS India Ltd.
• Immediate completion of evaluation of implementation level of HACCP standards emphasized ;	It has been assessed.
• Suggested to expose specifically the identified hazardous chemicals in different poultry feeds for better understanding;	Necessary step taken & this has been focused in them next progress reports & review workshops

Appendix D. Recommendation of the coordination meetings

Central Coordination meeting at BARC	
Recommendations	**Action taken**
• In addition to analysis of only carcass muscles of birds for antibiotic and heavy metal residue it should also cover various internal organs (like liver, kidney),skin and head parts of birds;	It requires a huge cost. Our major thrust was to analyze major edible parts.
• Depending upon the availability of fund, analysis of antibiotic and heavy metal residue level at various stages of grown up culture (like initial, mid and consumption stages) to understand the level of residue concentration pathway;	Actually it was our concern about the meat and eggs for table consumption. Thus, only one time analysis was considered.
• To attain desired progress as per activity plan of the HSTU component, emphasizing of acceleration of research progress in coordination with the other project component;	It has been completed.
• Meaning of ***"House Feed/Market Feed/Normal Feed"*** of poultry birds need to clarify;	It has been explained in respective study in the report
Two Other virtual Coordination meetings	
Component 1 (PSTU)	
• Necessary measures to complete the trials of previously identified hazards (antibiotics and heavy metals) in poultry feed and eggs within August'20;	Has been completed.
• Study on HACCP standards for poultry feed and broiler meet are to be carried out jointly for both the components (PSTU and HSTU) during the last three working months of the project;	Assessment has been included in the report.
• Finding out alternate way for involving manpower due to absence of working students because of long university holiday which has been hampering field data/sample collection etc.	The tasks were conducted with the technical assistance from Md. Shajadul Islam, Microbiologist, Indo-Bangla Pharmaceuticals Ltd., Barishal
Component 2 (HSTU)	
• Re-examination of baseline data before finalizing for presenting in report;	Has been done.
• Remaining two experiments on heavy metals and antibiotics analysis are to be completed by July'20;	It has been completed.
• Alternate way for involving of supporting technical persons need to be considered at project level by the PI considering project time length and volume of research to be done;	It has been done.
• Study on HACCP standards for poultry feed and broiler meet are to be carried out jointly for both the components (PSTU and HSTU) during the last three working months of the project;	It is assessed.

2. Component 2 (PSTU)

Appendix A: *Nutritive content of different types of meat (per 100 g)*

Nutrient	Chicken	Pork	Beef	Lamb
Energy/kcal	165	165	185	180
Water/g	65.26	65.75	64.83	64.92
Protein/g	31.02	28.86	27.23	28.17
Total fat/g	3.57	4.62	7.63	6.67
Saturated fatty acids	1.010	1.451	2.661	2.380
Monounsaturated fatty acids	1.240	1.878	3.214	2.920
Polyunsaturated fatty acids	0.770	1.066	0.285	0.440
Cholesterol (mg)	85	86	78	87
Calcium (mg)	15	16	6	8
Iron (mg)	1.04	0.97	2.40	2.06
Magnesium (mg)	29	27	18	26
Phosphorus (mg)	228	273	172	208
Potassium (mg)	256	425	222	342
Sodium (mg)	74	80	36	66
Zinc (mg)	1.00	2.48	4.74	5.02
Vitamin C (mg)	0.0	0.0	0.0	0,0
Thiamin (mg)	0.070	0.523	0.057	0.110
Riboflavin (mg)	0.114	0,408	0.170	0,280
Niacin (mg)	13.712	7.940	5.232	6.390
Vitamin B6 (mg)	0.600	0.538	0.380	0.170
Folate (µg)	4	0	9	24
Vitamin B12 (µg)	0.34	0.67	1.61	2.71
Vitamin A (µg)	6	1	0	0
Vitamin E (mg)	0.27	0.26	0.37	0.18
Vitamin D (D2 + D3) (µg)	0.1	0.3	-	-
Vitamin K (µg)	0.3	0.0	1.3	-

Source: USDA; National Nutrient Database for Standard Reference Release 28 [Internet]. 2016. Available from: https://ndb.nal.usda.gov/ndb/search/list [Accessed: 2017-09-25]

Appendix B: *Nutrients information of different birds eggs*

Nutrient	Quail	Duck	Goose	Turkey	Chicken	Ostrich	Emu
Average weight(g)	9	70	144	79	56	1400	550
Proximate							
Water	74.35	70.83	70.43	72.5	76.15	75.1	71.8
Protein	13.05	12.81	13.87	13.68	12.56	12.2	12.9
Total lipid (fat)	11.09	13.77	13.27	11.88	9.51	11.7	13.8
Ash	1.1	1.14	1.08	0.79	1.06	1.4	1.3
Carbohydrate	0.41	1.45	1.35	1.15	0.72	0.7	0.7
Energy (**Kcal**)	158	185	185	171	143	151	174
Minerals (mg)							
Calcium, Ca	64	64	60	99	56	64.7	63.9
Iron, Fe	3.65	3.85	3.64	4.1	1.75	2.51	2.43
Magnesium, Mg	13	17	16	13	12	13.92	12.5
Phosphorus, P	226	220	208	170	198	196.71	198.12
Potassium, K	132	222	210	142	138	122.5	120.7
Sodium, Na	141	146	138	151	142		
Zinc, Zn	1.47	1.41	1.33	1.58	1.29	1.34	0.98
Copper, Cu	0.062	0.062	0.062	0.062	0.072	0.54	0.43
Manganese, Mn	0.038	0.038	0.038	0.038	0.028	0.16	0.21

Nutrient	Quail	Duck	Goose	Turkey	Chicken	Ostrich	Emu
Selenium, Se (µg)	32	36.4	36.9	34.3	30.7	32.4	32
Vitamins (mg)							
Thiamin	0.13	0.156	0.147	0.11	0.04	0.15	0.15
Riboflavin	0.79	0.404	0.382	0.47	0.457	0.24	0.24
Niacin	0.15	0.2	0.189	0.024	0.075		
Pantothenic acid	1.761	1.862	1.759	1.889	1.533	0.75	0.75
Vitamin B-6	0.15	0.25	0.236	0.131	0.17		
Choline, total	263.4	263.4	263.4		293.8		
Vitamin E (alpha-tocopherol)	1.08	1.34	1.29	–	1.05	0.04	0.04
Vitamins (µg)							
Folate, total	66	80	76	71	47	48	48
Vitamin B-12	1.58	5.4	5.1	1.69	0.89		
Vitamin A, RAE	156	194	187	166	160	5.79	5.79
Retinol	155	192	185	166	160		
Carotene, beta	11	14	13		0		
Cryptoxanthin, beta	10	12	12		9		
Lutein + zeaxanthin	369	459	442	–	503		
Vitamin D (D2 + D3)	1.4	1.7	1.7	–	2	–	–
Vitamin K (phylloquinone)	0.3	0.4	0.4	–	0.3	–	–
Vitamins (IU)							
Vitamin A	543	674	650	554	540	383	383
Vitamin D	55	69	66	–	82	–	–
Lipids (g)							
Fatty acids, total saturated	3.557	3.681	3.595	3.632	3.126	–	31*
Fatty acids, total monounsaturated	4.324	6.525	5.747	4.571	3.658	–	58*
Fatty acids, total polyunsaturated	1.324	1.223	1.672	1.658	1.911	–	10*
Cholesterol (mg/gm)	844	884	852	933	372	10.3	15.7
Amino Acids (g)							
Tryptophan	0.209	0.26	0.282	0.219	0.167	–	–
Threonine	0.641	0.736	0.797	0.672	0.556	1.013	–
Isoleucine	0.816	0.598	0.647	0.855	0.671	0.672	–
Leucine	1.146	1.097	1.188	1.201	1.086	1.336	–
Lysine	0.881	0.951	1.03	0.924	0.912	0.947	–
Methionine	0.421	0.576	0.624	0.442	0.38	0.395	–
Cystine	0.311	0.285	0.309	0.326	0.272	–	–
Phenylalanine	0.737	0.84	0.91	0.773	0.68	0.6	–
Tyrosine	0.543	0.613	0.664	0.569	0.499	0.547	–
Valine	0.94	0.885	0.958	0.985	0.858	0.811	–
Arginine	0.835	0.765	0.828	0.876	0.82	0.527	–
Histidine	0.315	0.32	0.346	0.33	0.309	0.284	–
Alanine	0.762	0.631	0.683	0.799	0.735	0.316	–
Aspartic acid	1.294	0.777	0.841	1.357	1.329	–	–
Glutamic acid	1.662	1.789	1.937	1.742	1.673	–	–
Glycine	0.434	0.422	0.457	0.455	0.432	–	–
Proline	0.518	0.48	0.52	0.543	0.512	–	–
Serine	0.992	0.963	1.043	1.04	0.971	0.832	–

Source: *USDA Food Composition Databases (2018) Angel (1993), Du Preez (1991).*

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